Use of chewing gum containing 15% of xylitol and reduction in *mutans* streptococci salivary levels

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Abstract: Frequent use of Xylitol may decrease the *S. mutans* levels. However, very little is known about whether this effect on the levels of cariogenic bacteria is maintained after the interruption of short-term usage of xylitol. This study aimed at evaluating changes in *mutans* streptococci (MS) salivary levels after using a chewing gum containing xylitol. Twelve volunteers harboring ≥ 10^5 CFU MS/ml saliva levels were asked to chew Happydent-xylit® for 5 minutes, 5 X/day, for 30 days. Saliva samples were collected at baseline, at 30 days after xylitol usage began, and at 30 days beyond its interruption. MS salivary levels were estimated. The average salivary levels of MS in the ten subjects who completed the study were 13.17 (NL-CFU) at baseline (A). After the 30 days experimental period (B), this average decreased to 9.45 (NL-CFU). Nine of ten subjects studied showed a reduction in MS salivary levels in relation to baseline, whereas salivary levels were maintained in the remaining subject. At thirty days beyond the interruption of xylitol usage (C), the average levels of MS were still reduced to 10.31 (NL-CFU). Multiple sample comparison using the Bonferroni test revealed that the decrease in MS levels observed from baseline (A) to the time immediately after 30 days of xylitol usage (B) was statistically significant ($p < 0.05$), and those levels were still decreased between baseline and 30 days beyond the interruption of xylitol usage (C). So, the use of xylitol induced a reduction in MS salivary levels after a short period of usage which persisted beyond its interruption.

Descriptors: *Streptococcus mutans*; Saliva; Xylitol; Chewing gum.

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Introduction

Sucrose consumption in high frequency leads to higher levels of cariogenic bacteria such as lactobacilli and *mutans* streptococci (MS).[1,2] Studies using mixed cultures grown in chemostats have shown that the increase in cariogenic organisms in biofilm does not occur due to sugar availability *per si*, but in response to the low pH associated to sugar catabolism. It is the ability of acid-tolerant microorganisms to survive in the low-pH environments that promotes their selection of, and thriving in, such acidic environments.[3] In addition to MS and lactobacilli, other bacteria can also produce acids in the dental biofilm, but at a lower rate.[4] MS also uses sucrose to produce intra and extracellular glucans,[4] which play important roles in bacterial adherence and aggregation, as

sources of energy in fasting periods, and by altering the physio-chemical characteristics of the dental biofilm.[5] High sugar intake and low pH seem to be the primary mechanisms that disrupt homeostasis, leading to the development of dental caries. Thus, strategies aiming to prevent the disease should include the inhibition of acid production, decrease in sugar consumption (mainly between meals), and use of non fermentable sugars.[3]

Xylitol is a polyalcohol derivative for xylose that does not induce dental caries.[6,7] Substitution of sugar by xylitol is not only non-cariogenic but may be considered as anticariogenic,[8,9,10,11,12] and xylitol is mainly indicated for use as a sugar substitute between meals.[9] The use of xylitol chewing gum reduced caries incidence from 30 to 65%,[8] in spite of its effectiveness still being discussed in literature.[10,13,14,15,16] Besides not being metabolized by oral bacteria,[7,17] leading to no pH drop in the biofilm,[18,19] xylitol penetrates into the bacterial cytoplasm and accumulates as xylitol 5-phosphate inside the cell. This impairs glycolysis and ATP production, and results in cell growth inhibition.[7,17]

Xylitol use leads to a reduction in salivary and plaque levels of MS;[1,12,20,21,22] and the use of xylitol gums by mothers was able to reduce transmission of MS to their children.[10,14,16,23,24,25]

Thus, the present study aimed at evaluating the effect of short-term use of a chewing gum available in Brazil containing 15% xylitol on MS salivary levels, and whether the expected decrease in MS levels was maintained beyond the interruption of xylitol usage.

Material and Methods

Subject population

Twelve adult potential subjects, dental students at the University of São Paulo, were selected as volunteers for this study. Inclusion criteria were high MS salivary levels (> 10^5 CFU/ml), absence of dental cavities and periodontitis, and no report of use of antibiotics in the 3 months prior to the beginning of the study. Screening saliva samples were taken for *mutans* streptococci quantification using the spatula method. This study was approved by the ethics committee for research involving human subjects, of the University of São Paulo (n.212/02). The 12 participants had a mean age of 27 years, ranging from 21 to 35, and 75% were women.

