THE EFFECT OF DIETARY CARBOHYDRATES ON THE IN-VITRO ADHESION OF CANDIDA ALBICANS TO EPITHELIAL CELLS

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SUMMARY. The effects of dietary carbohydrates on the adherence of Candida albicans to HeLa epithelial monolayers and buccal epithelial cells were compared by two assay systems. Candida preincubated in 0.5M glucose, sucrose, galactose, xylitol or maltose medium produced a significant enhancement in adhesion to both types of epithelial cells. Maltose was the most effective sugar and glucose the least effective in promoting adhesion, while lactose had no significant effect. A clinical isolate of C. albicans demonstrated a greater overall enhancement in adhesion from preincubation with glucose, sucrose and maltose, when compared with a reference laboratory strain of Candida. These results imply that exogenous or endogenous carbon sources may affect the oral and vaginal carriage of C. albicans, by modifying their adhesive properties.

INTRODUCTION

Various observations indicate that microbial adhesion to a host surface is the first event in colonisation and infection (Gibbons, 1980; Ofek and Beachey, 1980). Although the adhesion of bacteria to host surfaces has been studied in detail, there are only a few reports dealing with the adhesion of Candida albicans to epithelial cells (Liljemark and Gibbons, 1973; Kimura and Pearsall, 1978 and 1980; King, Lee and Morris, 1980; Samaranayake and MacFarlane, 1981; Sobel et al., 1981). It is known that the adhesion of yeasts is markedly influenced by various factors that operate within a given host ecosystem (Samaranayake, McCourtie and MacFarlane, 1980; Sobel et al., 1981; Samaranayake and MacFarlane, 1982). For example, the adhesion of C. albicans to denture acrylic and epithelial cells is significantly enhanced when the yeasts are grown in sucrose-containing media (Samaranayake and MacFarlane, 1980, 1981). The aim of the present investigation was to study, further, the effect of dietary carbohydrates, such as glucose, sucrose, lactose, galactose, maltose and xylitol, on the adhesion of C. albicans to HeLa epithelial monolayers and to desquamated buccal epithelial cells.
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MATERIALS AND METHODS

Strains of Candida

C. albicans strain MRL3153 was used throughout; strain GDH1957, from a patient attending Glasgow Dental Hospital with chronic atrophic candidosis, was included in some experiments. Both strains were identified and tested by germ-tube production (Mackenzie, 1962) and sugar-assimilation and fermentation techniques (Lodder, 1970). Stock cultures were maintained on Sabouraud's dextrose agar slopes at 4°C and subcultured every 2 weeks. A loopful of stock culture was incubated on a Sabouraud's slope for 18 h at 37°C. Four standard loopfuls of this culture were inoculated into 30 ml of mycological peptone with or without added sugar and incubated for the same period; the resultant suspension was centrifuged at 1700 g for 20 min and the deposit was washed once with phosphate-buffered saline (PBS, pH 7.2, 0.1M). A final yeast suspension of c. $4.3 \times 10^7$ yeasts/ml was prepared by adding 100 µl of this deposit to 10 ml of PBS.

Carbohydrate media and culture of yeasts

Molar solutions of sucrose, glucose, lactose, galactose, xylitol and maltose were sterilised by tyndallisation during a period of 3 days. Appropriate volumes of these stock solutions were added aseptically to 2% mycological peptone (Oxoid) to give a 500mM concentration of each carbohydrate.

The test yeasts were grown in the carbohydrate media for 18 h at 37°C. Their adhesion was compared with that of control cultures grown in mycological peptone alone. Control and test cultures were washed twice in PBS with centrifugation at 1700 g for 20 min before the assay.

