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Xylitol and Erythritol Decrease Adherence of Polysaccharide-Producing Oral Streptococci

Eva M. Söderling · Aija-Maaria Hietala-Lenkkeri

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Abstract Xylitol consumption decreases counts of mutans streptococci. However, the mechanism behind this decrease is not well understood. We studied not only type strains and clinical isolates of mutans streptococci, but also other polysaccharide-forming oral streptococci. Growth inhibition and adherence of cells to a smooth glass surface—reflecting synthesis of water-insoluble polysaccharides were studied in the presence of 2% (0.13 mol/l) and 4% (0.26 mol/l) xylitol. The effect of xylitol was compared to a novel polyol sweetener, erythritol. Except for *Streptococcus mutans* 10449 and *S. sobrinus* OMZ 176, the glass surface adhesion of most polysaccharide-forming streptococci was reduced by the presence of both 4% xylitol and erythritol. For the *S. mutans* and *S. sobrinus* type strains, the growth inhibition with 4% xylitol and erythritol was 36–77% and for the clinical *S. mutans* isolates 13–73%. Of the other oral streptococci, only *S. sanguinis* was inhibited with 4% xylitol (45–55%). For both polyols, the magnitude of the growth inhibition observed was not associated with the magnitude of the decrease in adherence (xylitol: $r = -0.18$; erythritol: $r = 0.49$). In conclusion, both xylitol and erythritol can decrease polysaccharide-mediated cell adherence contributing to plaque accumulation through a mechanism not dependent on growth inhibition.

E. M. Söderling (✉)
Institute of Dentistry, University of Turku, Lemminkäisenkatu 2, 20520 Turku, Finland
e-mail: eva.soderling@utu.fi

A.-M. Hietala-Lenkkeri
Kuusankoski Rehabilitation Centre, 45740 Kuusankoski, Finland

Introduction

Streptococcus mutans is regarded as a primary etiological agent in the pathogenesis of dental caries although additional microorganisms may be involved (for reviews, see [2, 20, 21]). Glucosyltransferases (GTF) and glucan-binding proteins are involved in the sucrose-dependent adhesion of *S. mutans* (for review, see [1]). The cariogenicity of *S. mutans* is related, in part, to its ability to colonize tooth surfaces in the presence of sucrose. The surface-absorbed GTFs synthesize glucans from sucrose, which are essential for bacterial accumulation on tooth surfaces and contribute to the bulk and integrity of biofilms formed on the teeth [1].

Xylitol, a 5-carbon polyol, inhibits the growth and acid production of mutans streptococci (MS; for review, see [17]). Clinical studies have also shown xylitol to decrease counts of MS, the amount of plaque, and the incidence of caries in children (for reviews, see [8, 17]). Apart from MS, the inhibition of streptococci by xylitol is poorly known. A previous study suggested that some *S. sanguinis* and *S. salivarius* strains may be inhibited by xylitol [23]. Xylitol has either inhibited the growth of *S. mitis* [6] or shown no effect [16]. In clinical studies, however, xylitol consumption has not affected the total counts of streptococci, while the MS counts have decreased (Söderling, unpublished findings).

Erythritol, a tetritol, has been suggested to be caries-preventive, but few studies have so far been published on its effects even on risk factors of caries [9–11]. Xylitol and most other polyols used as bulk sweeteners may have laxative effects and consequently they are suitable only for small-size products like chewing gums. Erythritol is not laxative and thus its food applications could be much broader.

This study was designed to compare the effects of xylitol and erythritol on glass adhesion of polysaccharide-

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forming oral streptococci and to relate these effects to the growth inhibition found for the polyols.

Materials and Methods

Microorganisms

The following type strains of streptococci were used: *S. mutans* 10449, *S. mutans* Ingbritt, *S. sobrinus* OMZ 176, *S. salivarius* 13419 and 25975, and *S. sanguinis* 10904 and BAA 1455. The clinical *S. mutans* isolates came from nonconsumers of xylitol and expressed various degrees of growth inhibition with xylitol. The origin, isolation, and identification of the clinical isolates (CIs) have been described earlier [19].

