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ORIGINAL ARTICLE

Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli

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Abstract The aim was to evaluate the effect of xylitol and probiotic chewing gums on salivary mutans streptococci (MS) and lactobacilli (LB). The material consisted of 80 healthy young adults (21–24 years) who volunteered after informed consent. They were assigned by random into one of four parallel study groups: A, probiotic gum group; B, xylitol gum group; C, probiotic + xylitol gum group; and D, placebo gum group. The gums were taken three times daily after meals, and the intervention period was 3 weeks. The probiotic gums contained two strains of *Lactobacilli reuteri* (ATCC 55730 at a dose of 1×10^8 CFU/gum and ATCC PTA 5289 at a dose of 1×10^8 CFU/gum), and each pellet of the xylitol gum contained ≈1.0 g xylitol as single sweetener. Pretreatment and posttreatment samples of stimulated whole saliva were collected and quantified for MS and LB with chair-side kits. A statistically significant reduction ($p<0.05$) of salivary MS was displayed in group A and B after the intervention when compared with baseline. A similar but nonsignificant tendency was seen in group C. No alterations of salivary LB was demonstrated in any group. In conclusion, daily chewing on gums containing probiotic bacteria or xylitol reduced the levels of salivary MS in a significant way. However, a combination of probiotic and xylitol gums did not seem to enhance this effect.

Keywords Bacteriotherapy · Cariogenic bacteria · Chewing gums · Oral ecology · Whole saliva

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Introduction

Dental caries forms through a complex interaction over time between acid-producing aciduric bacteria and fermentable carbohydrates (for a review, see [23]). Thus, actions taken towards microorganisms and sugars are important strategies to prevent the disease. It has however become increasingly clear that measures directed at eliminating specific caries-associated microorganisms, which are members of the endogenous microflora, have been proven difficult and maybe also unwise [28]. Consequently, alternative ways to affect the oral ecology have emerged such as bacteriotherapy and sugar substitution. Bacteriotherapy, or replacement therapy, is an alternative way to combat infections, and the administration of probiotic bacteria is the most common of its clinical applications. Probiotics are live microbial feed supplement that beneficially affects the host by improving its intestinal microbial balance as documented in clinical trials [3, 9, 19]. Previous clinical studies have indicated that lactobacilli-derived probiotics in dairy products may hamper the growth of salivary mutans streptococci [1, 21, 22]. Furthermore, our research group has shown similar results from daily intakes of yoghurt containing bifidobacteria and tablets with *Lactobaccillus reuteri* [4, 5]. Xylitol is a natural sugar alcohol commonly used as sweetener in chewing gums and

candy (for reviews, see [2, 18]). Adequate daily doses of the pentitol may affect the oral ecology by decreasing plaque acidogenicity and suppressing the proportion of oral mutans streptococci [11, 16, 20]. To our knowledge, no study has hitherto evaluated the effect of a combined supplementation of xylitol and probiotic bacteria on the levels of salivary mutans streptococci and lactobacilli. The aim of the present investigation was therefore to compare the effect of xylitol and probiotic chewing gums, as well as a combination thereof, on the levels of mutans streptococci and lactobacilli in the saliva of young adults. The null hypothesis was that the bacterial levels would remain unchanged in all study groups.

Materials and methods

Study group

The material consisted of 80 healthy young adults (44 women, 36 men), 21–24 years of age, who volunteered after receiving verbal and written information. Subjects with a history of systemic antibiotic or topical fluoride treatments within a 4-week period before baseline were not included nor were individuals with a habitual use of dairy probiotics or xylitol chewing gums. The subjects had a noncompromised oral health, and none exhibited untreated caries lesions or clinical signs of either gingivitis or periodontal disease.