Adhesion assays

HeLa-cell monolayers. The method was based on that of Samaranayake and MacFarlane (1981). HeLa cells were maintained in tissue-culture flasks as monolayers in the Glasgow modification of Eagle's Minimum Essential Medium (Gibco Bio-Cult, 3 Washington Road, Paisley PA3 4EP, Scotland) supplemented with fetal-calf serum (Gibco) 10%(v/v), sodium bicarbonate 0.2%(w/v), L-glutamine 20 µg/ml, penicillin 100 µg/ml and streptomycin 100 µg/ml. To obtain HeLa-cell suspensions the nutrient medium was discarded and the cells were washed once with PBS and treated with 0.25% trypsin solution. The resulting cells were suspended in Eagle's medium, supplemented as above, to obtain an approximate concentration of $10^5$ cells/ml. Three ml of cell suspension were then seeded aseptically into each well of a multiwell tissue-culture tray (Sterlin, 43 Broad Street, Teddington, Middlesex TW11 8QZ) containing sterile cover slips, and incubated for 3 days at 37°C in an atmosphere of 95% air and 5% carbon dioxide to give confluent monolayers. Immediately before the assay, the nutrient medium was discarded and the monolayer was washed once with PBS. To each well containing a washed monolayer, 2.5 ml of the yeast suspension were added and the tray was incubated at 37°C for 30 min on an orbital shaker (Lukham Ltd, Victoria Gardens, Burgess Hill, West Sussex RH15 9QN) at 60 rpm.

The coverslips with the monolayers were then put in a coverslip carrier and washed for 45 s by gentle manual agitation in PBS, fixed in 10% formalin in balanced salt solution for 30 min, and air dried. The cells were then gram stained and mounted on glass slides with Harleco synthetic resin medium. A stratified sampling technique (Weibel, 1969) was used for counting the yeasts on each monolayer. A graticule of equal squares mounted in the ocular lens of a microscope being used for this purpose. The samples were set up in duplicate for each experiment and the mean number of attached yeasts per monolayer was expressed as yeasts/mm² of epithelial cells. Each experiment was done at least eight times.

Buccal epithelial cells. The method of preparation was that of Kimura and Pearsall (1978). Buccal epithelial cells were collected from two healthy human subjects by gently rubbing the inside of the cheeks with a sterile wooden spatula which was then agitated in 10 ml of PBS. Epithelial cells were washed twice in PBS to remove attached micro-organisms and were
resuspended in PBS at a concentration of $2 \times 10^5$ cells/ml. For the assay, 0.25 ml of epithelial-cell suspension and 0.25 ml of the yeast suspension ($10^7$ cells/ml) were incubated on an orbital shaker at 37°C for 1 h. The cells were then collected on 17-μm-pore polycarbonate filters (Millipore GMBH, Germany) and washed with 70 ml of PBS to remove unattached yeasts. The washed epithelial cells were then air dried, methanol fixed, and gram stained. One hundred randomly selected epithelial cells were examined and the yeasts attached to each epithelial cell were counted. Each experiment was done on at least 8 days with duplicate determinations on each day.

**Statistical analysis**

The results obtained by the sampling technique used in the two assays were not normally distributed. Hence, non-parametric statistical analysis, by the Wilcoxon test for matched pairs, signed-ranks (Siegel, 1956) was applied to determine levels of significance.

**RESULTS**

**Adhesion to HeLa-cell monolayers**

Incubation of C. albicans strain MRL3153 in 500mM glucose, sucrose, galactose, xylitol or maltose for 18 h significantly enhanced their adhesion, compared with that of the control yeasts (table I). Maltose-grown yeasts demonstrated a more than threefold increase in adhesion when compared with that of the yeasts in control media, while glucose-grown yeasts elicited only a marginal increase. In contrast, the lactose-grown yeasts did not show a significant variation in adhesion compared with that of the controls.

