Influence of Maternal Xylitol Consumption on Acquisition of Mutans Streptococci by Infants
E. Soderling, P. Isokangas, K. Pienihäkkinen and J. Tenovuo

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ABSTRACT

Xylitol is effective as a non-cariogenic sugar substitute. Habitual xylitol consumption appears to select for mutans streptococci (MS) with impaired adhesion properties, i.e., they shed easily to saliva from plaque. One hundred sixty-nine mother-child pairs participated in a two-year study exploring whether the mothers’ xylitol consumption could be used to prevent mother-child transmission of mutans streptococci. All mothers showed high salivary levels of mutans streptococci during pregnancy. The mothers in the xylitol group (n = 106) were requested to chew xylitol-sweetened gum (65% w/w) at least 2 or 3 times a day, starting three months after delivery. In the two control groups, the mothers received either chlorhexidine (n = 30) or fluoride (n = 33) varnish treatments at 6, 12, and 18 months after delivery. The children did not chew gum or receive varnish treatments. MS were assessed from the mothers’ saliva at half-year intervals and from the children’s plaque at the one- and two-year examinations. The MS were cultured on Mitis salivarius agars containing bacitracin. The salivary MS levels of the mothers remained high and not significantly different among the three study groups throughout the study. At two years of age, 9.7% of the children in the xylitol, 28.6% in the chlorhexidine, and 48.5% in the fluoride varnish group showed a detectable level of MS. In conclusion, therefore, habitual xylitol consumption by mothers was associated with a statistically significant reduction of the probability of mother-child transmission of MS assessed at two years of age. The effect was superior to that obtained with either chlorhexidine or fluoride varnish treatments performed as single applications at six-month intervals.

KEY WORDS: xylitol, mutans streptococci, colonization, transmission, chlorhexidine varnish.

INTRODUCTION

Children whose teeth are colonized earlier by mutans streptococci (MS) show higher caries experience than those colonized later or not at all (Alaluusua and Renkonen, 1983; Köhler et al., 1984; Tenovuo et al., 1990). Most children appear to acquire the micro-organism from their mothers (Berkowitz and Jordan, 1975; Calfield et al., 1985, 1988). The acquisition of MS has been suggested to occur between 19 and 31 months of age (Caufield et al., 1993), though extensive MS colonization has also been described at younger ages (Berkowitz et al., 1980; Mohan et al., 1998).

Various methods to reduce mothers’ salivary MS counts have delayed their babies’ acquisition of MS (Köhler et al., 1983, 1984; Tenovuo et al., 1992). For example, Köhler and co-workers (1983) administered chlorhexidine gel (1%) in trays, with MS monitoring at four-month intervals, to mothers with high MS levels, resulting in long-term reduction of colonization of their children by these bacteria and reduced caries experience (Köhler and Andréen, 1994).

Xylitol shows specific MS-inhibiting effects (for review, see Trahan, 1995), suggesting permanent reductions in oral MS levels. In short-term habitual consumption, it reduces the levels of MS assessed both from plaque and from stimulated saliva (Loesche et al., 1984; Söderling et al., 1989). Habitual long-term xylitol consumption appears to select for natural mutant cells of MS which are “xylitol-resistant” (Trahan and Mouton, 1987). Xylitol-resistant mutants appear to shed more easily into saliva from plaque than do xylitol-sensitive parental strains (Trahan et al., 1992).

The aim of the present study was to explore whether mothers’ habitual xylitol consumption could prevent the mother-child transmission of MS. Xylitol consumption starting when the child was three months old was compared with biannual fluoride or chlorhexidine varnish treatments.

MATERIALS & METHODS

Subjects and Study Design

The study was carried out at Ylivieska, Alavieska, and Siievi Health Centers in the central part of Finland. In Finland, women are entitled to free dental care during pregnancy. In the selected health centers, the mothers and children participated in a free post-natal oral health care program, which included: examinations; advice on diet, oral hygiene, and use of fluorides; and, where necessary, restorative treatment. The regular oral health care program, summarized in Neuvolahammashoidon käsikirja (1980), was based on the instructions of the National Board of Health (later the National Agency for Welfare and Health).

All pregnant women (n = 338) who participated in dental examinations in the above three health centers during one calendar year were screened for their salivary levels of MS (see saliva sampling and microbiological analyses). Of these, 195 women showed high (≥ 105 CFU/mL) salivary levels of MS and were invited to take part in the study. The mothers received written information on the
study design (Table 1) and samples to be collected, and they were specifically instructed not to change their dietary habits during the study. The subjects participated after providing informed consent to the study protocol. The protocol was reviewed and approved by the Ethical Boards of both the Medical Faculty at Turku University and the three Health Centers.