Study design

The study protocol was approved by the School of Dentistry Ethics Committee at the University of Yeditepe, Istanbul, Turkey. The prospective investigation had a randomized placebo-controlled study design with four parallel arms and with an experimental period of 3 weeks. The size of the material was determined by a power calculation based on data from our previous trial [5]. The subjects were randomly assigned to one of four equally sized groups (n=20): group A consumed one chewing gum with probiotic bacteria three times daily; group B was given two chewing gums with xylitol three times daily; group C consumed two chewing gums with probiotic bacteria and four with xylitol daily; and group D chewed placebo gums without active ingredients three times a day. The use of the chewing gums was scheduled as shown in Table 1. Salivary samples were collected at baseline and 1 day after the final gum use. During the experimental period, the subjects were strongly encouraged to maintain their normal diet and continue to brush their teeth twice a day with fluoride-containing toothpaste.

Table 1 The 3-week schedule of chewing in the four randomized groups with 20 subjects in each

Group	Morning	Noon	Evening	Code
A	1 probiotic gum	1 probiotic gum	1 probiotic gum	Probi
B	2 xylitol gums	2 xylitol gums	2 xylitol gums	Xylit
C	2 xylitol gums	2 probiotic gums	2 xylitol gums	Xylit + Probi
D	1 placebo gum	1 placebo gum	1 placebo gum	Control

Chewing gums

The probiotic chewing gum (Biogaia, Stockholm, Sweden) contained two strains of *L. reuteri* (ATCC 55730 at a dose of 1×10^8 CFU/gum and ATCC PTA 5289 at a dose of 1×10^8 CFU/gum) and utilized sorbitol and sucralose as sweeteners. The xylitol chewing gum (Xylimax, Fennobon Oy, Karkkila, Finland) contained 77% xylitol corresponding to 1.03 g per pellet. The ingredients of the test gums are listed in Table 2. The participants were instructed to actively chew on the assigned gums during 10 min after the meals; in the morning (8:00–9:00 A.M.), at noon (12:00–1:00 P.M.), and evening (7:00–8:00 P.M.). The test and placebo products, provided by the manufacturer, were identical in size, form, and taste but without probiotic bacteria or xylitol. The sweetness and taste varied somewhat between the test and control gums, but the participants were not beforehand informed on its content. A 1-week supply was handed out to the subjects at the time to

Table 2 Ingredients of the chewing gums according to the manufacturer's declaration

	Probiotioc gum	Xylitol gum
Active strains	*L. reuteri* ATCC 5730	–
	L. reuteri ATCC PTA5289	–
Sugar/ sweetener	Sorbitol, sucralose	Xylitol
Miscellaneous	Gum base	Gum base
	Isomalt	Malic acid
	Flavors	Flavors
	Hydrogenated palm oil	Gum arabic
	Hydrogenated cotton seed oil	Soy lecithin
	Talc	Glycerol
	Silicon dioxide	Carnuba and bees wax
	Magnesium stearate	Shellac

continuously check the compliance. No toothbrushing was allowed for at least 1 h after the use of the chewing gums.

Saliva collection and microbial enumeration

Samples of paraffin-stimulated whole saliva were collected in the morning (10:00–11:00 A.M.) on the day before onset and 1 day after the intervention period. After a thorough rinse with water, the saliva was expectorated directly into a graded test tube during a 5-min chewing period, and the flow rate was calculated as milliliter per minute. The counts of salivary mutans streptococci and lactobacilli were evaluated using Dentocult SM (Strip Mutans®) and Dentocult LB® chair-side kits (Orion Diagnostica, Espoo, Finland) as described earlier [12, 15]. After cultivation at 37°C for 48 h and 96 h, respectively, the colony forming units (CFU) were identified on the basis of their morphology and counted in a stereomicroscope with 12–25 times magnification. The results were categorized in four scores as shown in Tables 3 and 4. The laboratory staff and the clinicians evaluating the test kits were blinded to the subject's group assignment.

Statistical methods

The data were processed with the SPSS software (version 14.0, Chicago, Ill., USA). Posttreatment and pretreatment values within each regimen were compared with a two-tailed marginal homogeneity test for categorical data. The baseline distribution and transitions between bacterial scores was tested between the groups with a two-tailed chi-square test. A p value <0.05 was considered statistically significant. The groups were coded for the investigators, and the code was not broken until the statistical calculations were finalized.

