Effect of mouthrinses with SnF₂, LaCl₃, NaF and chlorhexidine on the amount of lipoteichoic acid formed in plaque

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Abstract - The in vivo effect of some cations on the amount of lipoteichoic acid (LTA) formed in plaque was investigated in two studies. In the first part five students followed six mouthrinsing programs, each of which lasted 4 d. Rinsing was performed for 1 min every second hour with a test solution (0.05% stannous fluoride (SnF₂); 0.05% chlorhexidine gluconate; 0.3% lanthanum chloride (LaCl₃) and 0.05% sodium fluoride (NaF)), followed 5 min later with a rinse of a 15% sucrose solution. Sucrose alone and xylitol rinses were used as controls. The second part involved a group of 10 students rinsing for 1 week four times daily with a sucrose solution, and for another week four times daily with the same sucrose solution and in addition, three times daily with a 0.05% SnF₂ solution. The individual plaque samples were collected after each rinse program, made into a suspension and then divided for protein analysis, carbohydrate analysis and estimation of LTA by phenol extraction and indirect hemagglutination against a specific antiserum. The total amount of plaque formed and the LTA content were reduced in sucrose plaque by the chlorhexidine and SnF₂ rinse programs, or when xylitol replaced sucrose as the main sugar source. There was no significant effect with LaCl₃ or NaF. The rinse programs produced changes in the clinical appearance of the plaque.

Key words: chlorhexidine; dental plaque; lipoteichoic acid; stannous fluoride; xylitol.

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Extracellular lipoteichoic acids (LTA) are polymers of glycerol and phosphate with a strong negative charge (37). They also contain a lipid moiety which readily forms hydrophobic bonds with cell surfaces (3, 22), and has a high affinity for enamel and hydroxyapatite (8).

One area of research has shown that some species of oral streptococci, including Streptococcus mutans, produce significant amounts of extracellular LTA (16, 21). Plaque grown in the presence of sucrose is particularly adhesive (26) and has been shown to contain significant amounts of extracellular LTA (15, 27). It has been suggested that LTA/glucan complexes in the plaque matrix are the chemical basis for the high adhesiveness of sucrose plaque (8, 26).

Another area of research has reported the effect of SnF₂, SnCl₂, AlCl₃, ZnCl₂ and MgCl₂ on the quantity, appearance and consistency of
dental plaque. These substances seem to induce in many cases a less adhesive plaque with a dry, granular appearance (32, 34, 35).

The present study has attempted to bridge these two areas of research by investigating the in vivo effect of some cations, including stannous fluoride, on the amount of LTA formed in plaque.

Material and methods

The study was divided into two parts: the first part involved a group of five dental students (Group 1) while the second part involved a group of 10 dental students (Group 2). All the students had full dentitions (excluding 3rd molars) and no clinical signs of gingivitis. Each student was the subject of a thorough prophylaxis immediately before commencement of each of the rinsing programs described below. All forms of oral hygiene were suspended after the initial prophylaxis until plaque samples had been collected. Test solutions were prepared immediately before each rinse.

GROUP 1

All of the first group followed six rinsing programs, each of which lasted 4 d. Each program involved rinsing for 1 min every second hour with 10 ml of a test solution, followed 5 min later with a 1 min rinse of 10 ml of a 15% w/v sucrose solution.

Each test solution was made up of distilled water and one of the following substances: stannous fluoride (0.05%); chlorhexidine gluconate (I.C.I.) (0.05%); lanthanum chloride (0.3%); sodium fluoride (0.05%); xylitol (15% w/v); and sucrose (15% w/v).

A distilled water rinse was used as a placebo in the sucrose and xylitol rinse programs. The students were requested to reduce dietary sugars especially when rinsing with xylitol.

At the end of each rinse program the individual plaque indices (18) were recorded and all of the plaque formed on the smooth tooth surfaces from each individual was collected with curettes and placed into 0.5 ml of distilled water, care being taken to avoid gingival bleeding and to minimize water loss.

A further 3.5 ml of distilled water was added to each sample and mechanically vibrated into a uniform suspension. Each analysis was carried out on separate volumes of this original suspension.

