# Xylitol and the bactericidal effect of chlorhexidine and fluoride on *Streptococcus mutans* and *Streptococcus sanguis*

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The present study was made to investigate the effect of xylitol on the bactericidal and bacteriostatic action of chlorhexidine diacetate (CHX) and sodium fluoride (F) in ATCC strains of *Streptococcus mutans* and *S. sanguis*. Standardized bacterial cell suspensions were used in tests for bactericidal effect and for inhibition of growth and sucrose fermentation. The results showed no interference of xylitol with the antibacterial effect of CHX and F combinations. Xylitol did not show any additive effect either but appeared inert in the combinations used. □ *Chlorhexidine; fluoride;* Streptococcus mutans; Streptococcus sanguis; *xylitol*

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In the chemotherapy of caries and gingivitis an ideal agent would be a chemical that is efficient against pathogenic microorganisms but lacks all side effects (1). So far, no single agent has fulfilled these criteria (2). A combination of two or more chemicals in one preparation might prove beneficial if their mode of action is additive or even synergistic so that the concentrations could be kept as low as possible.

Chlorhexidine (CHX) and fluoride (F) were shown to exert a synergistic mode of action against *Streptococcus mutans* cells (3). This combination was originally introduced by Luoma (4), and clinical studies have been carried out in which the concomitant use of CHX and F solutions has proved beneficial with regard to caries and gingivitis (5–9). Xylitol, a non-cariogenic sweetener (10), was shown to act synergistically with F (11, 12). It also causes membrane alterations in oral streptococci, and the resulting net effect is an inhibition of bacterial growth (13).

We have suggested an antiplaque tablet preparation containing CHX and F in which xylitol forms the bulk of the tablet (14). In clinical testing, the combination appeared effective in reducing plaque and gingival indexes of the test subjects (15).

The aim of this in vitro study was to investigate more specifically how the addition of xylitol to the previously known combination of CHX and F interacts with the bactericidal and bacteriostatic effect of this combination against *S. mutans* and *S. sanguis*. Xylitol in water is an uncharged molecule, and it can be assumed that chemical interactions would normally not occur between xylitol and chlorhexidine and fluoride.

## Materials and methods

### *Test solutions*

The chemicals and their combinations studied are shown in Table 1. Hibitane-Dental solution was included as a positive control and phosphate-buffered saline (PBS) as a negative control. Combination no. 10 was F and xylitol, to which CHX was added to obtain the same CHX concentration as that in Hibitane®.

In the growth inhibition study the CHX concentration varied from 0.1 mg/ml to 1.0 mg/ml and the F concentration from 0.05 mg/ml to 0.5 mg/ml, and the xylitol concentration was 60.9 mg/ml.

*Bacterial strains, growth conditions, and bactericidal effect*

Stock cultures of *S. mutans* ATCC 27351 and *S. sanguis* ATCC 10556 were grown in thioglycollate broth. After incubation for 18 h at 37°C the cells were harvested by centrifugation (9000 *g*, 15 min at 4°C), washed three times with 0.1 M PBS, and suspended in the same buffer to a turbidity of 175 in a Klett–Summerson colorimeter (filter 62; range, 590–660 nm). This corresponded to a level of approximately $10^8$ cells/ml.

Aliquots of 2 ml of the test solutions were added to equal volumes of bacterial suspensions, mixed thoroughly, and incubated in a water bath at 37°C for 1 h with gentle agitation. The cells were then washed three times (9000 *g*, 15 min at 4°C) with the 0.1 M PBS and suspended in 2 ml of buffer (which equaled the original volume). Bacterial dilutions were plated on blood agar, on duplicate plates. After 36 h of incubation at 37°C the bactericidal effect on resting cell suspensions was determined as CFU/ml. The experiment was carried out three times.

*Growth inhibition study*

Aliquots of bacterial suspensions (200 µl) which had been grown for 20 h at 37°C in the thioglycollate broth were spread on Müller–Hinton blood agar plates (Orion Diagnostica, Espoo, Finland). PDM non-impregnated antibiotic sensitivity discs, with a diameter of 6 mm (AB Biodisk, Solna, Sweden), were moistened with 12 µl of the test solutions and placed on the agar. The plates were incubated for 24 h at 37°C, after which the zones of inhibition were read (the diameter of the disc included) by using a Peak Light scale magnifying glass with ×7 magnification and ±0.1 mm reading accuracy. The experiment was carried out three times, and two parallels were used.