**Adhesion to buccal epithelial cells**

Adhesion of the reference-laboratory strain of C. albicans to buccal epithelial cells was markedly enhanced after incubation in glucose, sucrose, galactose, xylitol or

<table>
<thead>
<tr>
<th>Preincubation of Candida in</th>
<th>Number of experiments</th>
<th>Mean number of yeasts/mm² (± SEM)</th>
<th>Test Control</th>
<th>Wilcoxon’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP + lactose</td>
<td>10</td>
<td>125 (±16) 187 (±17)</td>
<td>0.7</td>
<td>NS</td>
</tr>
<tr>
<td>MP control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP + glucose</td>
<td>8</td>
<td>216 (±16) 179 (±8)</td>
<td>1.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MP control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP + sucrose</td>
<td>8</td>
<td>238 (±27) 118 (±23)</td>
<td>2.0</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>MP control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP + galactose</td>
<td>10</td>
<td>365 (±38) 166 (±11)</td>
<td>2.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>MP control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP + xylitol</td>
<td>8</td>
<td>310 (±52) 119 (±23)</td>
<td>2.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MP control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP + maltose</td>
<td>10</td>
<td>430 (±55) 114 (±17)</td>
<td>3.8</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>MP control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MP = mycological peptone; NS = not significant. SEM = standard error of the mean.
TABLE II
The effect of dietary carbohydrates on the adhesion of C. albicans strains MRL3153 and GDH1957 to human buccal epithelial cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Preincubation of <em>Candida</em> in</th>
<th>Mean number of yeasts/mm² (± SEM)</th>
<th>Test Control</th>
<th>Wilcoxon’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRL3153</td>
<td>MP + glucose</td>
<td>146 (± 18)</td>
<td>1.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MP control</td>
<td>118 (± 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MP + sucrose</td>
<td>180 (± 18)</td>
<td>1.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MP control</td>
<td>118 (± 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MP + galactose</td>
<td>261 (± 31)</td>
<td>2.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>MP control</td>
<td>118 (± 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MP + xylitol</td>
<td>319 (± 33)</td>
<td>2.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MP control</td>
<td>118 (± 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MP + maltose</td>
<td>408 (± 60)</td>
<td>3.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MP control</td>
<td>118 (± 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDH1957</td>
<td>MP + glucose</td>
<td>211 (± 18)</td>
<td>1.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MP control</td>
<td>151 (± 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MP + sucrose</td>
<td>377 (± 36)</td>
<td>2.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MP control</td>
<td>151 (± 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MP + galactose</td>
<td>998 (± 72)</td>
<td>6.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>MP control</td>
<td>151 (± 15)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean of eight experiments done in duplicate.

maltose medium (table II). As in the HeLa-cell studies, yeasts grown in maltose media elicited more than a threefold increase in adhesion while yeasts preincubated with the rest of the tested carbohydrates demonstrated similar degrees of enhancement in adhesion.

The adherence of *C. albicans* strain GDH1957, a fresh clinical isolate, was also greatly promoted by preincubation with glucose, sucrose or maltose. This strain produced a sixfold enhancement in adhesion to buccal epithelial cells, after incubation with maltose, in contrast to the threefold enhancement observed with the similarly incubated MRL strain of *C. albicans*.

Comparison of the HeLa and buccal-epithelial-cell techniques

Comparison of test/control values for strain MRL3153 (tables I and II) shows that the two methods gave similar results.

DISCUSSION

Two methods have been used in the present study to determine the effects of dietary carbohydrates on candidal adhesion: the HeLa-cell monolayer assay which was described by us (Samaranayake and MacFarlane, 1981) and the buccal-epithelial-cell system used by Kimura and Pearsall (1978). Although both assay methods yielded similar results, the HeLa-cell system gave a significantly lower percentage coefficient of variation between replicate experiments than the buccal-epithelial-cell system (Samaranayake, 1981). This may be due to the prior in-vivo exposure of the buccal cells to bacteria, saliva and serum and to variations in the quality of cells obtained from
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different donors (King *et al.*, 1980; Sklavounou and Germaine, 1980; Varian and Cooke, 1980) in contrast to uncontaminated, standardised cell populations used in the HeLa-cell assay.

