EFFECT OF CHLORHEXIDINE GLUCONATE ON THE ADHERENCE OF *CANDIDA* SPECIES TO DENTURE ACRYLIC

JANE McCOURTIE, T. W. MACFARLANE AND L. P. SAMARANAYAKE

Oral Microbiology Unit, Department of Oral Medicine and Pathology, University of Glasgow Dental Hospital and School, 378 Sauchiehall Street, Glasgow G2 3JZ

SUMMARY. The effect of pretreatment of denture acrylic with chlorhexidine gluconate on the subsequent adherence of *Candida albicans* GDH 2346 was measured *in vitro*. Adherence was significantly reduced by pretreatment with chlorhexidine; maximal inhibition was achieved by incubation at room temperature for 30 min in 2% chlorhexidine. Inhibition of adherence was greatest when the organisms were grown in conditions that enhanced adherence the most, i.e., growth to stationary phase in high concentrations of galactose and sucrose. Yeasts grown in high concentrations of galactose, which were the most adherent to acrylic, were also the most sensitive to the fungicidal action of chlorhexidine gluconate, whereas those grown in a low concentration of glucose were the least adherent and also the most resistant. Adherence to acrylic of seven strains of *C. albicans* isolated from active infections (I strains) and grown in medium containing 500 mM sucrose was significantly higher than that of four strains obtained from asymptomatic carriers (C strains). A spectrum of adherence values was obtained when various yeasts other than *C. albicans* were tested.

INTRODUCTION

Chronic atrophic candidosis (CAC) is the commonest form of oral candidosis (Odds, 1979). The fitting surface of the upper denture is the main reservoir of infection, and the ability of *Candida* species, especially *C. albicans*, to adhere to and to colonise denture acrylic is thought to be an important factor in pathogenesis. The main aim of treatment of CAC is to eliminate yeasts from the denture surface and both antifungal agents and antiseptics have been used to achieve this (Odds, 1979). Although CAC has been treated successfully with chlorhexidine gluconate (Budtz-Jørgensen and Løe, 1972; Olsen, 1975*a* and *b*), there is little specific information regarding its mode of action.

In the present study, the effect of pretreatment of denture acrylic with chlorhexidine gluconate on the subsequent attachment *in vitro* of various yeasts isolated from carriers or patients with candidosis was investigated.

MATERIALS AND METHODS

Organisms. Eleven strains of *C. albicans*, three each of *C. tropicalis* and *C. glabrata*, and one each of *C. parapsilosis* and *C. krusei* were used in this study. Eight of the *C. albicans* isolates have previously been described (McCourtie and Douglas, 1984), and have been deposited with the National Collection of Yeast Cultures (NCYC), Food Research Institute, Norwich. *C. albicans* MRL 3153 was from the Mycological Reference Laboratory at the London School of Hygiene and Tropical Medicine. All other strains were isolated at the Glasgow Dental Hospital. *C. albicans* GDH 3212 and GDH 3339 were isolated from paraffin-stimulated saliva obtained from asymptomatic children; *C. tropicalis* GDH 870, *C. glabrata* GDH 820 and *C. parapsilosis* GDH 1218 were from tongue lesions; *C. tropicalis* GDH 1160 and GDH 819 and *C. krusei* GDH 901 were from patients with CAC; *C. glabrata* GDH 879 was from a patient with recurrent oral candidosis; *C. glabrata* GDH 1269 was isolated along with *Staphylococcus aureus* and *C. albicans* from a tongue lesion in a patient on radiotherapy. All isolates were identified by testing for germ-tube production and sugar-assimilation and -fermentation patterns (Lodder, 1970). The organisms were maintained on slope of Sabouraud Dextrose Agar (Difco) and subcultured monthly. Every 2 months the cultures were replaced by new ones freshly grown from freeze-dried stock.

Growth conditions. The yeasts were grown in Yeast Nitrogen Base Medium (YNB; Difco) containing 50 mM glucose or 500 mM glucose, sucrose, galactose, maltose or fructose as previously described (McCourtie and Douglas, 1981). They were harvested after incubation for 24 h (stationary growth phase) and washed twice in 0.15 M phosphate-buffered saline (pH 7.2; PBS).

