Effect of Xylitol on Growth of *Streptococcus pneumoniae* in the Presence of Fructose and Sorbitol

TERHI TAPIAINEN,\(^1\,*\) TERO KONTIOKARI,\(^1\) LAURA SAMMALKIVI,\(^1\) IRMA IKÄHEIMO,\(^2\) MARKKU KOSKELA,\(^2\) AND MATTI UHARI\(^1\)

Department of Pediatrics, University of Oulu,\(^1\) and Clinical Microbiology Laboratory, Oulu University Hospital,\(^2\) Oulu, Finland

Received 5 June 2000/Returned for modification 5 July 2000/Accepted 14 October 2000

Xylitol is effective in preventing acute otitis media by inhibiting the growth of *Streptococcus pneumoniae*. To clarify this inhibition we used fructose, which is known to block similar growth inhibition observed in *Streptococcus mutans*. In addition, we evaluated the efficacy of sorbitol in inhibiting the growth of pneumococci, as sorbitol is widely used for indications similar to those for which xylitol is used. The addition of 5% xylitol to the growth medium resulted in marked growth inhibition, an effect which was totally eliminated in the presence of 1, 2.5, or 5% fructose but not in the presence of 1 or 5% glucose, 1% galactose, or 1% sucrose. This finding implies that xylitol-induced inhibition of pneumococcal growth is mediated via the fructose phosphotransferase system in a way similar to that in which mutants group streptococcal growth is inhibited. The addition of sorbitol at concentrations of 1, 2.5, or 5% to the growth medium did not affect the growth of pneumococci and neither inhibited nor enhanced the xylitol-induced growth impairment. Thus, it seems that xylitol is the only commercially used sugar substitute proven to have an antimicrobial effect on pneumococci.

Yield of *Streptococcus pneumoniae* was monitored over 24 h to determine the effects of sorbitol and fructose. The OD of each tube was measured at a wavelength of 650 nm with an SFM spectrophotometer. The OD results were calculated as the means of triplicate measurements. The OD results were calculated as the means of triplicate measurements.

**MATERIALS AND METHODS**

Twenty strains of pneumococci were isolated from consecutive routine patient samples taken from middle-ear effusions, and one pneumococcal strain was isolated from a sinus aspirate. Strain ATCC 49619 was used as a commercially available reference strain. The pneumococcal strains were identified from their colony morphologies and optochin sensitivities. The strains were kept frozen at −20°C in skim milk broth until they were used. They were serotyped at the National Health Institute Laboratory in Oulu, Finland. Strains of serotypes 6A, 6B (two strains), 7 (sinus aspirate), 9V (two strains), 14, 15 (four strains), 16, 19A (two strains), 23F (two strains), 38 (two strains), and pool G (serotype 29, 35, or 42) were used for the purposes of the xylitol and fructose studies (Table 1). One strain was not viable at the time of serotyping after storage. Ten of these strains (serotypes 6A, 6B [two strains], 9V, 14, 15 [three strains], 19A, and 38) were used in complementary studies and in the xylitol and sorbitol studies (Table 1). The serotype of strain ATCC 49619 was 19F (serotype 59 in the U.S. nomenclature according to the American Type Culture Collection [ATCC]).

Pneumococci were cultured in brain heart infusion broth (BHI) (Difco Laboratories, Detroit, Mich.) containing 0.2% glucose. To ensure optimal growth, 10% (vol/vol) heat-inactivated fetal calf serum (PAA Laboratories, Linz, Austria) was added. Xylitol (Sigma Chemical Co., St. Louis, Mo.), D(+)-fructose (BDH Laboratory Supplies, Poole, England), D(+)-glucose (anhydrous; Fluka Biochimika, Buchs, Switzerland), D(+)-galactose (Merck, Darmstadt, Germany), sucrose (BDH Laboratory Supplies), or D(−)-sorbitol (Merck) was added to the basic medium, and the mixture was sterilized by filtration (Minisart 0.2-μm pore-size filter; Millipore Corp., Bedford, Mass.). The test media contained the sugar or sugar alcohol concentrations indicated in Table 1. Each strain was cultured aerobically in BHI at 35°C in a 5% CO₂ atmosphere up to the exponential phase of growth (optical density [OD], 0.2 to 0.3). Three hundred microliters of this suspension was transferred into 3 ml of test medium containing various sugar alcohol or sugar concentrations (Table 1). All the test tubes with medium also contained 10% fetal calf serum at a volume of 3 ml. The test tubes were incubated at 35°C for 24 h. Each test was carried out in triplicate.