GROUP 2

Using a double-blind crossover approach, the second group rinsed for 1 week four times daily with a 15% w/v sucrose solution, then for a second week four times daily with a sucrose solution of the same concentration and, in addition, three times daily with a 0.05% stannous fluoride solution.

At the end of each week plaque samples were collected with a flat plastic instrument, care being taken to avoid gingival bleeding. The samples were frozen at −18°C until all the samples had been collected. Each sample was then made into a uniform suspension with 4 ml of distilled water by mixing on a mechanical vibrator.

EXTRACTION OF LTA FROM PLAQUE

The LTA present in the 1 ml of each of the plaque samples was extracted with phenol (36) using the following method: equal volumes of 90% phenol (Baker Chemicals, Deventer, Holland) and the plaque suspension were mixed and placed in a water bath at 70°C for 30 min. The mixture was then cooled in an ice bath for 5 min and centrifuged at 11000 x g for 20 min at 4°C. The water phase containing the extracted LTA was pipetted off and dialyzed, once against running water for 24 h, twice against distilled water at 4°C for 12 h and finally once against saline at 4°C for 24 h.

One ml of each sample was treated with an equal volume of 90% phenol to extract the LTA and the quantity of LTA was assayed by indirect hemagglutination titration (36).

A further 1 ml of each plaque sample was treated with alkali and protein analyses were conducted using the same procedures as were used for Group 1.

ASSAY OF LTA BY HEMAGGLUTINATION TITRATION

Once extracted, the LTA content of each of the plaque samples was estimated by the use of the passive hemagglutination (HA) method (13). Each extract was incubated at room temperature for 3 h and overnight at 4°C with 0.25 ml fresh, packed sheep erythrocytes. Serial twofold dilutions of antiserum in saline and equal volumes of the 0.5% incubated sheep erythrocyte were mixed in Titertek disposable trays and the HA titers were read after 3–4 h at room temperature. The results are expressed as the last dilution of antiserum which produces hemagglutination and is a reflection of the amount of LTA present.
REDUCTION OF LTA IN PLAQUE

Table 1
Plaque indices from Group 1. P.I., Plaque Index according to Löe (19)

<table>
<thead>
<tr>
<th>Test subject</th>
<th>Sucrose + SnF₂</th>
<th>Sucrose + LaCl₃</th>
<th>Sucrose + chlorhexidine</th>
<th>Sucrose + Xylitol</th>
<th>Sucrose + NaF</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>F 1</td>
<td>2.01</td>
<td>1.13</td>
<td>1.90</td>
<td>0.65</td>
<td>1.45</td>
<td>1.83</td>
</tr>
<tr>
<td>F 2</td>
<td>2.00</td>
<td>1.26</td>
<td>1.88</td>
<td>0.52</td>
<td>0.89</td>
<td>1.88</td>
</tr>
<tr>
<td>M 3</td>
<td>2.00</td>
<td>1.27</td>
<td>1.88</td>
<td>0.79</td>
<td>1.07</td>
<td>1.77</td>
</tr>
<tr>
<td>F 4</td>
<td>1.96</td>
<td>0.91</td>
<td>1.77</td>
<td>0.62</td>
<td>1.61</td>
<td>1.24</td>
</tr>
<tr>
<td>F 5</td>
<td>1.96</td>
<td>1.16</td>
<td>1.85</td>
<td>0.52</td>
<td>1.45</td>
<td>1.62</td>
</tr>
</tbody>
</table>

in the plaque samples. To serve as a control, serial twofold dilutions of the SnF₂, chlorhexidine gluconate, LaCl₃, and NaF test solutions and equal volumes of 0.5% sheep erythrocytes were mixed in Titertek trays and the HA titers read after 3–4 h at room temperature.

The titrations for the whole of each group were carried out at the same time using the same reagents, so that a direct comparison of the results within each group could be made. But since the titrations of Group 1 were carried out on a different date from those in Group 2, a direct numerical comparison between the two groups could not be made, because variations occur when different batches of erythrocytes are being used. The antiserum was specific for the glycerol-phosphate backbone of LTA and was kindly provided by Dr. K. W. Knox.