The subjects were randomly divided into three study groups: a xylitol (Xyl) chewing gum group, a chlorhexidine (CHX) varnish group, and a fluoride (F) varnish group. The mothers in all study groups received basic prevention and restorative treatment within the post-natal oral health care program. One of the aims was a longitudinal study of the effect of xylitol on the transmission of both S. mutans and S. sobrinus. Since S. sobrinus occurs in only approximately 10% of Finnish children (Tenovuo et al., 1992), the xylitol group was deliberately made the largest in the randomization. At the start of the study, there were 127 subjects in the Xyl group, 36 in the F group, and 32 in the CHX group. In this region of Finland, daily habitual consumption of xylitol by adults was rare. The seven subjects who at screening reported a history of regular xylitol consumption were assigned to the Xyl group. At each child’s two-year examination at the end of the study, the groups consisted of 169 mother-child pairs (106/Xyl, 33/F, 30/CHX). The distribution of boys and girls was similar in the three groups. The main reason for subjects’ interrupting the study was the family moving away from the area. No subjects interrupted the study due to presumed side-effects of xylitol gum or of the chlorhexidine or fluoride varnish treatments. The percentage of dropouts was fairly low in all three groups (16.5%/Xyl, 8.3%/F, 6.7%/CHX). The dropouts did not differ significantly with respect to DMFT indices from those subjects who completed the study.

In the Xyl group, the mothers were recommended to use xylitol chewing gum at least 2 or 3 times a day. The chewing gum contained xylitol as the only sweetener (65% w/w), and it was supplied for the study by Leaf (Turku, Finland). The mothers were interviewed about their gum usage at each examination, i.e., when the child was six months, one, and two years of age. The consumption was registered as ≤ 1, 2-3, 4-5, and > 5 times a day. The number of chewing gum pieces used at a time was also recorded.

In the CHX and F groups, the mothers received CHX or F varnish treatments at 6, 12, and 18 months after delivery. These varnish treatments were added to the oral health care program and administered under it. The varnish treatments (chlorhexidine varnish—EC 40, Certichem, Nijmegen, The Netherlands; and fluoride varnish—Duraphat® R, Rhône-Poulenc Rorer GmbH, Cologne, Germany) were performed in accordance with the manufacturers’ instructions, after clinical examination and collection of plaque and saliva samples.

At the six-month, one-, and two-year examinations, the mothers were interviewed about various possible confounding factors: new pregnancies, type of daycare, acute and chronic illnesses, and medication of either the mother or the child. The children did not consume xylitol gum or lozenges or receive varnish treatments.

About one-fifth of the two-year-old children had been mainly cared for, during weekdays, by someone other than their biological mother for more than half a year (20%/Xyl, 19%/F, 20%/CHX).

Saliva Sampling and Microbiological Analyses
The mothers were requested not to brush their own or their children’s teeth on the morning of the saliva sampling. They were also instructed not to eat or drink for at least one hour before the appointment.

At the one- and the two-year examinations, plaque samples were collected from the children by means of toothpicks (Tenovuo et al., 1992). All together, five children would not cooperate in plaque collection, and thus no sample was obtained. Visible plaque was collected from all tooth surfaces, particularly from the cervical margins and interproximal regions, with 1 to 4 toothpicks, the ends of which were cut and pooled in 0.5 mL transport medium containing Tryptic Soy Broth (Difco, Detroit, MI, USA) with 10% glycerol (v/v). The transport tubes were stored frozen at -70°C before microbiological analysis. The mothers provided 2 mL of paraffin-stimulated whole saliva at each appointment. A 100-μL quantity of saliva was immediately transferred to 900 μL of transport medium and stored frozen before microbiological analyses.

For the microbiological analyses of plaque and saliva, the transport tubes were thawed and vortexed thoroughly for 1 min. To detach the plaque from the toothpicks and disrupt bacterial aggregates, we also treated the plaque samples with mild 10-second sonication at +4°C (MSE, London, UK; amplitude, 3; end diameter of the probe, 3 mm). Our earlier studies (Trahan et al., 1992) showed that the MS levels are not affected by the laboratory methods used in this study. After serial ten-fold dilutions, the bacteria were plated on Mitis salivarius agars (Difco) containing bacitracin (MSB) (Gold et al., 1973). The plates were incubated for two days in a 7% CO2 atmosphere at 37°C. The numbers of MS were identified based on colony morphology and counted by means of a stereomicroscope. The identification of Streptococcus mutans and Streptococcus sobrinus was performed as described earlier (Fujisawa et al., 1991). Classification of S. mutans was based on: consistent findings of “rough” colony morphology on the MSB plate; positive fermentation with sorbitol, mannitol, raffinose, and melibiose; and negative dextran agglutination. The identification of S. sobrinus was based on “smooth” colonies on the MSB plate, positive fermentation with mannitol but negative fermentation with raffinose and melibiose, and positive dextran agglutination. For