Preparation of acrylic strips. Acrylic strips were prepared as described by Samaranyake and MacFarlane (1980) with the following modifications. Acrylic monomer and polymer (Simplex Rapid; Howmedica International Ltd, 622 Western Avenue, London) were mixed on aluminium foil-covered glass slides and polymerised in a water bath (40°C). The aluminium foil aids recovery of the acrylic from between the slides, while the lower temperature for polymerisation reduces bubbling within the acrylic and increases clarity.

Adherence assay. Acrylic strips were preincubated in either chlorhexidine gluconate (ICI Pharmaceuticals Ltd) or sterile distilled water at room temperature before performing the adherence assay as previously described (McCourtie and Douglas, 1981). Briefly, transparent acrylic strips were incubated in standardised yeast suspensions (2.5×10^9 cells/ml in PBS) and the number of adherent organisms determined by microscopy. All adherence values quoted were obtained by counting 30 fields on each one of duplicate strips from ten independent assays.

Sensitivity to chlorhexidine gluconate. To determine the fungicidal concentrations of chlorhexidine for the yeast isolates, 1-ml volumes of standardised yeast suspensions (5×10^9 cells/ml in distilled water) were added to 1-ml volumes of various concentrations of chlorhexidine. After 5 min at room temperature, the reaction was stopped by tenfold dilution in chlorhexidine neutraliser—3% L- α -phosphatidylcholine (lecithin) in 10% polyoxyethylenesorbitan mono-oleate (Tween 80) (Sigma Chemical Co. Ltd, Poole). Each suspension was further diluted 100-fold in PBS before being spotted on to plates of Sabouraud Dextrose Agar. Growth patterns were recorded after incubation for 48 h at 37°C. The fungicidal concentration was taken as the lowest percentage of chlorhexidine at which no growth was detected. Three independent determinations were made.

Statistical analyses. Student's *t* test was used to evaluate differences in yeast adherence. A *p* value < 0.05 was considered significant.

RESULTS

Effect of chlorhexidine on adherence

Adherence of *C. albicans* GDH 2346 grown in 500 mM sucrose to denture acrylic preincubated with chlorhexidine gluconate was significantly reduced when compared with adherence to acrylic similarly treated with sterile distilled water. Fig. 1 shows the

FIG. 1.—Effect of pretreatment of acrylic with different concentrations of chlorhexidine gluconate on the subsequent adherence of *C. albicans* GDH 2346 grown in medium containing 500 mM sucrose. Vertical bars represent the standard error of the mean.

percentage inhibition of adherence achieved with preincubation for 30 min at room temperature in concentrations of chlorhexidine from 0.2 to 3.0%. Inhibition was greatest at concentrations greater than about 1.5%, when the number of adherent yeasts was reduced by *c.* 85%.

The effect of length of preincubation of acrylic in 2% and 0.2% chlorhexidine on adherence is shown in fig. 2. Adherence was reduced by half when the acrylic was exposed to 2% chlorhexidine for as little as 30 s. The greatest effect was found after about 20 min when adherence was reduced to about 20% of that to untreated acrylic. Adherence was similarly affected when 0.2% chlorhexidine was used to pretreat the acrylic, but to a lesser extent. In all cases the reduction in adherence was highly significant ($p < 0.001$). In the light of these results, the acrylic was preincubated with 2% chlorhexidine gluconate for 30 min at room temperature in all subsequent experiments.

Adherence to acrylic after growth in different sugar media

We have shown previously that adherence of *C. albicans* GDH 2346 to denture acrylic is enhanced by growth of the yeast in 500 mM concentrations of various sugars (including sucrose), when compared with cells cultured in medium containing a relatively low concentration (50 mM or 0.9%) of glucose (McCourtie and Douglas, 1981). The adherence of yeasts grown in these conditions is shown in table I. The

FIG. 2.—Effect of time of pretreatment of acrylic with 0.2% (○) and 2.0% (●) chlorhexidine gluconate on the subsequent adherence of *C. albicans* GDH 2346 grown in medium containing 500 mM sucrose. Vertical bars represent the standard error of the mean.

presence of the disinfectant reduced adherence in all cases ($p < 0.001$) but to different extents. Once more, adherence was most inhibited when the organisms were grown in conditions that promote adherence to the greatest extent, i.e., in the presence of 500 mM sucrose or galactose. Inhibition of adherence was also greater when the yeasts were in stationary phase compared with the mid-exponential phase of growth (results not shown).