PROTEIN ANALYSIS
The estimation of protein was carried out by mixing 0.5 ml of the original suspension with 0.5 ml 1 M NaOH and heating the mixture in boiling water for 30 min. Protein analyses were conducted according to Lowry et al. (20) using bovine serum albumin (Sigma Chemical Co) as a standard.

CARBOHYDRATE ANALYSIS
(GROUP 1 ONLY)
Using the method of Dubois et al. (10) the total carbohydrate was determined from 2-ml samples of each original suspension, using dextran (Grand Island Biol. Co., NY) as a standard.

Table 2
Protein and carbohydrate values from Group 1. The results are expressed in µg/ml. Protein: Lowry et al. after alkali hydrolysis (20). Carbohydrate: Dubois et al. (10)

<table>
<thead>
<tr>
<th>Test subject</th>
<th>Sucrose + SnF₂</th>
<th>Sucrose + LaCl₃</th>
<th>Sucrose + chlorhexidine</th>
<th>Sucrose + Xylitol</th>
<th>Sucrose + NaF</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>F 1</td>
<td>87</td>
<td>2067</td>
<td></td>
<td>13</td>
<td>58</td>
<td>1092</td>
</tr>
<tr>
<td>F 2</td>
<td>66.5</td>
<td>640</td>
<td>12</td>
<td>125</td>
<td>70</td>
<td>1132</td>
</tr>
<tr>
<td>M 3</td>
<td>42</td>
<td>460</td>
<td>23</td>
<td>265</td>
<td>60</td>
<td>440</td>
</tr>
<tr>
<td>F 4</td>
<td>16</td>
<td>310</td>
<td>8</td>
<td>220</td>
<td>54</td>
<td>740</td>
</tr>
<tr>
<td>F 5</td>
<td>23</td>
<td>493</td>
<td>12</td>
<td>245</td>
<td>46</td>
<td>412</td>
</tr>
</tbody>
</table>
Table 3
Lipoteichoic acid in plaque (in vivo) in Group I. Indirect hemagglutination. The results expressed as the last dilution of antiserum which produces hemagglutination

<table>
<thead>
<tr>
<th>Test subject</th>
<th>Sucrose</th>
<th>Sucrose</th>
<th>Sucrose</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SnF₂</td>
<td>LaCl₃</td>
<td></td>
</tr>
<tr>
<td>1 F</td>
<td>128</td>
<td>8</td>
<td>256</td>
<td>8</td>
</tr>
<tr>
<td>2 F</td>
<td>256</td>
<td>16</td>
<td>256</td>
<td>&lt;4</td>
</tr>
<tr>
<td>3 F</td>
<td>128</td>
<td>16</td>
<td>128</td>
<td>8</td>
</tr>
<tr>
<td>4 F</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>5 F</td>
<td>128</td>
<td>8</td>
<td>128</td>
<td>&lt;8</td>
</tr>
</tbody>
</table>

Results

GROUP I

The statistical significance of the results in Group I was tested by analysis of variance with a pooled standard deviation (7).

The plaque indices and protein analyses from Group I have shown that there is a significant reduction in the quantity of plaque formed when rinsing during the chlorhexidine, stannous fluoride and xylitol rinse programs (Tables 1 and 2). The HA titers for LTA (Table 3) were low with the chlorhexidine, SnF₂ and xylitol rinse programs and, in addition, the LTA-to-protein ratios (Table 4) for these three rinse programs showed significantly low values. The plaque indices and protein analyses obtained from the LaCl₃ rinse program were similar to those of sucrose alone. The LTA titers were high and in some cases higher than those of sucrose. There was no significant difference in the LTA-to-protein ratios compared with the sucrose control. The effect of the sodium fluoride rinse program was variable and the results were not significant. When the control test solutions were titrated against the sheep erythrocytes the polyvalent ions were shown to cause agglutination of the red blood cells.