Table 1. Study Design Used for Comparison of the Effect of Xylitol Consumption with Fluoride or Chlorhexidine Varnish Applications on Mother-Child Transmission of Mutans Streptococci

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Selection of pregnant women with high levels of salivary MS&lt;sup&gt;a&lt;/sup&gt; [saliva sample].</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery</td>
<td></td>
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<tr>
<td>Child 3 months</td>
<td>The consumption of xylitol starts in Xyl&lt;sup&gt;b&lt;/sup&gt; group.</td>
</tr>
<tr>
<td>Child 6 months</td>
<td>Saliva sample from the mother. Recording of caries/mother, count of teeth/child. F&lt;sup&gt;c&lt;/sup&gt; or CHX&lt;sup&gt;d&lt;/sup&gt; varnish treatment of the mother in the F and CHX groups.</td>
</tr>
<tr>
<td>Child 1 yr</td>
<td>Saliva sample from the mother and pooled plaque sample from the child. Recording of the child’s number of teeth. F or CHX varnish treatment of the mother in the F and CHX groups.</td>
</tr>
<tr>
<td>Child 1.5 yrs</td>
<td>F or CHX varnish treatment of the mother in the F and CHX groups.</td>
</tr>
<tr>
<td>Child 2 yrs</td>
<td>Saliva sample from the mother and pooled plaque sample from the child. Recording of the child’s number of teeth. Consumption of xylitol discontinues in Xyl group.</td>
</tr>
</tbody>
</table>

<sup>a</sup> MS = mutans streptococci.
<sup>b</sup> Xyl = xylitol.
<sup>c</sup> F = fluoride.
<sup>d</sup> CHX = chlorhexidine.
Table 2. DMFT Index\(^a\) and Age\(^b\) of Mothers in the Three Study Groups (child 6 months old)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>DMFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>F varnish</td>
<td>31.6 (29.9-33.3)</td>
<td>18.5 (15.6-21.4)</td>
</tr>
<tr>
<td>CHX varnish</td>
<td>28.8 (27.2-30.4)</td>
<td>17.7 (14.9-20.5)</td>
</tr>
<tr>
<td>Xylitol gum</td>
<td>29.3 (28.3-30.2)</td>
<td>17.5 (16.1-18.9)</td>
</tr>
</tbody>
</table>

\(\text{\(^{a}\) Mean (95\% confidence intervals) is given for the mothers who completed the study (n = 169).}\)

saliva, the detection limit of the MS assay was 250 CFU/mL, and for plaque, 20 CFU/sample.

Dental Examinations

The numbers of children’s teeth were recorded at each examination. The teeth of the mothers were examined clinically under standardized conditions at the child’s six-month examination. Caries was recorded as decayed, missing, and filled teeth (DMFT) according to the WHO (1979) criteria.

Statistical Analyses

Analysis of variance (ANOVA) was used for comparison of salivary MS levels of mothers among the groups, and Student’s \(t\) test for comparison of dropouts and subjects (DMFT). The Wilcoxon Matched Pairs test was used in the analysis of change in the consumption of chewing gum. The proportion of children in each group with detectable levels of MS was used as a measure of transmission. The differences among the groups were analyzed by relative risk (RR) and its confidence intervals (CI) (Rothman, 1986). The \(\chi^2\) test was used for analysis of the association of xylitol frequency, daycare type, or antibiotic usage with MS colonization in children. Statistical computation was carried out with the CSS: \textsc{Statistica} Program (1991).

RESULTS

At baseline, the caries experiences of the mothers were similar in the Xyl, CHX, and F groups (Table 2). The mean levels (± SD) of salivary MS of the mothers during the study are shown in Fig. 1. There were no significant differences in the salivary MS levels among the three groups at pregnancy or at any of the three other examinations. The percentages of mothers with high levels of MS (CFU ≥ 10^3/mL) in their saliva at all four examinations (pregnancy, 6 mos, 1 yr, 2 yrs) were 87.0% in the Xyl group, 78.8% in the F group, and 75.0% in the CHX group. Less than 10% of the mothers in the three groups showed detectable levels of \textit{S. sobrinus} in their saliva at any examination.