The fungicidal concentrations of chlorhexidine gluconate for *C. albicans* GDH 2346 grown with different carbon sources are shown in table I. Organisms with the greatest adhering capacity were killed by lower dilutions of chlorhexidine and were, therefore, more sensitive to the disinfectant than organisms that were weakly adherent.

Effect of chlorhexidine on yeasts

Adherence of all strains of *C. albicans* to denture acrylic was significantly reduced by prior treatment of the acrylic with 2% chlorhexidine gluconate at room temperature for 30 min (table II). The strains originally isolated from active infections (I strains; the first seven strains in table II) were affected most, with adherence reduced to between 10 and 30% of that to untreated acrylic. On the other hand, with the four strains from asymptomatic carriers (C strains; *C. albicans* GDH 3339, GDH 3212, GRI 681 and GRI 682) adherence to chlorhexidine-treated acrylic was proportionately higher,

TABLE I

Effect of chlorhexidine gluconate on the adherence to acrylic of C. albicans GDH 2346 grown with different carbon sources

Carbon source*	Mean number of adherent yeasts/mm ² of acrylic $\pm$ SEM		Relative adherence $\ddagger$	Fungicidal concentration $\S$
	Control	Chlorhexidine treated $\dagger$		
Glucose (50 mM)	78 $\pm$ 6	36 $\pm$ 4	0.5	0.5
Fructose	84 $\pm$ 9	39 $\pm$ 4	0.5	0.2
Glucose	177 $\pm$ 16	62 $\pm$ 8	0.3	0.07
Maltose	201 $\pm$ 17	64 $\pm$ 5	0.3	0.07
Sucrose	327 $\pm$ 18	63 $\pm$ 8	0.2	0.05
Galactose	719 $\pm$ 82	90 $\pm$ 18	0.1	0.02

* Carbon sources were present at 500 mM concentrations, except where indicated.

$\dagger$ Acrylic strips were preincubated for 30 min at room temperature in 2.0% chlorhexidine; control strips were similarly treated with water.

$\ddagger$ Adherence is expressed relative to that to untreated acrylic.

$\S$ Organisms were exposed to chlorhexidine for 5 min, and the fungicidal concentration taken as the lowest percentage of chlorhexidine required to kill all the yeast cells

ranging from 40 to 80% of that to untreated acrylic. The fungicidal concentrations of chlorhexidine for the I strains of *C. albicans* were fairly constant, either 0.05 or 0.07% chlorhexidine being required to kill the organisms. However, the C strains were more resistant to chlorhexidine, in particular strains GRI 681 and GDH 3339 which were killed by 1.0%.

Adherence of the three strains of *C. tropicalis* was inhibited by chlorhexidine, but not as effectively as that of the I strains of *C. albicans*. These strains were also sensitive to chlorhexidine, as shown by the fungicidal concentrations (table II). The fungicidal concentrations for the *C. glabrata* isolates were more varied, and the adherence to acrylic less affected by treatment with chlorhexidine. Indeed, there was no significant difference between adherence to chlorhexidine-treated and untreated acrylic with *C. glabrata* GDH 820. *C. parapsilosis* GDH 1218 and *C. krusei* GDH 901 were more resistant to chlorhexidine and adherence of both strains was not significantly affected by pretreatment of the acrylic with 2.0% chlorhexidine gluconate.

DISCUSSION

The results of the present study indicate that the adherence of *C. albicans* to acrylic surfaces is substantially reduced by pretreatment of the acrylic with chlorhexidine gluconate. Inhibition of adherence increased rapidly during the first 20 min of exposure of the acrylic to chlorhexidine. Indeed, Bonesvoll and Olsen (1974) showed that most chlorhexidine was adsorbed on to dentures during the 16-min period after a 0.2% mouth-rinse. These findings may explain in part the results of previous studies on the use of chlorhexidine as an anti-candidal agent. Budtz-Jørgensen and Løe (1972) found that disinfection of dentures with 2.0% chlorhexidine gluconate for 15 s was an effective treatment for CAC. Similar results were reported by Olsen (1975*a* and *b*) who showed that chlorhexidine reduced the number of organisms on the mucous membrane and on the denture, and also reduced inflammation of the palate. Also, Langslet *et al.*

TABLE II

Effect of chlorhexidine gluconate on the adherence of C. albicans and other yeast isolates to acrylic after growth in medium containing 500 mM sucrose