The OD of each tube was measured at a wavelength of 650 nm with an SFM 35 spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) against the standard medium, with the measurements being performed every 1 to 2 h during the logarithmic phase of growth. The OD results were calculated as the means of three measurements. To confirm the relationship between the OD and the total number of viable bacterial cells, viability counts were made by the standard dilution method on sheep blood agar plates at the beginning, at the mid-phase of logarithmic growth, and at the end of the logarithmic phase and also during the stationary phase. At the end of the experiment (24 h) the samples were cultured on sheep blood agar plates and in the basic medium to ensure the presence of viable pneumococci.
TABLE 1. Growth media used in the experiments*

<table>
<thead>
<tr>
<th>Control medium</th>
<th>Test medium</th>
<th>No. of strains^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHI only</td>
<td>BHI + 2.5% xylitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI only</td>
<td>BHI + 5% xylitol</td>
<td>21</td>
</tr>
<tr>
<td>BHI + 5% fructose</td>
<td>BHI + 5% fructose + 5% xylitol</td>
<td>21</td>
</tr>
<tr>
<td>BHI + 2.5% fructose</td>
<td>BHI + 2.5% fructose + 5% xylitol</td>
<td>21</td>
</tr>
<tr>
<td>BHI + 1% fructose</td>
<td>BHI + 1% fructose + 5% xylitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI + 5% glucose</td>
<td>BHI + 5% glucose + 5% xylitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI + 1% glucose</td>
<td>BHI + 1% glucose + 5% xylitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI + 1% galactose</td>
<td>BHI + 1% galactose + 5% xylitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI + 1% sucrose</td>
<td>BHI + 1% sucrose + 5% xylitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI only</td>
<td>BHI + 1% sorbitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI only</td>
<td>BHI + 2.5% sorbitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI only</td>
<td>BHI + 5% sorbitol</td>
<td>11</td>
</tr>
<tr>
<td>BHI only</td>
<td>BHI + 2.5% xylitol + 2.5% sorbitol</td>
<td>11</td>
</tr>
</tbody>
</table>

*All concentrations are shown as weight/volume.
^b Including strain ATCC 49619; each test was carried out in triplicate for each strain.

Statistical analyses were performed with the Arcus QuickStat biostatistical program. One-way analysis of variance was used to test the differences between the groups at each point in time. When there was a significant difference, the groups were further compared by the t test with the Bonferroni correction because of multiple comparisons.

RESULTS

Marked growth inhibition was detected in the presence of 5% xylitol in the basic medium with 0.2% glucose, as in our previous studies (6). The addition of 1, 2.5, or 5% fructose eliminated the effect of the xylitol (Fig. 1). None of the differences was statistically significant. Xylitol-induced growth inhibition was prevented by all fructose concentrations used.

The addition of 1% glucose, 1% galactose, or 1% sucrose did not alter the effect of xylitol, and growth inhibition was detected (Fig. 2). The difference was greatest 5 h after the beginning of the observation when glucose or galactose was used, whereas it was 0.34 (SD, 0.07) in the control culture containing 5% xylitol, whereas it was 0.18 (SD, 0.06) in the medium containing 2.5% xylitol combined with 0.2% glucose, as in our previous studies (6). The addition of 1, 2.5, or 5% fructose did not alter the effect of xylitol, and growth inhibition was prevented by all fructose concentrations used.

Xylitol at concentrations of 1, 2.5, and 5% had no effect on the growth of pneumococci. Xylitol at a concentration of 2.5% was as effective alone as it was in combination with 2.5% sorbitol (Fig. 3). The mean OD for the pneumococci after 5 h of observation was 0.40 (SD, 0.07) in the control medium (BHI), whereas it was 0.19 (SD, 0.06) in the medium containing both 2.5% sorbitol and xylitol and 0.22 (SD, 0.07) in the medium containing 2.5% xylitol. The OD values for the cultures grown on the experimental media differed significantly from those for cultures grown on the control medium. Growth systematically seen with strain ATCC 49619 and with all except one of the pneumococci isolated (n = 21). The serotype of this exceptional strain was 7, and it was the only strain isolated from a sinus aspirate.

Sorbitol at concentrations of 1, 2.5, and 5% had no effect on the growth of pneumococci. Xylitol at a concentration of 2.5% was as effective alone as it was in combination with 2.5% sorbitol (Fig. 3). The mean OD for the pneumococci after 5 h of observation was 0.40 (SD, 0.07) in the control medium (BHI), whereas it was 0.19 (SD, 0.06) in the medium containing both 2.5% sorbitol and xylitol and 0.22 (SD, 0.07) in the medium containing 2.5% xylitol. The OD values for the cultures grown on the experimental media differed significantly from those for cultures grown on the control medium. Growth
inhibition of 52% (95% CI, 35 to 68% [P < 0.0001]) and 46% (95% CI, 28 to 60% [P < 0.0001]), respectively, were observed.