Cultivation of the Microorganisms

The cells were cultured in 5 ml BHI overnight at +37°C from stocks kept frozen to produce log-phase cells, and then transferred to fresh BHI supplemented with 1% sucrose the following morning. The growth media contained 2% (0.13 mol/l) or 4% (0.26 mol/l) xylitol (Oriola, Espoo, Finland), or 2% (0.16 mol/l) or 4% (0.33 mol/l) erythritol (Sigma, St. Louis, MO, USA). We chose the polyol concentrations (w/v%) based on studies in which polyol concentrations have been measured in saliva in connection with use of polyol-containing products. Since we did not combine xylitol and erythritol in any of the experiments, it was not necessary to use them in equimolar concentrations. The polyols were added to the sterile media using filter sterilization. The control medium contained no added polyols. The cells were cultured in a shaking water bath at +37°C. Growth was followed by measuring the absorbance at a wavelength of 550 nm. All experiments were repeated at least twice. The inhibition percentages were calculated from the growth curves at late log-phase.

Adhesion Tests

The ability of cells to adhere to a smooth glass surface was determined according to Mattos-Graner et al. [13]. We estimated the adhesion percentage from the cell density of adherent cells. The adherence tests were performed in triplicate.

Statistics

The growth was compared at each examination point using independent samples *t*-test (SPSS 14.0 for Windows). Spearman's Rank Correlation was used to study associations between different variables. The level of statistical significance was set at $P < 0.05$.

Results

The glass surface adhesion of most oral polysaccharide-forming streptococci was reduced by the presence of both 4% (0.26 mol/l) xylitol and 4% (0.33 mol/l) erythritol. The adhesion of the type strains *S. mutans* 10449 and *S. sobrinus* OMZ 176 was, however, not affected by the presence of either xylitol or erythritol (Fig. 1). Adhesion of *S. mutans* Ingbritt was decreased by xylitol ($P < 0.05$), but not erythritol (Fig. 1). With one exception (CI 668/erythritol), the six CIs of *S. mutans* showed a statistically significant decrease in glass surface adhesion for both 4% xylitol and erythritol (for CIs 195, 2366, 668, see Fig. 1). A linear response in the reduction of adhesion was seen when the results were compared for 2 and 4% added polyol (not shown).

The glass surface adhesion of *S. sanguinis* 10904 was reduced by xylitol, but not by erythritol (Fig. 1). Surprisingly, the initially low adhesion of *S. sanguinis* BAA 1455 was increased by xylitol (not shown; erythritol, not tested). The glass surface adhesion of both *S. salivarius* strains was

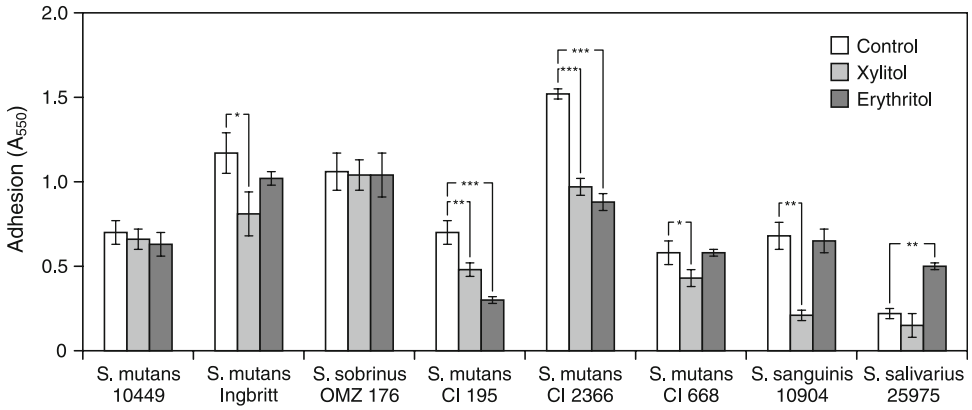

Fig. 1 The adhesion (A550) of eight streptococci grown in the presence of 4% (0.26 mol/l) xylitol or 4% (0.33 mol/l) erythritol to a smooth glass surface. Significant differences shown between control versus xylitol or versus erythritol: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

