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Efficacy of xylitol and fluoride mouthrinses on salivary mutans streptococci

Malee Arunakul[1*], Boonyanit Thaweboon[2], Sroisiri Thaweboon[2], Yuwadee Asvanund[1], Kesinee Charoenchaikorn[3]

[1]*Department of Pedriatic Dentistry, Faculty of Dentistry, Mahidol University 6 Yothi Road, Rajthevee, Bangkok 10400, Thailand*
[2]*Department of Oral Microbiology, Faculty of Dentistry, Mahidol University 6 Yothi Road, Rajthevee, Bangkok 10400, Thailand*
[3]*Private Clinic*

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ABSTRACT

Objective: To evaluate the level of salivary *Mutans streptococci* (MS) after rinsing with xylitol, fluoride, and a combination of xylitol and fluoride solutions, compared with distilled water. **Methods:** Eighty healthy 8-9 years old subjects with high level of MS (> 10^5 CFU/mL) were equally divided into 4 groups. Subjects rinsed their mouths for 1 min with 10 mL of 0.05% (w/v) sodium fluoride (NaF), 12.5% (w/v) xylitol or 0.05% (w/v) NaF + 12.5% (w/v) xylitol 3 times daily over 10 weeks. Distilled water rinsed group served as a control. Paraffin-stimulated whole saliva samples were collected at baseline, 5 weeks, and 10 weeks after rinsing to determine the level of salivary MS by culturing on Mitis Salivarius Bacitracin agar. The statistical significance was calculated by Kruskal Wallis, Mann Whitney U, and Wilcoxon signed-rank tests at a significant level of $P< 0.05$. **Results:** Significant reductions in MS count were observed in subjects using 0.05% NaF + 12.5% xylitol over other groups within 5 weeks and after 10 weeks and 12.5% xylitol alone after 10 weeks compared with baseline. **Conclusions:** The present study provides evidence for the inhibitory effect of xylitol, used in combination with fluoride, delivered in the form of mouthrinse, on salivary MS in the group of schoolchildren.

1. Introduction

Dental caries is an infectious disease commonly found in the oral cavity. It is well established that mutans streptococci (MS), particularly *Streptococcus mutans* (*S. mutans*), are considered to be an important caries-associated member of microorganisms in dental plaque. Besides utilizing dietary sucrose as an energy source and producing organic acids, MS possess the ability to synthesize extracellular glucans forming plaque matrix. To prevent dental caries, mechanical methods of oral hygiene, brushing and flossing, are considered gold standard methods of plaque control. However, despite the emphasis on mechanical methods of plaque control, the prevalence of dental caries is still high[1,2]. Therefore, other oral hygiene agents such as mouthrinses with antimicrobial properties that can add to the effects of mechanical plaque control may have clinical value.

Xylitol is a five-carbon sugar alcohol, which occurs naturally in low concentrations in a variety of fruits and vegetables. It is used extensively in a great number of sugar-free products particularly chewing gum and lozenges. A wide range of experiments *in vitro* has shown that the majority of oral microorganisms cannot metabolize xylitol to acidic products[3-5]. In addition, xylitol has been reported to have growth and adherence inhibitory effects on some oral bacteria[6]. Several studies have shown that xylitol has anti-caries activity most notably in chewing gum and candy vehicles. Frequent use of xylitol chewing gum has been shown to prevent dental caries[7, 8]. Nevertheless, xylitol in the forms of chewing gum and candy may not be practical for young children or elderly adults. In these situations, an aqueous solution or mouthrinse may be useful.

Xylitol has been incorporated into fluoride-containing mouthrinses. *In vitro* studies have suggested that fluoride and xylitol exert an additive inhibitory effect on growth and acid fermentation by *S. mutans* and *Streptococcus sobrinus*[9,10]. However, clinical trials of xylitol effectiveness in the form of mouthrinse are contradictory and very limited[11,12]. The aim of the present investigation was therefore to evaluate the level of salivary MS after rinsing with xylitol, fluoride, and a combination of xylitol and fluoride solutions, compared with distilled water.