Some clinical variations were noted with the different test solutions. The consistency of the sucrose plaque was adhesive and it was difficult to collect from the tooth surfaces, whereas the xylitol plaque and the plaques formed with the LaCl₃ or NaF rinse programs were loose, less

Table 4
Ratio of lipoteichoic acid to protein in plaque (in vivo) in Group I. LTA, indirect hemagglutination. Protein: LOWRY et al. after alkali hydrolysis. P < 0.05 for SnF₂, chlorhexidine and xylitol compared with sucrose

<table>
<thead>
<tr>
<th>Test subject</th>
<th>Sucrose</th>
<th>Sucrose</th>
<th>Sucrose</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SnF₂</td>
<td>LaCl₃</td>
<td></td>
</tr>
<tr>
<td>1 F</td>
<td>1.47</td>
<td>0.62</td>
<td>4.41</td>
<td>0.94</td>
</tr>
<tr>
<td>2 F</td>
<td>3.85</td>
<td>1.33</td>
<td>7.31</td>
<td>0.53</td>
</tr>
<tr>
<td>3 M</td>
<td>3.05</td>
<td>0.70</td>
<td>2.14</td>
<td>0.53</td>
</tr>
<tr>
<td>4 F</td>
<td>0.50</td>
<td>1.00</td>
<td>0.30</td>
<td>0.67</td>
</tr>
<tr>
<td>5 F</td>
<td>5.57</td>
<td>0.67</td>
<td>2.78</td>
<td>0.50</td>
</tr>
</tbody>
</table>
Table 5
Lipoteichoic acid in plaque (in vivo) in Group 2. 15% sucrose rinses 4 x daily, 15% sucrose 4 x daily + 3 x 0.05% SnF₂ daily

<table>
<thead>
<tr>
<th>Test subject</th>
<th>LTA*</th>
<th>Pr**</th>
<th>Ratio†</th>
<th>LTA</th>
<th>Pr</th>
<th>Ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 F</td>
<td>512</td>
<td>22</td>
<td>23.2</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2 M</td>
<td>4096</td>
<td>15</td>
<td>273</td>
<td>8</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>3 F</td>
<td>4096</td>
<td>13</td>
<td>315</td>
<td>8</td>
<td>13</td>
<td>0.62</td>
</tr>
<tr>
<td>4 F</td>
<td>8</td>
<td>19</td>
<td>0.42</td>
<td>4</td>
<td>27</td>
<td>0.15</td>
</tr>
<tr>
<td>5 F</td>
<td>8</td>
<td>12</td>
<td>0.67</td>
<td>4</td>
<td>6</td>
<td>0.67</td>
</tr>
<tr>
<td>6 M</td>
<td>16</td>
<td>14</td>
<td>1.14</td>
<td>2</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>7 M</td>
<td>4096</td>
<td>18</td>
<td>228</td>
<td>4</td>
<td>12</td>
<td>0.33</td>
</tr>
<tr>
<td>8 F</td>
<td>4096</td>
<td>26</td>
<td>158</td>
<td>64</td>
<td>18</td>
<td>3.6</td>
</tr>
<tr>
<td>9 M</td>
<td>2048</td>
<td>9</td>
<td>228</td>
<td>16</td>
<td>13</td>
<td>1.23</td>
</tr>
<tr>
<td>10 M</td>
<td>2048</td>
<td>18</td>
<td>114</td>
<td>16</td>
<td>13</td>
<td>1.23</td>
</tr>
</tbody>
</table>

* Indirect haemagglutination
** Lowry et al. after alkali hydrolysis (18).
† P < 0.02 applying Sign test (31).

adhesive and whiter in color. Plaques formed with the chlorhexidine or stannous fluoride rinse programs were reduced in quantity, granular in appearance, less adhesive and golden in color.

The plaque in all cases was found mainly in areas of minimal mechanical interference, along the posterior gingival margins and interproximally. The relative proportion of interproximal plaque increased following the chlorhexidine and stannous fluoride rinse programs.

One student had several areas of exposed cementum and complained of acute hypersensitivity following the two sucrose control rinse programs. Another student observed an increase in salivary flow when rinsing with the stannous fluoride test solution. There were no other comments concerning the test solutions.

