

## Antiadhesive effects of xylitol on otopathogenic bacteria

Tero Kontiokari<sup>a\*</sup>, Matti Uhari<sup>a</sup> and Markku Koskela<sup>b</sup>

<sup>a</sup>Department of Paediatrics and <sup>b</sup>Division of Microbiology, University of Oulu, FIN-90220 Oulu, Finland

The exposure of either epithelial cells or pneumococci or both to 5% xylitol reduced the adherence of pneumococci. Exposure of epithelial cells or bacteria alone to xylitol did not reduce the adherence of *Haemophilus influenzae*, although the exposure of both cells and bacteria to xylitol reduced the adherence significantly. The adherence of *Moraxella catarrhalis* remained low irrespective of the exposure.

### Introduction

*Streptococcus pneumoniae* binds to the carbohydrate sequence GlcNAc $\beta$ 1-3Gal on human pharyngeal cells and on GalNAc $\beta$ 1-4Gal or GalNAc $\beta$ 1-3Gal on type-II pneumocytes.<sup>1</sup> *Haemophilus influenzae* expresses adhesins to the GalNAc $\beta$ 1-4Gal receptor of human lung tissues.<sup>2</sup> The binding of *Moraxella catarrhalis* may also target carbohydrates.<sup>3</sup>

In an earlier study we found that xylitol, a five-carbon sugar alcohol, was effective in preventing acute otitis media in children.<sup>4</sup> Previous studies have shown that xylitol inhibits the adhesion of cariogenic *Streptococcus mutans*.<sup>5</sup> We hypothesized that xylitol may also affect the adhesion of otopathogens and we tested this hypothesis *in vitro*.

### Materials and methods

Ten strains of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were isolated from middle ear aspirates, subcultured on sheep blood agar plates or on chocolate agar plates (*H. influenzae*) and incubated overnight at 37°C with 5% CO<sub>2</sub>. Strains were transferred to brain heart infusion medium (BHI, Difco Laboratories, Detroit, MI, USA) supplemented (BHIs) with haemin, NAD (SR 158, Oxoid, Unipath Ltd, Basingstoke, UK), and 10% (v/v) fetal bovine serum (GibcoBRL, Life Technologies, lot 40G5159F, Eggenstein, Germany) and incubated to logarithmic phase. Two hundred microlitres of the suspension was transferred to 5 mL of BHIs and incubated for 9–11 h.

The epithelial cells were obtained from ten healthy adult donors by scraping the oropharynx with a cotton swab, then released by stirring the swabs in 2 mL of saline, washed twice by centrifugation at 600g for 15 min, and

resuspended in saline at a concentration of 10<sup>4</sup> cells/mL as counted in a Bürker chamber. Half of the suspension was supplemented with 5% (w/v) of xylitol (Sigma Chemical Co., St Louis, MO, USA) diluted in 0.1 mL of saline, while the other half was supplemented with the same amount of pure saline.

Adherence testing was performed using a method described by Andersson *et al.*<sup>6</sup> The bacteria were used at concentrations of 10<sup>7</sup>–10<sup>9</sup>/mL as counted with the plate dilution. Two 1 mL aliquots were drawn, one was supplemented with 5% (w/v) of xylitol dissolved in 0.1 mL of BHIs and the other with 0.1 mL of BHIs. Pneumococci were additionally supplemented with 1% (w/v) choline (Sigma Chemical Co.). The bacteria (50  $\mu$ L) were mixed with 50  $\mu$ L of cell suspension. The final concentrations (w/v) of xylitol were 0%, 2.5% (cells or bacteria exposed to xylitol) and 5% (both exposed to xylitol). Suspensions were centrifuged at 500g for 3 min and incubated at 37°C for 30 min. Unattached bacteria were removed by six cycles of centrifugation at 500g for 15 min. Control suspensions for each experiment treated with BHIs had a mean of four bacteria per cell, unchanged when 5% of xylitol was added. The samples were fixed with methanol and stained with acridine orange. The number of each strain of bacteria adherent to 40 epithelial cells was counted with a fluorescence microscope at 500  $\times$  or 1000  $\times$  magnification. In total, 400 cells/bacteria were counted. Diplococci were counted as one bacterium. The numbers of damaged cells and cells with more than 300 attached bacteria were counted. Adherence was given as a mean number of bacteria per cell after background adhesion had been deducted.

A non-parametric Kruskal–Wallis variance analysis was used to test the differences between the different exposures and between the different bacteria. The

\*Corresponding author. Tel: +358-08-3155101; Fax: +358-08-3155559.

significance of the results was assessed with the Mann-Whitney U-test. The cells with more than 300 bacteria were analysed assuming the count followed Poisson's distribution.

## Results and discussion

*S. pneumoniae* had a stable adhesion pattern of 32–47 bacteria per cell (mean 41) without xylitol, except for one highly adherent strain with 89 bacteria per cell. Incubation of epithelial cells on xylitol reduced the adherence to 21 bacteria per cell ( $P = 0.001$ ). The exposure of bacteria to xylitol reduced the adherence to 25 bacteria per cell ( $P = 0.05$ ). Addition of xylitol to both cells and bacteria reduced the adherence to 13 bacteria per cell ( $P < 0.001$ ) (Figure).