Sucrose and glucose are dietary sugars commonly consumed in the western world and the results reported here imply that diets rich in these sugars may facilitate candidal adhesion and colonisation of the oral mucosa. We have previously demonstrated a similar relationship between glucose, sucrose and candidal adhesion to denture acrylic surfaces *in vitro* (Samaranayake, McCourtie and MacFarlane, 1980; Samaranayake and MacFarlane, 1980) and these findings have been confirmed by McCourtie and Douglas (1981). The present observations lend support to the findings of Russell and Jones (1973) who showed that rats fed on sucrose-rich diets carried *C. albicans* for a longer period than the control rats. In clinical terms, Ritchie *et al.* (1969) reported aggravation of symptoms in patients with oral candidal infections when their carbohydrate intake was raised above normal levels. Also, Olsen and Birkeland (1976) found that repeated sucrose rinses initiated and aggravated chronic atrophic candidosis, which is the most prevalent form of oral candidal infection, commonly seen in elderly denture wearers. Also, it is known that the oral carriage of *Candida* in diabetics is higher than in normal individuals (Weinstein *et al.*, 1960; Barlow and Chattaway, 1969; Tapper-Jones *et al.*, 1981) and slightly higher concentrations of salivary glucose in diabetics may induce proliferation of *Candida* (Knight and Fletcher, 1971). Therefore, our results partially explain the clinical relationship reported by other workers between candidosis and intra-oral concentration of glucose and sucrose.

The phenomenon of glucose-induced adhesion of *Candida* could also play a contributory role in the pathogenesis of vaginal thrush, which is frequently seen during the last trimester of pregnancy and in diabetic women. Both of these conditions are associated with a massive impregnation of the vaginal mucosa with glycogen (Hurley *et al.*, 1974) and a consequent liberation of free glucose (Stewart-Tull, 1964). Moreover, the acidic vaginal conditions that prevail during the later stages of pregnancy may further promote the vaginal carriage of the yeast because it is known that low pH favours *in-vitro* candidal adhesion (Samaranayake and MacFarlane, 1982) and growth (Odds, 1979).

Lactose is the second most common dietary sugar consumed in the western world (Page and Friend, 1975), and it does not appear to influence the adhesion of *Candida* to epithelial surfaces, at least *in vitro*. A possible reason for this result is the inability of *C. albicans* to metabolise lactose. However, *C. albicans* incubated in galactose, which it ferments, elicited a significant twofold increase in adhesion and Douglas, Houston and McCourtie (1981) have obtained similar results with galactose-grown *C. albicans* exposed to acrylic surfaces and epithelial cells.

Maltose and xylitol induced maximal candidal adhesion to HeLa and buccal epithelial cells compared with that induced by the other tested sugars. Maltose is widespread in natural foods and significant amounts are found in bread starch (Birkhed and Fuchs, 1975). The latter could be rapidly hydrolysed, intra-orally, into glucose and maltose by the *α* amylase of saliva (Mörmann and Mühlemann, 1981) and it is tempting to speculate that soft, starchy diets frequently consumed by denture wearers may play a significant role in the pathogenesis of chronic atrophic candidosis by facilitating candidal adhesion to the oral mucosa and acrylic denture surfaces (McCourtie and Douglas, 1981). Xylitol is a pentitol that has been suggested by
various workers as a possible substitute for dietary sucrose because of its anti-cariogenic effect (Koulourides et al., 1976; Scheinin et al., 1976). It is already incorporated in confectionary in some Scandinavian countries, but our results suggest that this procedure may promote the carriage of potentially pathogenic Candida species in the oral cavity and perhaps in the gastro-intestinal tract.

In the present study, with all the tested carbohydrates, a clinical isolate from a patient with chronic atrophic candidosis showed greater enhancement in adhesion, than the reference strain C. albicans MRL 3153. Similar observations have been made in studies on adherence to acrylic surfaces (Samaranayake and MacFarlane, 1980; McCourtie and Douglas, 1981; Samaranayake, 1981), indicating that the organism from an active infection may possess a greater potential to colonise surfaces than one repeatedly cultured in laboratory media over prolonged periods. Similar variations in the adhesion of bacteria to epithelial cells have been previously described (Williams and Gibbons, 1975; Ofek and Beachey, 1980).

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REFERENCES


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