*Corresponding author: Dr. Boonyanit Thaweboon, Department of Oral Microbiology, Faculty of Dentistry, Mahidol University 6 Yoyhi Road, Rajthevee, Bangkok 10400, Thailand.
Tel: +66 (0)2 2036411
Fax: +66 (0)2 2036410
E-mail: boonitdt@yahoo.com
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2. Materials and methods

2.1. Subjects

A total of 180 healthy students (97 females and 83 males, aged 8-9 years) recruited from Saunmisakawan School in Bangkok, Thailand, volunteered to participate in this study. All the procedures were explained to their parents and the students. Informed consent was obtained and the Committee on Human Rights Related to Human Experimentation, Mahidol University, Thailand approved this study. The students and their parents were interviewed for medical conditions and antibiotic use. Individuals were excluded if they had taken antibiotics during the last 4 weeks or anticipated doing so during the study. Subjects with a history of gastrointestinal problems, having unrestored carious lesions or moderate to severe gingivitis were excluded. Screening saliva samples were taken to enumerate the level of MS from subjects who met the inclusion criteria. Of these, 80 subjects with $\geqslant 10^5$ CFU/mL in their saliva were invited to participate.

2.2. Test solutions

The following 3 solutions were tested: (1) 0.05% (w/v) sodium fluoride (NaF), (2) 12.5% (w/v) xylitol, and (3) 0.05% (w/v) NaF + 12.5% (w/v) xylitol. Distilled water served as a control solution. NaF and xylitol were obtained from Sigma (USA), and Danisco (Finland), respectively.

2.3. Study design

Subjects were allocated to 1 of 4 test groups balanced with respect to sex (12 females and 8 males in each group). The study was carried out double-blind and included a 10-week mouth rinsing period. During the study period, the subjects rinsed their mouths 3 times daily (at 8:30 am, 10:30 am, and 3:30 pm) for 1 minute with 10 mL mouthrinse solution or control solution on schooldays under supervision of their teachers.

At the beginning of the study period, subjects received a thorough professional mechanical tooth cleaning and instructions for tooth brushing. They brushed their teeth each morning and evening before mouthrinsing using non-fluoride toothpaste.

2.4. Saliva collection

On the day of appointment, subjects refrained from eating, drinking and having oral hygiene habit for at least 2 h before saliva collection. A volume of 3 mL of paraffin-stimulated whole saliva sample was collected from each subject at baseline, 5 weeks and 10 weeks after mouthrinsing.

2.5. Determination of microorganisms

Saliva was collected, kept on ice and immediately transferred to the laboratory. A volume of 0.5 mL from each sample was serially diluted in phosphate saline solution (pH 7.4). Then 25 μL of each dilution was plated in duplicate on Mitis Salivarius Bacitracin (MSB) agar and incubated at 37 ℃ in 5% CO_2 for 48 h. The number of MS was counted under stereo-microscope and calculated as colony forming unit (CFU)/mL saliva.

2.6. Statistical analyses

The number of microorganisms was converted logarithmically prior to statistical analyses. Four groups were compared with each other at various intervals by Kruskal Wallis and Mann Whitney U tests. Changes over time within a group were compared using Wilcoxon signed-rank test. The level of significance was set at P-value < 0.05. SPSS version 11.5 statistical software was used.

3. Results

The mean MS counts of all groups at baseline, 5-week and 10-week examinations were shown in Table 1. At baseline, MS levels among all groups were not significantly different. The longitudinal effects of the mouthrinses were observed when comparing the differences in MS counts between baseline and 5 weeks, baseline and 10 weeks and between 5 weeks and 10 weeks. Significant reductions in MS count were observed in subjects using 0.05% NaF + 12.5% xylitol within 5 weeks and after 10 weeks compared with baseline even though the result of 5 weeks was not different from that of 10 weeks. In addition, rinsing with 12.5% xylitol alone for 10 weeks exhibited a significantly reduced MS count compared with baseline.

Table 1
Salivary mutans streptococci (Log CFU/mL) in 4 groups of subjects at different time intervals (Mean ± SD) (n=20).