Table 3 Distribution of salivary mutans streptococci score at baseline and after 3-week consumption of different chewing gum regimens (n=80)

Time	n	Mutans streptococci score[a]				
		0	1	2	3	p
Group A (probi)						
Baseline	20	7	2	5	6	
3 weeks	20	12	5	3	0	<0.05
Group B (xylit)						
Baseline	20	6	3	4	7	
3 weeks	20	7	5	8	0	<0.05
Group C (xylit + probi)						
Baseline	20	5	5	7	3	
3 weeks	20	5	8	6	1	NS
Group D (control)						
Baseline	20	6	4	6	4	
3 weeks	20	7	5	4	4	NS

The values in the table denotes the number of subjects.
NS No statistically significant difference
[a] Score 0=0–10 CFU; Score 1=11–99 CFU; Score 2=100–500 CFU; Score 3=>500 CFU

Table 4 Distribution of salivary lactobacilli scores at baseline and after 3-week consumption of different chewing gum regimens (n=80)

Time	n	Lactobacilli (CFU/ml)				
		$\leq 10^3$	10^4	10^5	$\geq 10^6$	p
Group A (probi)						
Baseline	20	5	6	4	5	
3 weeks	20	8	6	3	3	NS
Group B (xylit)						
Baseline	20	5	8	2	3	
3 weeks	20	15	3	1	1	NS
Group C (xylit + probi)						
Baseline	20	2	4	8	6	
3 weeks	20	7	3	7	3	NS
Group D (control)						
Baseline	20	3	8	7	2	
3 weeks	20	4	9	5	2	NS

The values in the table denotes the number of subjects.
NS No statistically significant difference

Results

All subjects completed the trial, the compliance was excellent and no side effects were reported. The salivary secretion rates were within normal limits among the participants, ranging between 0.8 and 2.0 ml/min. The preintervention and postintervention levels of salivary mutans streptococci and lactobacilli are shown in Tables 3 and 4. All subjects had detectable levels of salivary mutans streptococci at baseline, and there were no statistically significant differences between the four groups concerning the distribution of scores. One day after the 3-week intervention period, significantly reduced levels (p<0.05) of mutans streptococci compared to baseline were displayed in group A and B. A similar but nonsignificant tendency was noticed in group C, while no changes were demonstrated among the subjects in the control group D. The levels of salivary lactobacilli were fairly stable on the individual level, and no statistically significant alterations were found in any of the four experimental groups.

The individual transitions between the pretreatment and posttreatment mutans streptococci scores are illustrated in Fig. 1. In group A, 11 subjects exhibited decreased scores (one to three steps) and 9 subjects had unchanged scores; the corresponding figures in group B were 11 and 8 subjects while one subject displayed a one-step increased score. In group C, decreased scores were registrated in eight

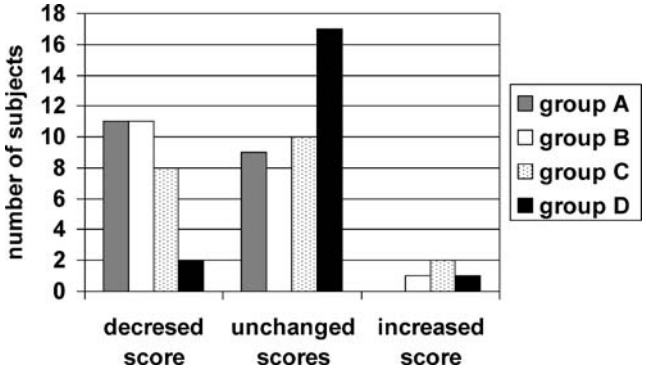

Fig. 1 Transition of salivary mutans streptococci scores when posttreatment levels were compared with the baseline in the different experimental groups. Decreased scores=1–3 steps under baseline; unchanged score=same as baseline; increased score=1 step over baseline. The distribution in group D (control) was significantly different ($p<0.05$) from the distributions in groups A–C

subjects, ten subjects had unchanged levels, and two subjects displayed a one-step increased score. The mutans streptococci transitions in all these three groups were significantly different ($p<0.05$) from the transitions in the control group.

