EFFECTS OF SHORT-TERM USE OF XYLITOL CHEWING GUM AND MOLTITOL ORAL SPRAY ON SALIVARY *STREPTOCOCCUS MUTANS* AND ORAL PLAQUE

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Abstract. The purpose of this study was to investigate the short-term effects of xylitol chewing gum and maltitol spray on the concentration of salivary mutans streptococci (MS) and on the plaque index. Eighty-one second, third and fourth year dental and dental assistant students with a salivary MS concentration > 10^3 CFU/ml cultured on mitis salivarius bacitracin (MSB) agar were included in the study. The age range of subjects was 18-23 years. The participants were divided into 3 groups: control, xylitol chewing gum and maltitol spray groups. Each subject brushed their teeth with fluoridated toothpaste (1,000 ppm). Each subject in the xylitol chewing gum group was told to chew 2 pieces, 6 times a day (total xylitol dose=7.3 g/day) for 4 weeks. Each subject in the maltitol spray group was told to spray one puff twice daily (morning and evening) for 4 weeks. A dental examination and saliva samples to determine the salivary MS concentration were collected at baseline and at 2 and 4 weeks after experiment initiation. The nonparametric Mann–Whitney *U* test was used to analyze differences among groups. The mean ages in the control, xylitol chewing gum and maltitol spray groups were 22±1, 20±1 and 20±1 years, respectively. The mean MS concentrations at the beginning of the study and after 2 weeks in the control, and xylitol chewing gum and moltitol oral spray groups were not significantly different from each other. There was a significantly lower MS concentration in the moltitol oral spray group than in the control group by 4 weeks (p=0.045) but no significant difference between the control group and the xylitol gum group by 4 weeks. There were no significant differences in the mean plaque index at baseline among the control group, the xylitol chewing gum group and the moltitol oral spray group. The plaque index was significantly lower in the xylitol chewing gum group than the control group (p=0.003) at 2 weeks but not 4 weeks. There was no significant difference in the mean plaque index between the control group and the moltitol oral spray group at any time. Using the maltitol spray significantly reduced the MS level in the saliva by 4 weeks use but using the xylitol gum did not. However, using the xylitol chewing gum did reduce the mean plaque by 2 weeks use but the effect did not

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last; by 4 weeks there was no difference from control. The moltitol spray provided no benefit over the control in reducing the mean plaque index.

Keyword: dental plaque, maltitol spray, mutans streptococci, cylitol chewing gum

INTRODUCTION

Dental caries is an infectious, multifactorial disease resulting from interaction among acidogenic bacteria, carbohydrate substrate, mostly sucrose, and host susceptibility (Berkowitz, 2003, Tanzer *et al*, 2001). Mutans streptococci (MS), *Streptococcus mutans* and *Streptococcus sobrinus* are the causative pathogens of dental caries (Tanzer *et al*, 2001). A reduction in MS concentrations may help prevent dental caries (Tanzer *et al*, 2001; Söderling, 2009).

Xylitol, a five-carbon non-cariogenic natural sugar alcohol, has been used for caries prevention. A previous study found xylitol inhibits the growth and metabolism of several bacterial species, including MS (Bradshaw and Marsh, 1994). Studies have found routine xylitol use decreases MS counts (Maguire and Rugg-Gunn, 2003; Ly *et al*, 2006; Söderling, 2009). There are three properties of xylitol that promote it for preventive use: 1) xylitol is not readily fermented by oral bacteria, such as streptococci; 2) it has been shown to reduce the numbers of *S. mutans* in the oral cavity by limiting the source of fermentable substrates for their survival; and 3) xylitol can induce the production of salivary enzymes which lead to growth inhibition of bacteria in plaque (Söderling, 2009).

The beneficial amount of xylitol for dental health has been reported to be 5-7 g of xylitol at least three times a day (Milgrom *et al*, 2009a,b). The recommended dose for dental caries prevention is 6-10 g/day (Söderling, 2009). Lower doses of xylitol have also been shown to be effective in caries prevention (Alanen *et al*, 2000; Thorild *et al*, 2004). Chewing xylitol gum regularly may be difficult for those with temporomandibular joint dysfunction and difficulty in chewing. For people who cannot chew gum, xylitol candy, xylitol syrup, xylitol wipes and other polyol products may be useful (Alanen *et al*, 2000; Ly *et al*, 2006).

Lactitol and maltitol have been tested mainly on laboratory animals (Grenby and Phillips, 1989). Lactitol, a lactose-based sugar alcohol, has anti-dental caries properties similar to xylitol (Grenby and Phillips, 1989). Maltitol is not used by MS, so it does not produce sufficient acid to demineralize tooth enamel (Grenby and Phillips, 1989). Replacement of sucrose with maltitol in the diet can result in caries reduction (Ooshima *et al*, 1992).