Mouthrinses	baseline	5 weeks	10 weeks
control	5.98±0.55	6.12±0.49	5.94±0.72
0.05% NaF	6.03±0.62	5.60±1.55	5.82±1.53
0.05% NaF ± 12.5% xylitol	6.02±0.60	5.34±0.63 [a,b]	5.39±0.59 [a,b]
12.5% xylitol	5.87±0.57	5.68±1.06	5.45±0.76 [a]

a: significant difference from baseline
b: significant difference from data at the same time interval

Comparing MS counts of the 4 groups at various time intervals, it was found that rinsing with 0.05% NaF+12.5% xylitol showed a significantly decreased MS count over other groups in both after 5 weeks and 10 weeks. Subjects using distilled water, 0.05% NaF and 12.5% xylitol did not show any significant difference among each other during the various times of the study.

4. Discussion

It is well known that mouthrinses are used as adjuncts to mechanical oral hygiene. Mechanical control alone for reducing microorganisms in oral biofilm has been challenged because it is considered to be rather time-consuming and most importantly insufficient for effective oral hygiene. This study was assessed to evaluate the potential of 0.05% NaF, 12.5% xylitol and 0.05% NaF + 12.5% xylitol mouthrinses on salivary MS compared with distilled water, which was used as a control. The main findings were that rinsing with 0.05% NaF + 12.5% xylitol solution showed a significant

reduction in salivary MS within 5 weeks. Our results are in accordance with a study performed by Goncalves *et al*[13], which demonstrated a reduction of salivary *S. mutans* after using 0.05% NaF solution containing either 2.5% or 12.5% xylitol twice daily for 28 days, but contradict the findings of Giertsen *et al*[14]. In the latter study, they concluded that using 0.025% NaF + 20% xylitol did not affect MS levels in dental plaque and saliva.

It should be worth noting that the amount of xylitol administered in mouthrinse in this study was as little as 12.5% or equivalent to a dose of 3.75 g/day. At this concentration, a significant decrease was also observed after 10 weeks compared with baseline. However, the reduction was not different from those in other groups. Hildebrandt *et al*. [11] demonstrated that 4.4 g/day of xylitol mouthrinsing did not show a significant decrease of MS level although a 1 log unit reduction was observed whereas our previous report revealed a significant change of MS scores following chewing xylitol gum at a dose of 5.8 g/day for 3 months. To our knowledge, the prophylactic amount of xylitol has not been established but the most suitable form of delivery vehicle is proposed to be chewing gum.

Xylitol has been shown to have a bacteriostatic effect on MS. The inhibitory effect is due to the uptake of xylitol via fructose phosphotransferase system and xylitol-5-phosphate formed in the cells. Subsequent accumulation of xylitol-5-phosphate interferes with carbohydrate metabolism and inhibits bacterial growth[15, 16]. In the case of fluoride, antimicrobial effects have been demonstrated by the disturbance of carbohydrate metabolism and macromolecules (*i.e.*, peptidoglycan, lipoteichoic acid) synthesis[17]. The key factor in the additive effect of xylitiol and fluoride as revealed by Maehara *et al* [9] was the intracellular accumulation of xylitol-5-phosphate, which increased their inhibitory effect on growth and acid production in the cells.

Kaneko *et al*[18] demonstrated that long-term use of mouthrinse with NaF 500 ppm daily as a preventive program followed by a weekly NaF mouthrinse with 2 000 ppm contributed to the reduction of salivary MS in schoolchildren. The result from this study showed a slight tendency for 0.05% NaF (225 ppm) to inhibit MS in saliva even though the effect was not significantly different from baseline or distilled water. A possible explanation for the lack of statistical significance is the lower daily dose of fluoride applied in this study compared with that used in their study.

In conclusion, the present study gave evidence for the inhibitory effect of xylitol, used in combination with fluoride, delivered in the form of mouthrinse, on salivary MS in the group of schoolchildren. However, the effect of larger amounts or more frequent applications as well as in another study population such as the elderly should be investigated.

Conflict of interest statement

We declare that we have no conflict of interest.

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