At one year of age, 6.8% of the children in the Xyl group showed detectable mother-child transmission of MS; in the F varnish group, the percentage was 18.2%, and in the CHX varnish group, 3.6%, respectively. No significant differences among the groups were detected. At two years of age, 9.7% of the children in the Xyl group were colonized with MS (Fig. 2). The corresponding figures were 28.6% in the CHX and 48.5% in the F groups (Fig. 2). Thus, the children’s risk of MS colonization in their dentition, compared with that of those in the Xyl group, was five-fold in the F group (95% CI, 2.3-11.0) and three-fold in the CHX group (95% CI, 1.2-7.7). The differences between the F and CHX groups were not significant. When only those mothers whose salivary MS count was consistently high (CFU/mL ≥ 10^3) were analyzed, the difference between mother-child transmission of MS in the Xyl group and that in the F group was even higher. The two-year colonization percentages were 10.0% in the Xyl, 61.5% in the F, and 28.6% in the CHX groups. Compared with the Xyl group, the risk of MS colonization was 6.2-fold in the F group (95% CI, 2.7-13.9) and 2.9-fold in the CHX group (95% CI, 1.0-8.0). The differences between the F and CHX groups were, again, not significant. All children who were colonized with MS at one year of age also harbored MS at two years. None of the children was colonized with \textit{S. sobrinus} at two years of age. No differences among the groups were detected in the number of children’s teeth at six months, one, or two years of age.

All mothers from the Xyl group included in the analyses were classified as being daily, habitual xylitol consumers. The consumption frequency of the experimental gum decreased during the study (6 mos vs. 1 yr, \(p = 0.037\); 6 mos vs. 2 yrs, \(p = 0.008\)); however, at the two-year examination, a daily xylitol gum consumption of 2 or 3 was still reported by approximately 40% and 4 or 5 by approximately 50% of the mothers. The number of xylitol gum pieces (approximately two) consumed at a time remained stable during the study. The colonization percentages in the children were not associated with their mothers’ frequency of using xylitol chewing gum. No significant associations between MS colonization and type of daycare, new pregnancies, or antibiotic usage by either the mother or the child could be detected.

DISCUSSION

This is the first report of a longitudinal study exploring the possible effects of xylitol consumption by mothers on mother-child transmission of MS, and on the children’s risk of future caries development. We found that habitual xylitol

\[\text{Figure 1. The mean levels (log CFU/mL ± SD) of maternal salivary MS at pregnancy and at the child’s age six months, one, and two years. Groups: fluoride varnish, \(\bullet\); chlorhexidine varnish, \(\Delta\); and xylitol chewing gum, \(\nabla\). Data of the mothers who completed the study (n = 169) are shown.}\]
consumption by mothers drastically reduced the mother-child transmission of MS. In our study, protracted habitual xylitol consumption did not decrease salivary MS levels in the mothers, which is in agreement with some earlier results from long-term xylitol studies (Söderling et al., 1991; Mäkinen et al., 1996). In fact, the majority of the mothers showed high salivary MS levels at all examinations, and thus a high potential to transmit their MS to the children (Berkowitz et al., 1981). As a consequence of their habitual xylitol consumption during the study, most mothers in the xylitol group presumably already harbored predominantly xylitol-resistant MS within a few months after commencing xylitol consumption (Dréan and Trahan, 1990). Xylitol-resistant MS appear to be more easily shed into the saliva from plaque than the xylitol-sensitive parental strains (Trahan et al., 1992). Similarly, in vitro findings have suggested that the adhesion properties of xylitol-resistant MS cells are lower than those of xylitol-sensitive cells (Trahan, 1995). Thus, it can be speculated that the MS of the mothers in the xylitol group had impaired adhesion properties, leading to reduced mother-child transmission of MS.

Xylitol intervention was initiated when the children were three months old, and the first varnish treatments were performed at six months of age. Both animal (Ooshima et al., 1988) and human studies (Berkowitz et al., 1975; Catalanotto et al., 1975) indicate that the initial acquisition of MS coincides with the presence of newly emerged teeth. For infants, this could take place between approximately six months and two years of age (Mohan et al., 1998). In our study, the number of children’s teeth at six months, one, and two years of age did not differ among the study groups. Thus, the preconditions for colonization—i.e., mothers’ high salivary MS levels and available tooth surfaces—were similar in all three groups. The biannual varnish treatments were administered under the existing oral health care program. The xylitol intervention, however, was deliberately initiated earlier than the first varnish treatments. The effects of habitual xylitol consumption on MS levels, and the selection of xylitol-resistant MS cells, take from a few weeks to several months to become established (Wennergren and Emilson, 1989; Dréan and Trahan, 1990; Trahan et al., 1992), and it was important that stable levels both of xylitol-resistant MS and of MS in general should be established prior to the emergence of the first teeth of the children in the xylitol group. We found colonization percentages at one year of age ranging from 3.6% to 18.2% in the three groups. This finding is in accordance with the assumption that mother-child transmission starts when the first teeth emerge (Mohan et al., 1998).