*Fermentation test*

Bacteria were grown and washed as above. Resting cell suspensions were suspended in 10% sucrose in 0.9% NaCl to a turbidity of Klett 175. Equal volumes of bacterial suspension and the test solutions were used. The initial pH was measured. The test vials were incubated in a water bath at 37°C without agitation, and the pH was measured every 15 min for 2 h. The difference from the initial pH to the end pH was calculated. The experiment was carried out three times.

Table 1. The chemicals studied and their combinations

| No. and abbreviation of test chemicals used | Composition |
|---|---|
| 1. F | Sodium fluoride, 0.5 mg/ml, 10 mM (0.05%) |
| 2. F + CHX | Sodium fluoride, 0.5 mg/ml, and chlorhexidine diacetate, 1 mg/ml (1.6 mM chlorhexidine$^{2+}$) |
| 3. F + xyl | Sodium fluoride, 0.5 mg/ml, xylitol, 60.9 mg/ml (0.4 M) |
| 4. F + CHX + xyl | Sodium fluoride, 0.5 mg/ml, chlorhexidine diacetate, 1 mg/ml, and xylitol, 60.9 mg/ml |
| 5. CHX | Chlorhexidine diacetate, 1 mg/ml |
| 6. CHX + xyl | Chlorhexidine diacetate, 1 mg/ml, and xylitol, 60.9 mg/ml |
| 7. xyl | Xylitol, 60.9 mg/ml |
| 8. PBS | Phosphate-buffered saline, 0.1 M, pH 7.1 |
| 9. Hibitane | Hibitane-Dental® solution, ICI Ltd, Sussex, U.K. (2.23 mM chlorhexidine$^{2+}$) |
| 10. F + CHX + xyl conc. | Same as no. 4 except that chlorhexidine diacetate concentration was 11.3 mg/ml, corresponding to that of Hibitane |

Table 2. Bactericidal effect of *Streptococcus mutans* and *S. sanguis* after a 1-h incubation of resting cell suspensions with equal volumes of the chemicals listed

| Test chemicals* | Bacterial growth | |
|---|---|---|
| | *S. mutans*, $7.3 \times 10^8$ | *S. sanguis*, $3.6 \times 10^8$ |
| 1. F | $4.2 \times 10^8$ | $2.0 \times 10^8$ |
| 2. F + CHX | $1.0 \times 10^2$ | $4.0 \times 10^2$ |
| 3. F + xyl | $1.6 \times 10^8$ | $4.3 \times 10^8$ |
| 4. F + CHX + xyl | $1.0 \times 10^2$ | $7.5 \times 10^2$ |
| 5. CHX | $1.5 \times 10^2$ | $1.8 \times 10^3$ |
| 6. CHX + xyl | $4.0 \times 10^2$ | $2.6 \times 10^3$ |
| 7. xyl | $4.2 \times 10^8$ | $2.0 \times 10^8$ |
| 8. PBS | $5.2 \times 10^8$ | $4.4 \times 10^8$ |
| 9. Hibitane | No growth | $2.5 \times 10^2$ |
| 10. F + CHX + xyl conc. | $1.0 \times 10^2$ | $4.5 \times 10^2$ |

* See Table 1 for definition of chemicals.

### *Statistical methods*

Anova statistics of the Statistical Analysis System package (SAS Institute Inc., N.C., USA) was used to test the differences in results between the various combinations of the chemicals studied (16).

## Results

### *Bactericidal effect*

Sodium fluoride and xylitol, alone and combined, did not interfere with the viability of the bacterial strains, as expected. As shown in Table 2, the growth of both *S. mutans* and *S. sanguis* after incubation with F and xylitol was comparable to the input concentrations and the PBS series. All the series in which CHX was used showed a reduced viability in both study strains (Table 2). In general, *S. mutans* appeared more sensitive to CHX than *S. sanguis*. The combinations of F and xylitol with CHX showed the same growth reduction as those with CHX alone. The growth in these series was comparable with that of the Hibitane series with *S. sanguis*, whereas *S. mutans* did not grow in the presence of Hibitane.

The bactericidal effect caused by CHX and CHX combinations differed significantly from the results with the other chemicals ($P < 0.001$).

### *Growth inhibition study*

The effect of increasing concentrations of CHX and F on 0.4 M xylitol on the growth of *S. mutans* and *S. sanguis* is shown in Fig. 1. The F concentrations studied did not affect the growth of the streptococci, whereas the increase in CHX concentration distinctly inhibited both the bacteria.