The adherence of *H. influenzae* varied from 17 to 54 bacteria per cell (mean 31) without xylitol. Addition of xylitol either to cells or to bacteria did not reduce the adherence statistically significantly. The adherence declined to 16 bacteria per cell when both cells and bacteria were exposed to xylitol ( $P < 0.05$ ) (Figure).

*M. catarrhalis* adhered poorly to epithelial cells, with

2–15 bacteria per cell. Although some strains showed a slight decrease in adherence when exposed to xylitol, the mean difference was not statistically significant (Figure).

The counts of the cells with more than 300 pneumococci per cell differed significantly according to the different exposures to xylitol ( $P < 0.001$ ). Other bacteria showed no differences (Table).

We have earlier found that xylitol chewing gum reduces acute otitis media attacks by 50%.<sup>4</sup> The present finding of reduced adhesion of two important otopathogens supports this efficacy. The antiadhesive mechanism remains a matter for speculation, but blocking of bacterial lectins is possible.

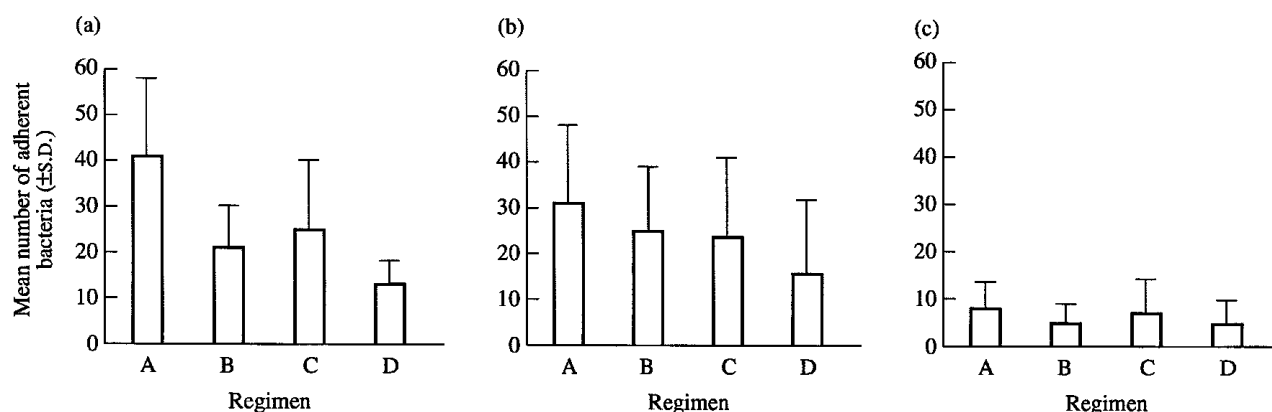
Several oligosaccharides can block bacterial adhesion, as can some monosaccharides and disaccharides.<sup>7</sup> Xylitol inhibits the adhesion of *S. mutans* at a concentration of 6%,<sup>5</sup> but not that of *H. influenzae* at a concentration of 1.5%.<sup>8</sup> These observations are consistent with the fact that monosaccharides are able to inhibit adherence only at the high concentrations, that are easily achieved in the oral cavity. The worldwide spread of penicillin-resistant strains of pneumococci substantiates the need for new approaches to preventing bacterial infections. Xylitol seems to be a promising agent for this purpose.

**Table.** Number of cells (percentage of all counted cells) not eligible for visual counting

Bacteria	>300 Bacteria/cell (%)				Damaged cells (%)			
	I	II	III	IV	I	II	III	IV
<i>S. pneumoniae</i>	22 (5.5)	6 (1.5)	6 (1.5)	0 <sup>a</sup>	13 (3.3)	9 (2.3)	6 (1.5)	10 (2.5)
<i>H. influenzae</i>	34 (8.5)	45 (11.3)	34 (8.5)	29 (7.3)	0	2 (0.5)	3 (0.8)	0
<i>M. catarrhalis</i>	3 (0.8)	9 (2.3)	7 (1.8)	7 (1.8)	32 (8.0)	27 (6.8)	44 (11.0)	36 (9.0)

I = Control; II = epithelial cells exposed to 5% xylitol; III = bacteria exposed to 5% xylitol; IV = cells and bacteria exposed to 5% xylitol.

<sup>a</sup>  $P < 0.001$ .



**Figure.** The mean number (S.D.) of bacteria adherent to human oropharyngeal cells. The reduction in adherence was statistically significant for *S. pneumoniae* (A vs B and D,  $P \leq 0.001$ ; A vs C,  $P \leq 0.05$ ) and for *H. influenzae* (A vs D,  $P < 0.05$ ) (Mann-Whitney U-test). (a) *S. pneumoniae*; (b) *H. influenzae*; (c) *M. catarrhalis*. A = Control; B = epithelial cells exposed to 5% xylitol; C = bacteria exposed to 5% xylitol; D = cells and bacteria exposed to 5% xylitol.

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