Effect of a high-molecular-weight component of cranberry on constituents of dental biofilm

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Background: Previous studies have shown that high molecular-weight non-dialysable material derived from cranberry juice (NDM) inhibits co-aggregation of a variety of oral bacteria.

Objectives: In the present study, we examined the effect of NDM on several constituents of the dental biofilm, glucosyltransferase (GTF) and fructosyltransferase (FTF), as well as on the adhesion of Streptococcus sobrinus.

Results: The activity of immobilized and soluble GTF and FTF was inhibited by NDM (P > 0.05). NDM also inhibited adhesion of S. sobrinus to hydroxyapatite (P < 0.05).

Conclusions: Our results indicate that NDM may affect biofilm formation. One of the proposed mechanisms is via inhibition of extracellular polysaccharide synthesis, which promote the sucrose-dependent adhesion of oral bacteria as S. sobrinus.

Keywords: mutans streptococci, anti-adhesion, glucosyltransferases, fructosyltransferases

Introduction

Dental biofilm is associated with the initiation and progression of tooth decay and periodontal diseases.1 Dental biofilm, found on hard surfaces in the oral cavity, harbours cariogenic bacteria, cell-free enzymes, polysaccharides and host constituents.2 The formation of biofilm on hard surfaces involves several stages.3 The primary coat of the hard surface is a conditioning film composed mainly of salivary proteins and cell-free enzymes. These include glucosyltransferase (GTF) and fructosyltransferase (FTF), secreted by bacteria inhabiting the oral cavity. These extracellular bacterial enzymes synthesize fructans and glucans, which provide binding sites for a succession of bacteria on hard surfaces.4,5 The oral bacteria then adhere to, accumulate and propagate on these surfaces. Mutans streptococci are among the prominent bacterial serotypes proliferating in the dental biofilm plaque. Demineralization of the enamel is greatly enhanced by organic acids generated following carbohydrate metabolism by these biofilm streptococci.

It is believed that reducing the mass of mutans streptococci in dental biofilm could lower the incidence of dental carries. The use of anti-adhesion agents that disengage mutans streptococci from the dental biofilm or interfere with their adhesion, without affecting their viability, may prove clinically advantageous, as selective pressure and overgrowth of resistant bacteria would be avoided.

Cranberry juice is known to affect urinary tract infections. This effect is mediated by its action as an anti-adhesion agent.6,7 Different fractions of the juice were found to inhibit the adhesion of bacteria such as Escherichia coli8 and Helicobacter pylori9 to host cells. Furthermore, a high-molecular-weight, non-dialysable constituent (NDM) of cranberry juice was shown to inhibit or reverse the coaggregation of many oral bacterial pairs.10

The major pathogens responsible for biofilm formation belong to the mutans streptococci group. In the present study, we sought to determine the effect of NDM on mutans streptococci and their ability to form biofilm. The aim of the present study was to examine whether NDM inhibits the sucrose-dependent adhesion of Streptococcus sobrinus to hydroxyapatite (HA), and its influence on enzymatic activities of GTF and FTF, key enzymes in the formation of oral biofilm.

Materials and methods

Preparation of NDM

Concentrated juice from the American cranberry, Vaccinium macrocarpon, was obtained from Ocean Spray Cranberries, Inc. The juice was exhaustively dialysed at 4°C against distilled water in
15 000 MW cut-off dialysis bags and lyophilized. The non-dialysable material, designated NDM, exhibits tannin-like properties; it is highly soluble in water, devoid of proteins, carbohydrates and fatty acids and contains 56.6% carbon and 4.14% hydrogen. The effect of NDM on GTF in solution

The effect of NDM on cell-free GTF activity in solution was studied according to an assay described previously, with minor modifications. GTF, having activity of 0.02 μmol of glucose polymerized into glucan per minute as determined using [14C-glucose]sucrose, was isolated from S. sobrinus 6715, as described by Schilling & Bowen. The isolated GTF was incubated with 400 mM sucrose, supplemented with 0.1 μCi/mL [14C-glucose]sucrose (American Radiolabeled Chemicals, Inc., St Louis, MO, USA) in 5 mM phosphate buffer (pH 6.5) containing 80 mM dextran (MW 9300). NDM at concentrations of 0–2 mg/mL was added to the GTF solution. The reaction was terminated after 1 or 24 h of incubation at 37°C by adding ice-cold ethanol to a final concentration of 75%. The ethanol-insoluble polysaccharides were allowed to precipitate overnight at 4°C. The precipitate was collected and washed over a glass fibre filter, using a multi-sample vacuum manifold (Millipore Corporation, Bedford, MA, USA). The filters were dried, and the radioactively labelled glucans collected on the glass filter were counted in a scintillation counter. Results are presented as percent enzymatic activity from control (absence of NDM).