There are a number of studies regarding the effects of xylitol on salivary and plaque MS (Alanen *et al*, 2000; Ly *et al*, 2006; Zhan *et al*, 2012a,b). However, there are no published studies on the effect of moltitol spray on MS. The aim of this study was to evaluate the efficacy of short-term (4-weeks) daily use of xylitol-chewing gum and moltitol spray on the levels of MS and mean plaque index among adults.

MATERIALS AND METHODS

Study design

This cross sectional study was approved by the Ethics Human Research Committee, Faculty of Dentistry and the Faculty of Pharmacy, Mahidol University, Thailand (MU-DT/PY-IRB 2015/062.0912).

Each subject gave written informed consent prior to participation and was free to withdraw from the study at any time. The sample size, calculated based on a previous study, was determined to be a minimum of 22 subjects with significance set at p=0.05 (Zhan *et al*, 2012a,b). Sample size calculations were made using the software package Primer of Biostatistics (Glantz, 2012).

Subject selection and clinical examination

Subjects were recruited from second, third and fourth year dental and dental assistant students aged 18-23 years who were studying at the Faculty of Dentistry, Mahidol University, Bangkok, Thailand. Ninety students with high levels of salivary MS [> 10^3 colony-forming units (CFU)/ml] were selected to participate in this study. Salivary MS levels were determined using culture on MSB agar (Difco Laboratories, Detroit, MI) containing bacitracin (0.2 U/ml, Sigma Chemical, St Louis, MO), 0.001% (v/v) tellurite solution (Becton, Dickinson, Sparks, MD) and 15% (w/v) sucrose (Sigma Chemical). All subjects were otherwise healthy. None had professional prophylaxis or fluoride application during the 3 months prior to the study or took antibiotics during the study period. None of the subjects were regular users of xylitol products. Most admitted to infrequent intake of xylitol- or sorbitol-containing products.

Prior to beginning the study, all the subjects were taught proper tooth brushing techniques using the modified Bass Technique (Poyato-Ferrera *et al*, 2003). Before beginning the study, 4 undergraduate dental students attended lectures by a study researcher to familiarize themselves with the diagnostic criteria for dental caries and plaque deposition. The study examinations were performed by these 4 students, not the researchers. The oral examinations were conducted following the criteria published by the World Health Organization (Ismail, 1998). Kappa statistic values for the decayed, missing and filled teeth (DMFT) and plaque index were 0.83 and 0.87, respectively. The strength of agreement was good. The weighted kappa coefficient was 0.85 for the total score ($p < 0.001$) and showed excellent agreement. The plaque deposits were assessed visually using a simplified oral hygiene index (Greene and Vermillion, 1994). The six index teeth surfaces examined were the buccal surface of upper right first permanent molar (16B), the labial surface of the upper right permanent central incisor (11La), the buccal surface of the upper left first permanent molar (26B), the lingual surface of the lower left first permanent molar (36Li), the labial surface of the lower left permanent central incisor (31La) and the buccal surface of the lower right first permanent molar (46Li). The area of each tooth examined was given a score from 0 to 3 based on the following criteria. A score of 0 indicated no debris or stain present, 1 indicated soft debris covering not more than one third the tooth surface or the presence of extrinsic stains without other debris regardless of surface area covered, 2 indicated soft debris covering more than one-third but less than two-thirds the tooth surface and a score of 3 indicated soft debris covering more than two thirds the exposed tooth surface. To determine the total plaque index for each subject, the scores for each tooth were summed and divided by the number of teeth examined.

All participants were given an Mdent toothbrush and toothpaste with a fluoride (NaF) concentration of 1,000 ppm. The Mdent brand is a product of the Faculty

of Dentistry, Mahidol University, Bangkok, Thailand. The participants were randomly assigned to one of 3 treatment groups: control, xylitol chewing gum and maltitol spray.

Xylitol chewing gum regimen

Xylitol chewing gum is commercially available in Thailand (Xylitol®, Thai Lotte, Bangkok, Thailand). The ingredients of chewing gum are xylitol (42% by weight), moltitol (32%), gum base (21%) and artificial sweetener (0.14%). The mean weight of each piece of chewing gum is 1.45 g, of which xylitol and maltitol comprise 0.61 and 0.46 g, respectively. Subjects in this group were instructed to chew two pieces for 10 minutes six times a day after breakfast and toothbrushing, at 10:00 AM, after lunch, at 3:00 PM and in the evening after dinner (total xylitol dose=7.3 g/day) for 4 weeks.

Moltitol oral spray regimen

The moltitol oral spray used was a commercial product from Japan (Hamikea®; Tampei Phamaceutical, Osaka, Japan). The ingredients in the spray are moltitol, artificial flavor, ascorbic acid and glycerin-fatty acid ester. One bottle weighs 25 g and provides 80 doses (0.31 g/spray). These subjects were told to spray one puff twice daily, in the morning after breakfast and tooth brushing and in the evening after dinner and tooth brushing, for 4 weeks.