GROUP 2

The statistical significance of the results was tested by Sign test (31). The LTA titers and protein analyses were reduced when the SnF₂ rinse was used in addition to the sucrose rinse. There was a reduction in the LTA-to-protein ratio in all cases apart from one (5F) which remained unchanged. These results were significant when applying the Sign test.

As in Group 1, there was a reduction in the quantity of the plaque formed when the SnF₂ rinse was used in addition to the sucrose rinse. Again, the plaque was granular in appearance, less adhesive, and more golden in color than the plaque formed by sucrose alone.

Discussion

The results have shown that the use of certain cationic mouthrinses such as chlorhexidine or stannous fluoride in low concentrations can reduce the quantity of LTA produced in sucrose plaque. But the agglutination effect caused by polyvalent ions known to be retained in plaque (5, 33) suggests that the actual reduction in LTA is probably greater than the results have shown for the chlorhexidine and stannous fluoride rinsing programs. This agglutination effect may also explain the high LTA titers obtained from the lanthanum chloride rinse program.

Some of the students had low LTA to protein ratios after the sodium fluoride rinse program. This may indicate that the reduced metabolic
activity caused by the fluoride ions may also cause a reduction in the LTA content of plaque, although the fluoride concentration in the present study was not high enough to be effective in all individuals.

The results obtained from the one student forming a light plaque in Group 1 (4F) were not consistent with the results obtained from the rest of the group. This individual's greater resistance to plaque formation presumably masks the effect of other additional plaque inhibiting agents.

The large variations in the quantity of LTA found in Group 2 are presumably due to the more infrequent sucrose rinses in this study (four times daily versus once every second hour).

There appears to be no direct correlation between the amount of LTA in plaque and the amount of carbohydrate present (Tables 1 and 2). Other workers have suggested that LTA is retained through complex formation with the extracellular glucans (9) and this concept has been further supported by some in vitro observations (for review see ROLLA et al. (25)).

The low quantity of LTA produced in the presence of xylitol also confirms previously reported experiments (27). It is probably due to the lack of a suitable carbon source in the plaque, because any streptococci present in plaque are unable to metabolize xylitol (12).

The clinical appearance and physical properties of sucrose plaque changed in the presence of the stannous and chlorhexidine rinses, suggesting that LTA may be a factor in determining the physical properties and possibly the cariogenic potential of plaque. This hypothesis is supported by the evidence that chlorhexidine and stannous fluoride both reduce the incidence of caries (2, 19). Moreover, when xylitol replaces sucrose in the diet, the incidence of caries is also reduced (28, 29).

It is possible that the chlorhexidine and stannous ions interfere with plaque formation by altering the charge of the bacteria and thus their affinity for the tooth surfaces (23). It also appears possible that cations bind with the phosphate groups of LTA by electrostatic interaction and thus block the number of available sites for magnesium ions which are essential for membrane stability and bacterial metabolism (1, 3, 14). It is well established that about a third of all biologic enzymes require the presence of metallic ions either as an intrinsic part of their structure or to promote maximum activity (11).

"Foreign" cations retained in high concentrations in plaque may disturb such interactions. The plaque inhibiting cations may also react directly with susceptible groups in the enzymes present in the cell walls, causing an alteration in the rigid protein structure necessary for the catalytic activity of the enzyme.

The glucose molecule enters the streptococcal cell wall by phosphorylation to glucose-6-phosphate, the reaction being catalyzed by the enzyme hexokinase which requires the presence of magnesium ions for maximum efficiency (24). The enzyme hexokinase contains thiol groups which may be oxidized by a cation like the stannous ion, but not by lanthanum. Chlorhexidine can obviously reduce the bacterial metabolism by its bacteriostatic activity in low concentrations.

In accordance with recent data from SCHAMSCHULA et al. (30), which showed a negative relationship between certain metal ions in plaque and the incidence of caries in some native populations in the Pacific, it may be speculated from the present study that small amounts of stannous ions, and presumably other cationic substances in the oral cavity, may modify the chemical and physical properties of plaque thus leading to a reduced cariogenicity.

References

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