The effect of NDM on FTF activity in solution

The effect of NDM on FTF activity in solution was studied as previously described. FTF was prepared from S. mutans (V 1995), a gtf knock-out strain, as described by Rozen et al. The measured activity of the FTF preparation was 0.024 μmol of fructose polymerized into fructan per minute. FTF was incubated with 400 mM sucrose supplemented with [3H-fructose]sucrose (NEN, Boston, MA, USA) at 1 μCi/mL in 5 mM phosphate buffer (pH 6.5). The effect of NDM on fructan formation was studied as described above for GTF.

Effect of NDM on biofilm constituents

Effect of NDM on immobilized FTF and GTF. The effect of NDM on activity of cell-free GTF and FTF immobilized on HA was conducted according to an assay described previously. Three millilitres of GTF or FTF were each immobilized on HA as follows: 40 mg of HA beads, diameter 80 μm, surface area 40 m²/g (Bio-Rad Laboratories, Hercules, CA, USA) were equilibrated by three washes in the phosphate buffer. The beads were incubated with GTF or FTF as described above. Ninety-five percent of the introduced enzymes were adsorbed onto the beads. After 1 h of incubation, the HA beads were washed with phosphate buffer 5 mM (pH = 6.5). The enzyme-coated HA beads were incubated with sucrose supplemented with [H-fructose]sucrose (NEβ) for FTF activity, or with [14C-glucose]sucrose (American Radiolabeled Chemicals Inc.) for GTF activity, as described above, for 1 or 24 h in the absence and presence of various concentrations of NDM. The total amount of fructan or glucan synthesized by the respective immobilized enzyme was calculated from the radiolabelled glucans or fructans immobilized on the HA.

Bacterial adhesion

The effect of NDM on bacterial adhesion was tested as described by Rozen et al. Radioactively labelled S. sobrinus (6715) were prepared by supplementing brain heart infusion broth with 0.005 mCi [3H]thymidine (NEN)/mL of bacterial suspension. After 24 h incubation at 37°C, in 5% CO₂-enriched atmosphere, the bacteria were washed three times with PBS. The turbidity of the suspension was adjusted with PBS to OD₅₄₀ = 1.0 ± 0.05. Forty milligrams of equilibrated HA beads were coated with GTF or FTF as described above. Sucrose (400 mM) was added to the radiolabelled bacterial suspension, which was supplemented with NDM at a concentration of 0–1.33 mg/mL. The suspension was then incubated with the FTF or GTF pre-coated HA beads. After 1 h of incubation, the coated HA were washed three times to remove loosely bound bacteria, and samples were counted using a scintillation counter.

Figure 1. Effect of NDM on GTF in solution after 1 and 24 h incubation. *P < 0.05 between NDM of various concentrations and control group of no NDM.

Figure 2. Effect of NDM on FTF in solution after 1 and 24 h incubation. *P < 0.05 between NDM of various concentrations and control group of no NDM.
Statistical analysis

Each experiment was repeated three times, in identical triplicates. Statistical analysis was conducted using the Student t-test with the Bonferroni corrections. Statistically significant values were defined as $P < 0.05$.

Results

NDM significantly reduced the activities of GTF and FTF in solution (Figures 1 and 2) or immobilized on HA (Figures 3 and 4). A 90% inhibition was recorded for both FTF and GTF in solution ($P < 0.05$), but the inhibitory effect of NDM on those enzymes immobilized on HA was much reduced ($P < 0.05$).

The inhibitory effect of NDM on GTF and FTF was dose dependent except for the inhibition of the soluble form of FTF pre-exposed for 1 h to NDM.

In addition to the enzymatic effect, NDM also influenced the sucrose-dependent and non-sucrose-dependent adhesion of S. sobrinus to HA beads. The effect was dose-dependent at NDM concentrations of 0.066–1.33 mg/mL (Table 1). A more than 95% reduction in S. sobrinus adhesion to glucan- or fructan-coated HA was recorded at 1.33 mg NDM/mL. However, the magnitude of the anti-adhesion effect of NDM was less pronounced in reaction mixtures devoid of sucrose, as compared with those in the presence of sucrose.