Microbiological procedures

Saliva samples were collected from each subject to determine bacterial counts at baseline and after 2 and 4 weeks. Prior to saliva collection, each subject avoided eating, drinking, or brushing their teeth for 1 hour. Five milliliters of saliva was collected each time. To determine the bacterial counts, 0.2 ml of saliva was diluted serially in 1.8 ml M-DIL (NaCl, KCl, Na_2HPO_4 . $2H_2O$, KH_2PO_4, sodium glycerophosphate . H_2O, $MgCl_2$. 6 H_2O) to obtain a bacterial count of 10^1-10^3 bacteria per 1 ml. The solution (0.1 ml) was then cultured on MSB agar and incubated at 37°C in an atmosphere containing 5% CO_2 for 48 hours. Each procedure was performed in triplicate.

Statistic analysis

All data were processed using SPSS, Version 14.0, computer software (SPSS, Chicago, IL). The percent reduction in MS concentration was calculated and compared to that of the control group. Differences in the DMFT scores and plaque indices by group were analyzed using the nonparametric Mann–Whitney *U* test. A *p*-value<0.05 was considered statistically significant.

RESULTS

Ninety participants were chosen, 81 completed the study (10% drop-out rate). The mean age and DMFT scores are shown in Table 1.

The mean salivary MS concentrations are shown in Table 2. The mean MS concentrations in each of the three groups (control, Xylitol, Moltitol) at the beginning of the study were not significantly different from each other. The mean MS concentrations in the Xylitol and Moltitol groups were not significantly different from the control at 2 weeks. The mean MS concentration in the Moltitol group was lower than the control at 4 weeks but the mean MS concentration in the Xylitol group was not.

The mean plaque indices are shown in Table 3. The mean plaque indices in the The control, Xylitol and Moltitol groups did not different from each other at baseline. The mean plaque index in the Xylitol group was lower than the control at 2 weeks but by 4 weeks, they were not significantly

Table 1
Mean age, gender and decayed, missing and filled teeth (DMFT) scores in each group.

Groups	No.	Participant gender		Mean DMFT scores± SD
		Male	Female	
Xylitol chewing gum	29	10	19	4.9 ± 4.2
Moltitol oral spray	26	3	23	5.1 ± 3
Control	26	7	19	5.5 ± 4.4

SD, standard deviation.

different. There were no differences in the plaque indices between the Moltitol group and control at 2 and 4 weeks.

DISCUSSION

We conducted this study to determine the effect of short-term daily use of xylitol gum and moltitol spray on the concentration of salivary MS and the plaque index. In our study, we found the mean salivary MS concentration in the Moltitol group was lower than the control at 4 weeks but the mean MS concentration in the Xylitol group was not. These findings differ from previous studies which showed that MS level were lower after long-term use of xylitol products (Söderling *et al*, 1997; Holgerson *et al*, 2008). Nayak *et al* (2014) found daily use of xylitol at a dose of 6.88-10.32 g significantly reduced MS concentrations. A mouth rinse containing chlorhexidine and xylitol had greater inhibition of MS concentration than chlorhexidine alone was reported by Decker *et al* (2008). Milgrom *et al* (2009b) found using xylitol syrup at 8 mg/day decreased caries prevalence. Toothpaste containing xylitol was found to reduce MS levels (Surdacka, 2005). Thabuis *et al* (2013) found chewing gum containing both xylitol and moltitol significantly reduced the concentration of 4 cariogenic bacteria species, including MS and reduced the plaque index compared to gum without xylitol and moltitol. In our study xylitol chewing gum reduced the plaque index at 2 weeks only but by 4 weeks there was not benefit compared to control. One study found xylitol chewing gum increased salivary flow improving saliva buffering and leading to lower plaque formation (Hildebrandt and Sparks, 2000).

Maltitol has been tested mainly in laboratory animals. Replacement of sucrose with maltitol in the diet has been found to reduce caries and acid production (Wursch and Koellreutter, 1982; Ooshima *et al*, 1992; van Loveren, 2004). Keukenmeester *et al* (2014) found chewing 2 tablets of moltitol containing chewing gum for 10 minutes 5 times a day (14 g/day) significantly reduced dental plaque formation and gingivitis compared to placebo. Thabius *et al* (2013) found xylitol and moltitol affect dental plaque formation, acid-base properties and salivary MS concentration. They also found chewing moltitol gum (10 g/day) for 30 days significantly reduced salivary MS concentrations similar to chewing xylitol gum.

In our study, chewing xylitol gum (7.3 g/day) did not affect salivary MS concentrations in contrast to a previous study by Solderling *et al* (2015) who found chewing 2 pieces of xylitol gum 3 times a day (6 g/day) for 5 weeks significantly

Table 2
Mean mutans streptococci concentrations among studied groups over time.