Our MS transmission interventions discontinued when the children were two years of age. At present, there is disagreement in the literature as to whether children are colonized during a discrete “window of infectivity” (Caufield et al., 1993) or also both before and after this “window” (Mohan et al., 1998; Strætemans et al., 1998). The intervention period chosen appears, however, to coincide closely with the general period of infectivity. The effect of xylitol on the composition of oral flora, especially the percentage of xylitol-resistant MS cells, has been estimated to last for several months or even years after the cessation of habitual xylitol consumption (Trahan et al., 1992). Similarly, the effects of CHX treatments on oral flora, including MS, are believed to last at least 1 to 3 months after treatment (Schaeken et al., 1989; Tenovuo et al., 1992; Piemihäkken et al., 1995).

In the F group, the mothers were treated biannually with a fluoride varnish. Fluoride varnish treatments do not reduce MS levels in the oral cavity (Schäeken et al., 1991); thus, such treatment should have no effect on mother-child MS transmission. MS colonization in the F group, at all examinations, was approximately 50% for all children and approximately 60% for children whose mothers showed high MS counts. Although the F group was relatively small, the colonization percentage findings closely agree with figures published earlier for children of mothers with high salivary MS levels (Berkowitz et al., 1981; Köhler et al., 1983).

In the CHX group, the mothers’ teeth were treated with CHX varnish at six-month intervals. According to the available data, a single application of the high-concentration CHX varnish used in our study reduces oral MS levels for several months (Schäeken et al., 1989). We found no differences in the maternal salivary MS levels among the three groups at any examination. In agreement with this finding, the varnish treatments did not significantly reduce the mother-child transmission of MS. Due to the small size of the CHX group, no drastic conclusions can be drawn from these results. A higher frequency of varnish treatments (Köhler et al., 1983) might have improved the results.

In addition to the mothers’ MS counts, other factors may also affect the mother-child transmission of MS, such as the infants’ feeding practices (Mohan et al., 1998) and dietary habits in the family, e.g., sucrose consumption by the parents (Grindeffjord et al., 1991). Feeding habits, diet, and socio-economic factors were not registered in the present study, but

![Figure 2. The percentage of children showing detectable colonization with MS at two years of age. Groups: F = fluoride varnish, CHX = chlorhexidine varnish, Xyl = xylitol chewing gum. The number of children’s plaque samples assessed per study group is shown at the bottom of the bar. Significant relative risks associated with group difference: F vs. Xyl, 5.0 (95% CI, 2.3-11.0); CHX vs. Xyl, 3.0 (95% CI, 1.2-7.7). The colonization percentages in F and CHX groups did not differ significantly [RR: F vs. CHX, 1.7 (95% CI, 0.7-4.0)].](http://jdr.sagepub.com)

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earlier studies made in the same rural area (Isokangas, 1988) have shown that they are relatively uniform. The mothers’ DMFT indices were similar in all study groups, and they were comparable with mean DMFT values for other people of similar age in the same area. This suggests similar dietary habits of the mothers in the three study groups at the study baseline. In general, dramatic dietary changes are necessary for a reduction in MS (Kristoffersson and Birkhed, 1987). The type of daycare could be a confounding factor, since infants cared for by someone other than their biological mothers are significantly less likely to be early colonized with MS (Caufield et al., 1993). In our study, about one-fifth of the children in all three groups had been cared for by someone other than their biological mother. The MS colonization percentages for these children were nevertheless similar to those of the rest of the group. This result may be explained by the fact that, in Finland, working days are relatively short, and in the study area the distances between home and work are also generally short, leading to a maximum eight-hour absence from home during the mothers’ five-day working week. This enables frequent salivary contacts between the mothers and their children to take place even for working mothers.

In conclusion, habitual xylitol consumption by mothers was associated with a significant reduction in the mother-child transmission of mutants streptococci when assessed at two years of age. The effect was superior to that obtained with biannual CHX or F varnish applications.

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