Table 1. Effect of NDM on adhesion of S. sobrinus to FTF-coated HA, fructan-coated HA, GTF-coated HA, glucan-coated HA. Results are presented as counts per minute (cpm)

<table>
<thead>
<tr>
<th>NDM, (mg/mL)</th>
<th>FTF-coated HA</th>
<th>Fructan-coated HA</th>
<th>GTF-coated HA</th>
<th>Glucan-coated HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32980±1823</td>
<td>42872±1729</td>
<td>29506±4885</td>
<td>177189±8765</td>
</tr>
<tr>
<td>0.066</td>
<td>17994±1348*</td>
<td>1228±1732*</td>
<td>10560±1719*</td>
<td>164932±4682</td>
</tr>
<tr>
<td>0.083</td>
<td>19035±213*</td>
<td>10958±2714*</td>
<td>10318±39*</td>
<td>159760±2634</td>
</tr>
<tr>
<td>0.13</td>
<td>18616±110*</td>
<td>11054±250*</td>
<td>2521±600*</td>
<td>120864±26604*</td>
</tr>
<tr>
<td>0.26</td>
<td>17578±957*</td>
<td>715±77*</td>
<td>924±135*</td>
<td>3408±1028*</td>
</tr>
<tr>
<td>1.33</td>
<td>15027±303*</td>
<td>529±86*</td>
<td>618±53*</td>
<td>618±72*</td>
</tr>
</tbody>
</table>

* $P < 0.05$ between NDM of various concentrations and control group of no NDM

Discussion

Numerous drugs and drug delivery systems have been tested for their effect on dental biofilm formation and maturation. The most common of them contain antibacterial agents, which reduce the number of viable microorganisms in the biofilm.\textsuperscript{15} Although effective, such antibacterial applications have several undesirable side effects. Manipulation of the oral bacterial ecology by altering bacterial adhesion in biofilm—without affecting their viability—represents a novel targeting approach. The anti-adhesion strategies are based on antibodies, adhesion site analogues and receptor analogues.\textsuperscript{16} Anti-adhesion agents reduce the total mass of causative microorganisms but do not affect the viability of the oral bacteria, thereby decimating the development of resistant strains or secondary infections. Thus, application of anti-adhesion agents appears to be a promising approach in oral hygiene.

The formation of dental biofilm on teeth is the primary step leading to oral diseases. The sucrose-dependent mechanism (via synthesis of glucans and fructans by GTF and FTF, respectively) is one of the most important means by which bacteria adhere to hard surfaces in the oral cavity.\textsuperscript{1} However, bacteria can also adhere to constituents of the acquired pellicles via non-sucrose-dependent mechanisms, such as hydrophobicity, surface tension, protein–protein and electrostatic interactions.\textsuperscript{17,2}

Cranberry juice has been used in herbal medicine as an anti-infection agent, especially for urinary tract infections. The NDM...
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constituent of the juice exhibits anti-coaggregation activity against a variety of oral bacteria.11 This fraction is highly soluble in water, devoid of proteins, carbohydrates and fatty acids.12 Because of its high molecular weight, no accurate information could be gained from NMR and mass spectra (neither MALDI nor ES ionization) or from chromatography procedures to resolve its precise structure, even after alkaline or acid treatments (unpublished observations). The specific structure of NDM is under investigation.

Here we have shown that NDM strongly affects biofilm formation via a sucrose-dependent mechanism. The effect is mediated by inhibiting the synthesis of the polysaccharides glucan and fructan by immobilized and soluble GTF and FTF. The inhibitory effect of NDM on the soluble forms of GTF and FTF was more pronounced than on the immobilized forms. Similarly, Wunder & Bowen18 previously showed that the effect of active agents on GTF activity in solution is greater than that on GTF immobilized on HA. Steinberg et al.14 also found that anti-plaque agents such as chlorhexidine and cetylpyridinium chloride are more efficient against FTF in solution than against immobilized FTF. The reduced inhibitory effect of the surface-bound enzymes is attributable to the protective polysaccharide extracellular matrix enveloping the immobilized enzymes, or to conformational changes of the enzymes due to immobilization.2

By inhibiting GTF and FTF activity, NDM reduces the concentration of polysaccharides that mediate the adhesion of S. sobrinus to biofilm. However, it should be noted that NDM exhibited an anti-adhesion effect also in a sucrose-free environment, but this was less effective than the sucrose-dependent adhesion, suggesting that it affects more than one mechanism of bacterial adhesion. It is not clear yet whether the inhibitory effect of NDM on GTF enzymes affects the formation of dextrans or mutants synthesis—this warrants further investigations.

Our finding, that NDM acts as an anti-adhesion agent, is consistent with previous observations showing that NDM displays a broad spectrum of activity.10 Our study revealed a new type of effect of the cranberry constituent, involving effects on biofilm formation and interaction with enzymes associated with the formation of the dental biofilm.

References