Experimental design

In the experimental period the volunteers were asked to use a chewing gum containing 15% xylitol (Happydent-xylit,® Perfetti Van Melle, Valinhos, São Paulo, Brazil) for 5 minutes, five times a day, for 30 days. This level of use leads to an ingestion of 1 g xylitol per day. Salivary levels of MS were determined at baseline (A), immediately at the conclusion of the experimental period (B), and 30 days beyond the interruption of xylitol usage (C). Stimulated saliva samples were obtained after chewing paraffin for 1 min (Orion Diagnostica, Helsink, Helsink, Finland). Saliva samples were immediately diluted in peptone water and aliquots of 100 µl were inoculated on the surface of Mitis Salivarius Bacitracin agar (Difco, Sparks, MD, USA). The plates were incubated for 48 h at 37°C in an atmosphere of 5% CO_2 (ShelLab, Cornellius, OR, USA). The colonies resembling MS were counted, and the salivary levels of MS estimated.

Statistics

The data concerning MS salivary levels at the three sampling phases were analyzed for a normal distribution. Then, the data were transformed using the Neperian logarithm and compared using multiple sample comparison. Differences between groups were assessed using the Bonferroni test. Significance was considered when $p < 0.05$, at the 95% confidence interval.

Results

The salivary levels of each subject at the three sampling occasions are shown in CFU/ml (Table 1).

Two subjects used antibiotics for medical reasons in the 30-day period after xylitol usage, and were excluded. Since bacterial concentrations data did not exhibit a normal distribution, the data were transformed to logarithms to confer homogeneity among the groups and then submitted to variance analysis with repeated measures.

As shown in Table 2, the average MS salivary

Table 1 - Salivary levels of MS at baseline (A), after 30 days of xylitol use (B), and 30 days beyond interruption of xylitol use (C).

Subject	(A)	(B)	(C)
T 1	1.83×10^6	3.80×10^4	*
T 2	2.70×10^5	3.20×10^4	4.50×10^5
T 3	1.97×10^6	4.70×10^4	*
T 4	8.20×10^5	1.50×10^4	4.20×10^4
T 5	2.70×10^5	1.80×10^4	3.60×10^4
T 6	1.90×10^5	6.00×10^3	1.90×10^5
T 7	6.70×10^5	3.00×10^3	4.00×10^3
T 8	1.20×10^5	7.40×10^4	9.00×10^3
T 9	1.08×10^6	1.97×10^6	2.76×10^6
T 10	1.64×10^6	3.00×10^2	1.50×10^4
T 11	1.75×10^6	5.80×10^4	6.70×10^3
T 12	5.60×10^5	3.00×10^2	5.00×10^3

* Excluded subjects due to use of antibiotics.

levels were 13.17 (NL-CFU) at baseline (A). After the experimental period (B) the average levels of MS in saliva decreased to 9.45 (NL-CFU). Nine of ten studied subjects showed a reduction in MS salivary levels in relation to baseline data, whereas higher salivary levels were detected in the one remaining subject. At thirty days beyond interruption of xylitol usage (C), the average levels of MS were still reduced to 10.31 (NL-CFU).

Variance analyses for repeated measures using the Bonferroni test revealed that the decrease in MS levels observed from (A) to (B) was statistically significant ($p = 0.008$). There was also a statistically significant difference between (A) and (C) ($p = 0.028$). However, there was no statistical difference between B and C.

Discussion

The effect of xylitol on oral bacteria is usually observed after prolonged usage,[1,6,14,18,21,24] differing from the use of concentrated antimicrobial agents, such as 1% chlorhexidine gel, in which the effect on MS salivary levels can be observed after a couple of applications.[26] The use of antimicrobial agents in the oral cavity may reduce caries risk by reducing MS salivary levels, as shown by long term studies.[27,28]

Table 2 - Average, median, variance and standard deviation of log CFU of MS salivary levels of 10 studied subjects in the three sampling occasions of the study.