Discussion

The present investigation was undertaken to investigate if a combination of two potential anticaries agents, probiotic bacteria and xylitol, would enhance the suppressive effect on caries-associated bacteria in saliva. The choice of chewing gums as vehicle for delivery was natural to obtain a comparable amount of saliva stimulation in all groups. The number of chewing gums differed, however, among the experimental groups and the practical reason was simply to obtain a xylitol dose in groups B and C close to the 6.0 g/day that is shown to be needed to affect the oral ecology [20]. It should however be stressed that all participants had normal saliva secretion and chewed 10 min, three times a day with good compliance, so it is less likely that the different pellet numbers had a major influence on the outcome. The chair-side tests were considered as robust endpoints as they have been validated and compared with conventional cultivation methods on selective agar plates in previous studies [8, 13].

The results in groups A and B were expected and reinforced previous findings. Thus, for the agents, one by one, the null hypothesis could be rejected. The novel approach in group A was that two strains of a probiotic bacterium were combined in a chewing gum, and the effect seemed comparable with the lozenges or preprepared straws that were evaluated before [4]. To our knowledge, only one previous trial has reported an oral outcome after probiotic chewing gum use [14], but that study did not investigate the suppressive effect on oral bacteria. In group B, the daily dose of xylitol equaled 6 g, and the results were in harmony with previous findings with that amount [11, 20]. Although the results looked similar, the mechanisms of antibacterial action are basically different for probiotics and xylitol. Probiotic bacteria can act through several pathways; they prevent cellular adhesion and invasion of pathogenic bacteria, modify the intestinal environment by a reduction in pH as a result of fermentation products, and they interact and modulate the local and systemic inflammatory immune response [9, 10]. *L. reuteri* has been shown to be an intestinal inhabitant of potential importance by secreting an antimicrobial compound, reuterin, which may partly be responsible for its positive effects [25]. The beneficial role of *L. reuteri* ATCC 55730 in general health, immunomodulation, and disease protection has been validated and described in earlier of studies [6, 7]. Xylitol, on the other hand, exerts its antibacterial action through hampering bacterial growth through metabolic reactions. Xylitol is incorporated in the cell with help of the fructose-specific phosphotransferase system and phosphorylated to xylitol-5-phosphate [27]. This substance inhibits further intracellular metabolism of the bacterial cell and the process consumes energy. After exposure to xylitol, a shift towards xylitol-resistant mutans streptococci has been shown in saliva [24], and it has been suggested that those strains have a reduced ability to adhere to the tooth surfaces [26].

The novel thinking with the present study was to examine the oral effects of probiotics and xylitol together. Interestingly, when the regimens were combined in group C, no significantly hampering effect was disclosed compared with baseline, albeit the distribution of microbial score transitions differed significantly from the control group. Thus, irrespective of the different actions of probiotics and xylitol, it seemed obvious that the two strategies together were not enhancing each other, at least not when ingested at different times of the day. The xylitol gums were chewed in the morning and the evenings while the probiotic gums were taken after lunch. It is, however, important to keep in mind that the combined regimen also meant lowered doses of both probiotics and xylitol intake, and whether or not this influenced the outcome is a question that still remains to be answered. The probiotic administration was according to the common recommendations, while the xylitol levels might, in the light of recent research, have been suboptimal or too infrequent in group C [17, 20]. Nevertheless, the next step would be to evaluate the products when taken together.

In conclusion, the results of this study suggest that a 3-week administration of probiotic bacteria or xylitol in chewing gums may reduce the levels of salivary mutans streptococci, but the combinations of these agents did not seem to enhance this effect. Further studies are, however, needed to elucidate this event.

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