The correlation between the OD values during the logarithmic phase and the growth of pneumococci was good and was independent of the medium used. An OD of 0.05 to 0.1 was equal to $1 \times 10^6$ to $2 \times 10^6$ CFU/ml, and a value of 0.4 corresponded to $1 \times 10^7$ CFU/ml. The pneumococci in the medium containing xylitol and sorbitol remained viable until the end of the test. The autolysis of pneumococci was seen in BHI and in the medium containing 1, 2.5, or 5% sorbitol, which is a typical phenomenon in pneumococcal cultures after rapid logarithmic growth and which is thought to be mediated by autolytic enzymes. In media containing xylitol the bacteria never achieved the highest point of logarithmic growth and autolysis was not seen. The bacteria in the media containing an extra carbon source for growth, i.e., 1, 2.5, or 5% fructose, 1 or 5% glucose, 1% galactose, or 1% sucrose, had high OD values at the end of the experiment, without autolysis. Growth of these bacteria was detected after transfer to fresh basic medium.

**DISCUSSION**

We were able to confirm our earlier finding that xylitol causes marked inhibition of pneumococcal growth (6). Extra glucose, galactose, or sucrose had no effect on this inhibition, but it was totally eliminated by fructose. This shows that the mechanism of growth inhibition in pneumococci is mediated by a system regulated by fructose. The phosphotransferase system of oral streptococci is a flexible mechanism capable of taking in different sugars depending on the current sugar environment (13). In mutants group streptococci the first step of xylitol metabolism is entry into the bacterial cell via the fructose phosphotransferase system, and xylitol does not cause growth inhibition in the presence of fructose (3, 10). Xylitol is then metabolized to xylitol-5-phosphate, which mutants group streptococci cannot utilize further and which may even be toxic to bacteria (10). It must therefore be expelled from the cell (9). This futile xylitol cycle consumes energy and results in growth inhibition. Our results show that inhibition of the growth of pneumococci exposed to xylitol is prevented by fructose, a finding that supports our hypothesis that this inhibition is attributable to the involvement of the fructose phosphotransferase system in a way similar to that of inhibition of growth of mutans group streptococci.

This finding that xylitol exposure restored the pneumococci to a viable state despite the impaired growth is in accordance with our observation that xylitol does not reduce the nasopharyngeal carriage of pneumococci (5, 11). Of the 14 serotypes that we tested, xylitol had no effect on the growth of strains of only 1 serotype, serotype 7. Although xylitol has been widely used as a sweetener in Finland for two decades, the results of our clinical trials indicate that most pneumococcal strains are sensitive to it (11, 12). Whether xylitol insensitivity results in a decrease in virulence in the case of pneumococci remains to be established.

Sorbitol, which has been shown to have minimal or no effects on the growth of mutans group streptococci in the presence of glucose, had no effect in the present study on the growth of pneumococci (2, 3). Sorbitol alone is also less effective than xylitol for the prevention of caries (4, 8). It may enhance the inhibitory potential of xylitol in mutans group streptococci by alternation of the intracellular metabolism of xylitol (1), but in pneumococci it neither enhanced nor inhibited the effect of xylitol on growth. It is unlikely that the combination of xylitol and sorbitol in chewing gums would provide any clinical benefit in the prevention of otitis media relative to the clinical benefits of chewing gums with pure xylitol. The dose of xylitol needed to prevent acute otitis media is in any case quite high, so that its partial replacement with sorbitol would be an illogical approach.

In conclusion, we found that the beneficial effect of xylitol in inhibiting the growth of pneumococci is totally eliminated by fructose. The underlying mechanism of xylitol-induced inhibition of growth of pneumococci may be mediated by a fructose phosphotransferase system in a manner similar to that of inhibition of growth of mutans group streptococci. In practice, this finding means that xylitol should not be combined with fructose in products intended to prevent acute otitis media.

ACKNOWLEDGMENT

This work was supported by a grant from the Maud Kuistila Foundation, Helsinki, Finland, and the Yrjo Johnsson Foundation, Helsinki, Finland.

**REFERENCES**

8. Mäkinen, K. K., C. A. Bennett, P. P. Hujoel, P. J. Isokangas, K. P. Isotupa,