Sampling	Average	Median	Variance	SD
Baseline (A)	13.17	13.32	0.84	0.92
30 days (B)	9.45	9.70	6.95*	2.63
60 days (C)	10.31	9.79	5.69*	2.38
Total	10.98	11.09	6.79	2.60

* Indicates statistical difference, $p < 0.05$.

However, the use of chlorhexidine in high concentrations (1% or 2% gel), or other forms of application, such as varnishes, requires professional procedures. On the other hand, the use of xylitol is also able to reduce MS levels and can be easily performed by the subject. As shown in the present study, reduction in MS levels by xylitol can be reached even in highly infected subjects. In addition, xylitol can be used continuously since it does not exhibit side effects as do other agents.[9,13,16]

The effect of xylitol use on caries initiation and progression may not be attributed only to its effect on MS, since several studies have also shown that other sugar substitutes as sorbitol, as well as placebo gum, can exhibit some effect, although not as relevant as xylitol.[29] The effect of chewing gums on caries may also be attributed to the stimulation and increase of salivary flow and its resultant increased buffer capacity, and to increased removal of bacterial substrates,[10,13,14,16] which suggests an influence in the remineralization process, promoted by the regular use of chewing gums.[3,13]

The effect on bacteria may also be influenced by the form of xylitol presentation in the diet. The use of xylitol in food as a sugar substitute does not result in decreased MS salivary levels.[27,28] Hence, the direct, frequent, and sustained contact of xylitol with the tooth surfaces occurring with the use of candies or gums is required in order to achieve the beneficial effect.[7,28,29] Thus, the proper xylitol concentration and frequency may have affected results from different studies, since the effect of xylitol on bacterial levels is not only dependent on its high frequency but also on the concentration of the agent.[1,8,10,13,15,16,18,19,20,21]

The effect on MS levels promoted by the use of xylitol may be attributed to its antimicrobial properties since several studies have shown that the reduction on MS salivary levels is not observed when other sugar substitute, or even placebo gums, were used.[1,8,19,20,22,30]

Decreased MS salivary levels after xylitol use were correlated with a reduction of these organisms in the dental biofilm.[12,14,19,21,22,26] Since xylitol may not reach the bacteria in biofilm as easily as in saliva, due to the requirement of xylitol diffusion through the biofilm, most studies analyze saliva and not biofilm samples. It has previously been shown that salivary levels of MS reflect their levels in the biofilm of the whole oral cavity, whereas sampling biofilm from certain surfaces may induce several errors due to plaque removal by mechanical measures and heterogeneity of biofilm composition within the same subject.

In the present study the use of a chewing gum (15% of xylitol, 5 X/day for 30 days) led to at least 10 times reduction in MS salivary levels in 9 of 10 subjects. Despite the low total intake of xylitol (1 g/day), the high frequency employed here (5 X/day) may possibly have influenced the MS salivary levels.[8,18,22] And, although other organisms are also sensitive to xylitol, the decrease in bacterial levels should be more intense over MS, due to a decrease in periods of acid pH in the dental plaque.[1,19,26]

Several studies have also indicated that, in addition to a reduction in MS levels promoted by xylitol, its use may induce the emergence of xylitol-tolerant strains which may exhibit a lower pathogenic potential than xylitol sensitive strains.[7,13,14,17] The high levels of MS did not decrease after use of xylitol in one of ten studied subjects, possibly due to selection of X^R isolates, but other hypotheses, such as low compliance or high sucrose intake leading to high glucose/fructose levels in saliva, should not be discarded. The observation that the low MS levels could be maintained in most subjects (six of ten subjects) even after one month of no exposure to xylitol indicated that the effect of xylitol may persist for longer periods. Studies using chlorhexidine gels have shown that MS levels in biofilm return to baseline levels in periods ranging from 8 to 12 weeks after the procedures.[30] Since MS are resident in the oral cavity and the conditions influencing their colonization were maintained stable in the studied subjects, such as keeping the same dietary habits, it is expected that the MS levels will return to its baseline levels within time.

Conclusion

In conclusion, the present data indicate that the use of 15% xylitol chewing gums, 5 times/day, even for short periods such as 30 days, resulted in reduced MS levels. The data also indicate that these levels were kept low for at least one month, suggesting that xylitol use should be encouraged, particularly in highly MS infected subjects.

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