We also carried out an extra experiment (results not shown in Fig. 1) in which the xylitol concentration varied from 0.05 M to 1.0 M, the CHX concentration was 1 mg/ml, and the F concentration was 0.5 mg/ml. The diameter of the inhibition zone was 15.5 mm for *S. mutans* and 11.0 mm for *S. sanguis*. The xylitol concentrations studied did not affect the diameter.

### *Fermentation test*

Results of the fermentations are shown in Table 3. Both the strains fermented sucrose when incubated in PBS. *S. sanguis* and *S. mutans* appeared to ferment sugar also when incubated with xylitol, a bit less than with PBS. The combination F + xylitol appeared to inhibit fermentation significantly ($P < 0.05$) by *S. sanguis* cells. The values from CHX and CHX combination experiments differed significantly from those of PBS values ($P < 0.001$).

## Discussion

Xylitol and F are efficient anticaries agents. F inhibits sugar fermentation (17–19). Xylitol has been shown to inhibit the growth of *S. mutans* in vitro (20), and the depression of *S. mutans* has also been shown in vivo (21).

However, we did not observe any reduction in the fermentation of sucrose in the reaction mixture containing xylitol, added to resting cells, compared with the PBS control. Xylitol alone did not show any effect on the microorganisms studied. It has been previously shown that much lower concentrations of xylitol than used in the present

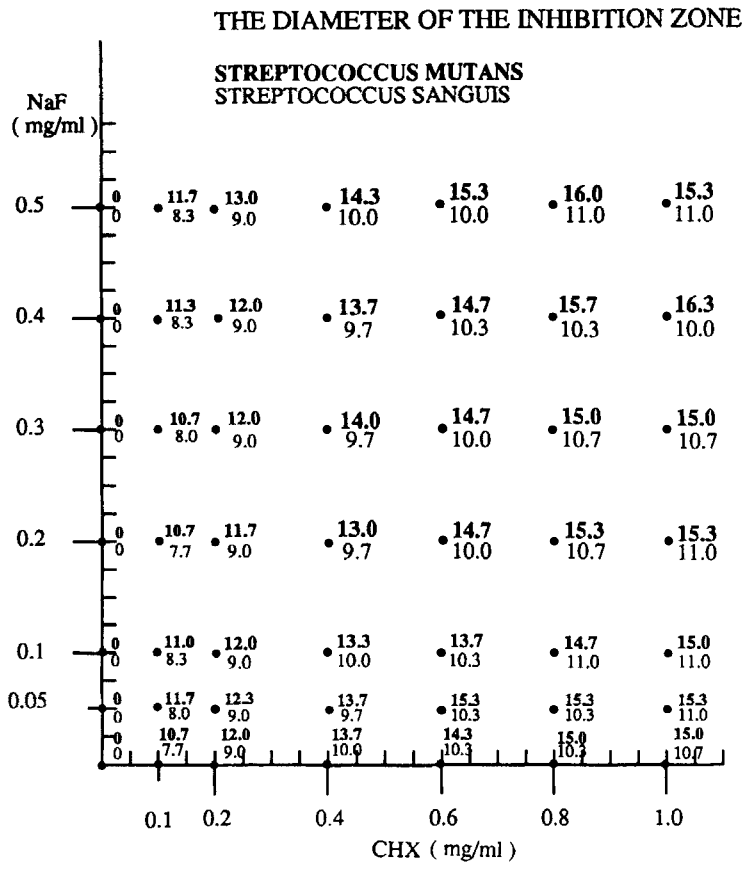


Fig. 1. Sensitivity of *Streptococcus mutans* (bold numbers) and *S. sanguis* (light numbers) bacteria to increasing concentrations of F and CHX in 0.4 M xylitol solution. Diameters of the inhibition zones are given in millimeters.

study may cause membrane alterations that can impair metabolic functions in *S. mutans* cells but do not affect their cultivability (13). On the other hand, xylitol did not seem to interfere with the bactericidal effect of CHX on *S. mutans* and *S. sanguis* cells.

The amount of xylitol appeared not to be critical with regard to the above results. Xylitol concentrations ranging from 0.05 M to 1.0 M were studied with the addition of CHX and F, and the effect on growth inhibition was nearly the same as with the 0.4 M concentration of xylitol.