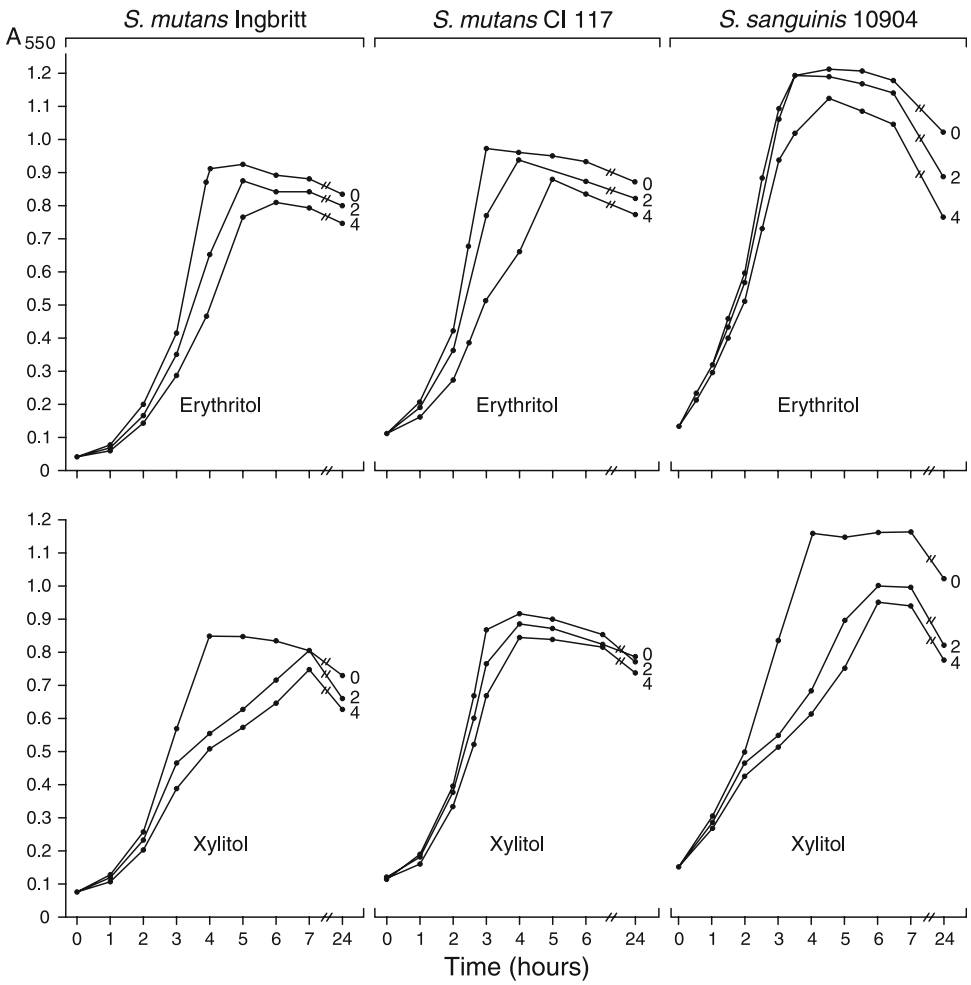

Fig. 2 Growth (A550) of *S. mutans* Ingbritt, *S. mutans* CI 117 and *S. sanguinis* 10904 in the presence of 2% (0.13 mol/l) and 4% (0.26 mol/l) xylitol and 2% (0.16 mol/l) and 4% (0.33 mol/l) erythritol

low (for *S. salivarius* 25975, see Fig. 1). Xylitol inhibited the adhesion, of *S. salivarius* 13419 significantly ($P < 0.01$), while erythritol significantly increased the glass surface adhesion of *S. salivarius* 25975 ($P < 0.01$; Fig. 1).

Both xylitol and erythritol inhibited the growth of all mutans streptococci studied. As a rule, 4% xylitol and erythritol inhibited the growth by more than 2% (Fig. 2). For the *S. mutans* and *S. sobrinus* type strains the growth inhibition with 4% (0.26 mol/l) xylitol and with 4% (0.33 mol/l) erythritol at late log-phase was high, between 36 and 77% (Figs. 2 and 3). For the clinical *S. mutans* isolates, the growth inhibition with 4% xylitol and erythritol ranged between 13 and 73%. Though both xylitol and erythritol inhibited the growth of the above-mentioned streptococci, the degree of inhibition, as well as the inhibitory pattern, differed for most streptococci. For example, the clinical *S. mutans* isolate 117 was strongly inhibited with erythritol but weakly inhibited with xylitol (Fig. 2).