	Control	Xylitol chewing gum	*p*-value[a]	Moltitol oral spray	*p*-value[b]	*p*-value[c]
Baseline	$1.95\times10^5 \pm 7.79\times10^5$	$2.94\times10^4 \pm 4.23\times10^4$	0.394	$9.36\times10^4 \pm 2.53\times10^5$	0.634	0.190
2-weeks	$2.05\times10^4 \pm 2.54\times10^4$	$3.26\times10^4 \pm 6.66\times10^4$	0.685	$3.08\times10^4 \pm 7.70\times10^4$	0.073	0.081
4-weeks	$3.50\times10^4 \pm 7.89\times10^4$	$3.46\times10^4 \pm 6.37\times10^4$	0.775	$1.60\times10^4 \pm 2.82\times10^4$	0.045[d]	0.064

CFU/ml = Colony forming unit per milliliter.
[a]*p*-value between control and xylitol chewing gum group.
[b]*p*-value between control and moltitol oral spray group.
[c]*p*-value between xylitol chewing gum and moltitol oral spray group.
[d]significantly different ($p<0.05$).

Table 3
Mean plaque indices among studied groups over time.

	Control	Xylitol chewing gum	*p*-value[a]	Moltitol oral spray	*p*-value[b]	*p*-value[c]
Baseline	1.23 ± 0.26	1.35 ± 0.32	0.329	1.31 ± 0.26	0.599	0.715
2-weeks	1.00 ± 0.19	1.20 ± 0.27	0.003[d]	1.10 ± 0.28	0.222	0.195
4-weeks	1.00 ± 0.18	1.13 ± 0.26	0.092	1.06 ± 0.18	0.400	0.346

[a]*p*-value between control and xylitol chewing gum group.
[b]*p*-value between control and moltitol oral spray group.
[c]*p*-value between xylitol chewing gum and moltitol oral spray group.
[d]significantly different ($p<0.05$).

reduced salivary MS concentrations. Thabius *et al* (2013) also found chewing 2 pieces of xylitol gum 5 times a day (10 g/day) for 1 month significantly reduced salivary MS concentrations. However, these two studies were conducted among subjects with salivary MS concentrations $>10^5$ CFU/ml, a higher MS concentrations than in our study. Starting with lower salivary MS concentrations might affect the results with no significant changes. In our study, salivary MS concentrations were assessed from stimulated saliva. The effect of habitual xylitol consumption on MS may be explained both by a decrease in total counts and by the adhesivity of MS on tooth surfaces. Xylitol may reduce the synthesis of extracellular polysaccharides *in vivo*, leading to a change in the plaque-saliva distribution of MS. The MS loosely bound to plaque are suggested to be easily shed to the saliva during mechanical saliva stimulation (Söderling, 2009). A previous study found xylitol use can change the plaque and salivary concentration of MS, but this can also be affected by oral hygiene, dietary habits, and the MS strain (Söderling, 2009). Further studies need to measure concentrations of MS in plaque and saliva and evaluate MS for xylitol resistance (Zhan *et al*, 2012a). Several of our test subjects found the chewing gum regimen difficult to follow and one subject reported stomach discomfort without diarrhea after using the xylitol chewing gum for 2-3 days.

In our study, MS concentrations decreased significantly among those using the moltitol spray by 4 weeks similar to a previous study using moltitol gum (Thabuis *et al*, 2013). Most of our subjects reported using the moltitol spray was easy; however, the dose of moltitol in each puff is unclear since the manufacturer does not disclose it. Another ingredient in the moltitol spray might have given a synergetic effect: ascorbic acid. Vaananen *et al* (1994) found ascorbic acid reduced dental plaque formation and caries incidence.

There were some limitations in our study. We only conducted the study for 4 weeks, which may not have been long enough to determine some of the effects of the studied products. Another factor was that subjects were studying in the school of dentistry, which could have affected their oral hygiene behavior and the results.

Xylitol may affect the oral microbes other than MS. One study found xylitol can have an *in vitro* affect on sugar metabolism among probiotic bacteria such as lactobacilli and bifidobacteria (Zhan *et al*, 2012a). Zhan *et al* (2012b) found using xylitol-wipe for a year significantly reduced new dental caries among young children without reducing the salivary MS concentration. This suggests further studies are needed to explore the various mechanisms for this occurrence.

In our study, xylitol gum reduced the plaque index by 2 weeks use but the effect did not last to 4 weeks. Moltitol spray reduced MS concentrations by 4 weeks. Moltitol spray had no affect on plaque index and xylitol gum had no affect on MS levels. Further studies are needed to determine if either of these products has an effect on dental cares, which is the main goal for both these products.

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