As shown in Fig. 1, the concentration of CHX should be kept close to 1 mg/ml in the preparation, to attain distinct growth inhibition of both bacterial strains. Concentrations of 1 mg/ml CHX and 0.5 mg/ml F are also clinically acceptable. In Finland, for example, 0.5 mg/ml F is the maximum concentration of fluoride allowed for daily topical use, and the 1 mg/ml of CHX is less than that in the commercially marketed Hibitane. The increase in CHX concentration did not seem to add any bactericidal effect to our preparation (combination no. 10 in Tables 2 and 3). Thus, the bactericidal effect of F + CHX + xylitol on the *S. mutans* and *S. sanguis* strains studied was of the same magnitude as that of Hibitane, although the chlorhexidine concentration in our preparation was only 1.6 mM, compared with 2.23 mM chlorhexidine in Hibitane. The lower chlorhexidine concentration may be anticipated to cause less side effects in the clinical situation than the higher concentration.

Our principal aim was to study whether xylitol interferes with the well-known bactericidal effect of CHX and F on oral strep-

Table 3. Fermentation of sucrose (10%) by *Streptococcus mutans* and *S. sanguis* incubated in the presence of the chemicals and their combinations studied. Results shown as pH change in the reaction mixture during a 2-h incubation

| Test chemicals* | $pH_{t\,0}$ | $pH_{t\,2h}$ | ΔpH |
|---|---|---|---|
| *S. mutans* ATCC 27351, 3.8 × $10^9$ CFU/ml | | | |
| 1. F | 7.2 ± 0.1 | 6.2 ± 0.2 | 1.0 |
| 2. F + CHX | 7.2 ± 0.1 | 7.2 ± 0.1 | 0 |
| 3. F + xyl | 7.3 ± 0.1 | 6.6 ± 0.3 | 0.7 |
| 4. F + CHX + xyl | 7.2 ± 0.1 | 7.2 ± 0.1 | 0 |
| 5. CHX | 7.3 ± 0.1 | 7.3 ± 0.1 | 0 |
| 6. CHX + xyl | 7.3 ± 0.1 | 7.3 ± 0.1 | 0 |
| 7. xyl | 7.3 ± 0.1 | 6.2 ± 0.1 | 1.1 |
| 8. PBS | 7.3 ± 0.1 | 6.0 ± 0.3 | 1.3 |
| 9. Hibitane | 7.3 ± 0.1 | 7.3 ± 0.1 | 0 |
| 10. F + CHX + xyl conc. | 7.3 ± 0.1 | 7.3 ± 0.1 | 0 |
| *S. sanguis* ATCC 10556, 3.9 × $10^9$ CFU/ml | | | |
| 1. F | 7.4 ± 0.02 | 6.7 ± 0.07 | 0.7 |
| 2. F + CHX | 7.4 ± 0.02 | 7.3 ± 0.03 | 0 |
| 3. F + xyl | 7.4 ± 0.01 | 7.2 ± 0.09 | 0.2 |
| 4. F + CHX + xyl | 7.4 ± 0.02 | 7.4 ± 0.02 | 0 |
| 5. CHX | 7.4 ± 0.02 | 7.4 ± 0.03 | 0 |
| 6. CHX + xyl | 7.4 ± 0.03 | 7.4 ± 0.02 | 0 |
| 7. xyl | 7.4 ± 0.06 | 6.6 ± 0.26 | 0.8 |
| 8. PBS | 7.4 ± 0.03 | 6.4 ± 0.10 | 1.0 |
| 9. Hibitane | 7.4 ± 0.02 | 7.4 ± 0.03 | 0 |
| 10. F + CHX + xyl conc. | 7.3 ± 0.03 | 7.3 ± 0.03 | 0 |

* See Table 1 for definition of chemicals.

tococci. Apparently, xylitol did not interfere with this effect, and it therefore seems to be a suitable but inert additive in the combination under the experimental conditions used.

In the clinical experiments our aim has been to replace a liquid with a convenient chewing tablet. In the tablet the concentrations of the effective constituents have equaled those used in the present study (the tablet stimulates the secretion of saliva; a rinsing solution of about 10 ml is produced, and rinsing takes place as with the use of Hibitane-Dental solution). The efficiency of the rinsing solution brought about by the chewing tablet has proved to be nearly as good as that of Hibitane-Dental in preventing bacterial growth on the surfaces of teeth (15, 22).

The in vitro system is a simplification compared with the in vivo circumstances. The in vitro chlorhexidine concentration we used was bactericidal; in our vivo studies it was partly bactericidal, partly bacteriostatic. In the complex ecosystem of the mouth the $F^-$ concentration we used might cause remineralization of enamel and to some extent prevent sucrose fermentation. Under these conditions xylitol might also inhibit bacterial growth and prevent fermentation (10, 20).

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