Xylitol also showed strong growth inhibition of *S. sanguinis* NCTC 10904 (for 4% xylitol 55%), while the inhibition with erythritol was weak (Fig. 2). *S. sanguinis* BAA 1455 also showed growth inhibition of 45% with 4% (0.26 mol/l) xylitol. Although both *S. salivarius* strains tested showed growth inhibition at log-phase with both xylitol and erythritol, this was only of transient nature.

The xylitol-induced decrease in the glass surface adherence of the tested polysaccharide-forming streptococci was not associated with the growth inhibition observed for xylitol (Fig. 3). For erythritol, there was a trend toward an association ($P = 0.12$), but it was not significant (Fig. 3). There was no association between the magnitudes of growth inhibition found for erythritol and xylitol. Moreover, no association was detected between the magnitudes of the decreases in glass adherence caused by the added xylitol versus erythritol.

Discussion

We have shown that both xylitol and erythritol inhibited the glass surface adherence of not only mutans streptococci, but also of other polysaccharide-producing oral

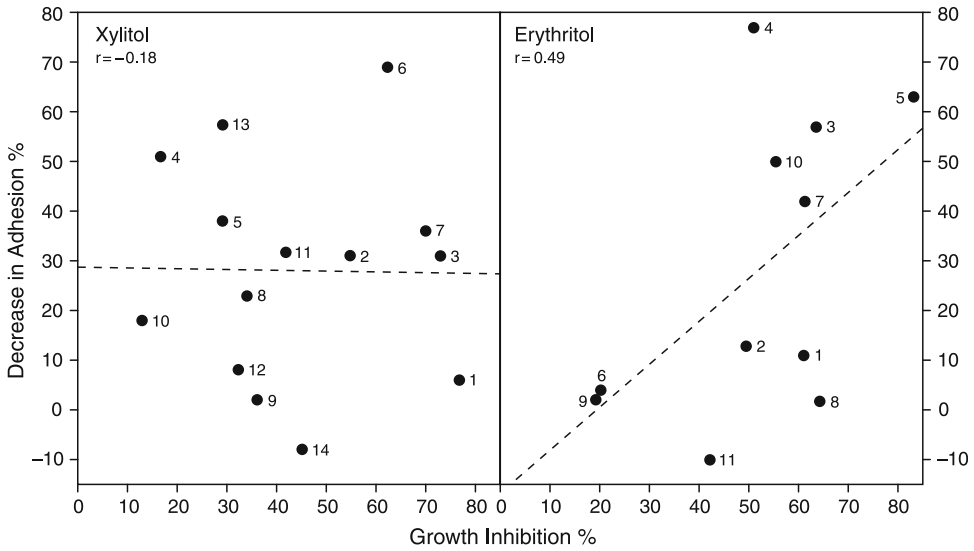

Fig. 3 Association of decrease in adhesion (%) to smooth glass surface and growth inhibition in the presence of 4% (0.26 mol/l) xylitol ($r = -0.18$) or 4% (0.33 mol/l) erythritol ($r = 0.49$). 1: *S. mutans* 10449, 2: *S. mutans* Ingbritt, 3: *S. mutans* CI 195, 4: *S. mutans* CI 117, 5: *S. mutans* CI 312, 6: *S. sanguinis* 10904, 7: *S. mutans* CI 2366, 8: *S. mutans* CI 668, 9: *S. sobrinus* OMZ 176, 10: *S. mutans* CI 113, 11: *S. salivarius* 25975, 12: *S. mutans* CI 199, 13: *S. salivarius* 13419, and 14: *S. sanguinis* BAA 1455

streptococci. The only exceptions were one strain each of *S. sanguinis* and *S. salivarius*. The adhesion of growing cells to glass surfaces in the presence of sucrose is associated with the production of water-insoluble glucans (WIG; [13]). A decrease in the WIG synthesis should lead to a decrease in plaque accumulation. For xylitol, this has been demonstrated in numerous studies [17]. As for erythritol, both a reduction in plaque accumulation [11] or no effect [10] have been reported. The adhesion inhibition was reached with realistic xylitol concentrations that can be found in the saliva when xylitol-containing products have been consumed [4]. Earlier, 6% xylitol was shown to decrease the synthesis of insoluble polysaccharides produced by *S. mutans* NCTC 10449 in vitro [18]. In that study, a significant decrease in the glass surface adherence of the cells was also observed. In a clinical study, habitual xylitol usage was suggested to reduce polysaccharides including WIG in plaque [12]. At present, there are no studies on the effects of erythritol on the WIG content of plaque.

As early as 1975, it was shown that xylitol inhibits the growth of *S. mutans* [5]. Apparently the magnitude of growth inhibition of MS with xylitol is related to the xylitol PEP:PTS activity [15]. Little is known about xylitol inhibition of other polysaccharide-forming streptococci than MS. We chose the clinical MS isolates so that isolates showing low and high xylitol inhibition were represented, although all MS strains showed some degree of xylitol inhibition. Our inhibition percentages for both the type strains and CIs agree with previous findings [19, 23]. Xylitol also inhibited the two *S. sanguinis* strains, but only transient inhibition of the *S. salivarius* strains was detected. One report showing no or low growth inhibition with xylitol for *S. sanguinis* and *S. salivarius* has been published earlier [23]. Erythritol inhibited the growth of most of the polysaccharide-forming streptococci. Two MS type strains and CIs have earlier been reported to show an atypical inhibition pattern for erythritol [11]. In our study, however, the inhibition found for xylitol and erythritol showed similar, typical growth inhibition patterns. No theories on the mechanism of growth inhibition by erythritol have been published so far. As for the effect on glass surface adherence, the magnitude of the growth inhibition also differed with xylitol and erythritol, suggesting that the mechanisms of inhibition of glass surface adherence and growth differed for the two polyols.

Growth inhibition of MS with xylitol is usually considered to be the reason for the xylitol-induced reductions in the MS counts observed in clinical studies. The finding that xylitol consumption selects for "xylitol-resistant" MS not inhibited by xylitol is convincing [22], but in practice it means that after the xylitol consumption has lasted for some time, xylitol should lose its ability to inhibit the majority of MS. However, decreased MS counts of plaque/saliva have been demonstrated for habitual xylitol consumption in both short- and long-term studies lasting from weeks to several months [14, 17]. Our study demonstrated that xylitol significantly reduced the glass surface adherence of even MS which showed only minor growth inhibition with xylitol. Thus, even the "xylitol-resistant" MS of habitual xylitol consumers may be less adherent compared to the MS of nonconsumers of xylitol [17]. A reduction in the adherence of the MS could explain why habitual long-term consumers of xylitol harbor reduced oral MS counts in spite of the fact that the majority of the MS may not show growth inhibition with xylitol.

In in vitro and in vivo studies involving other streptococci than MS, xylitol has only inhibited counts of MS [3, 7, 16]. Thus, MS have been suggested to be the target organisms of xylitol. The present finding that xylitol

inhibited both the growth and glass surface adherence of *S. sanguinis* and *S. salivarius* does not support this idea. We have conducted clinical studies using salivary total streptococci counts as a background variable and have only seen decreases in the MS counts, supporting the idea of MS being the target organisms of xylitol (Söderling, unpublished results). The few existing studies on the effects of erythritol consumption on counts of MS have revealed both a decrease [11] and no effect [10] on the MS counts. Since the mechanism of the erythritol inhibition is not known, there are no theoretical grounds to discuss whether the "resistance" phenomenon demonstrated for xylitol [22] could also be found for erythritol. More studies are clearly needed on both these topics.

In conclusion, both xylitol and erythritol inhibited glass surface adherence of polysaccharide-forming streptococci which are known to contribute to plaque accumulation. The magnitude of the decrease in the glass surface adherence did not appear to be associated with the growth inhibition exerted by these polyols. This may explain why xylitol decreases plaque and MS counts even in long-term xylitol consumers who harbor MS, the majority of which have lost the ability to be inhibited with xylitol.

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