Organisms		Mean number of adherent yeasts/mm ² of acrylic $\pm$ SEM		Relative adherence*	Fungicidal concentration
		Control	Chlorhexidine treated*		
<i>C. albicans</i> (I strains)	GDH 2023	582 $\pm$ 35	125 $\pm$ 13	0.2	0.05
	GRI 2773	444 $\pm$ 22	60 $\pm$ 5	0.1	0.07
	GRI 272	339 $\pm$ 12	70 $\pm$ 7	0.2	0.07
	GDH 2346	327 $\pm$ 18	63 $\pm$ 8	0.2	0.05
	GDH 2036	325 $\pm$ 33	84 $\pm$ 6	0.3	0.07
	BP 3968	273 $\pm$ 24	57 $\pm$ 7	0.2	0.05
	MRL 3153	234 $\pm$ 12	48 $\pm$ 7	0.2	0.05
<i>C. albicans</i> (C strains)	GDH 3339	146 $\pm$ 14	67 $\pm$ 4	0.5	1.0
	GRI 682	120 $\pm$ 8	65 $\pm$ 5	0.5	0.1
	GDH 3212	95 $\pm$ 6	76 $\pm$ 4	0.8	1.0
	GRI 681	88 $\pm$ 8	40 $\pm$ 5	0.4	0.1
<i>C. tropicalis</i>	GDH 870	220 $\pm$ 11	76 $\pm$ 5	0.3	0.05
	GDH 819	184 $\pm$ 9	104 $\pm$ 29	0.6	0.07
	GDH 1160	170 $\pm$ 18	67 $\pm$ 9	0.4	0.02
	GDH 879	144 $\pm$ 12	101 $\pm$ 11	0.9	0.2
<i>C. glabrata</i>	GDH 1269	113 $\pm$ 13	56 $\pm$ 11	0.5	0.05
	GDH 820	74 $\pm$ 5	62 $\pm$ 10	0.8	0.07
<i>C. parapsilosis</i>	GDH 1218	195 $\pm$ 17	146 $\pm$ 23	0.7	0.2
<i>C. krusei</i>	GDH 901	122 $\pm$ 14	137 $\pm$ 29	1.1	0.2

* See footnote to table I.

(1974) reported that a 0.2% solution of chlorhexidine gluconate was effective in the treatment of children with acute hyperplastic candidosis when it was either used as a mouthwash or was painted directly on to the lesions. Animal experiments substantiating these data have shown that induced palatal candidosis in rats is cured or prevented when they are fitted with chlorhexidine-containing acrylic plates (Lamb and Martin, 1983).

Some of the results obtained from this study were unexpected and it is interesting to speculate on the mechanisms involved. When *C. albicans* GDH 2346 was grown in media containing high concentrations of sugar, notably sucrose or galactose, the attachment of the organisms to acrylic was inhibited by chlorhexidine to a greater extent than that of yeasts grown in medium containing 50 mM glucose. In the same growth conditions, this organism was more sensitive to the antifungal activity of chlorhexidine (table I). There is evidence from electronmicroscopy studies that yeasts cultivated in 500 mM sucrose or galactose possess an additional surface layer when compared with cells grown in 50 mM glucose (McCourtie and Douglas, 1981). This layer is believed to promote adherence to acrylic surfaces (McCourtie and Douglas, 1981) and to buccal epithelial cells (Douglas *et al.*, 1981). The extracellular polysaccharides of certain oral streptococci appear to retain chlorhexidine and Davies (1973) has suggested that this property may explain the high sensitivity of these bacteria to chlorhexidine. A local retention and perhaps build up of chlorhexidine in the surface layer of candida may occur, and could explain in part these unexpected results.

McCourtie and Douglas (1984) have shown that strains of *C. albicans* isolated from active infections (I strains) were more adherent to acrylic than were strains obtained

from asymptomatic carriers (C strains) especially when they had been grown in media containing 500 mM sucrose or galactose. Similar results were obtained in this study with a mixture of the strains used by McCourtie and Douglas (1984) and fresh strains isolated in the Dental Hospital. It is interesting that I strains were more sensitive to chlorhexidine than C strains.

Compared to I strains of *C. albicans*, the other *Candida* spp. tested were generally less sensitive to the antifungal action of chlorhexidine, less adherent to acrylic and the adhesion was less affected by chlorhexidine. However, the importance of yeasts other than *C. albicans* in oral infections is uncertain. Budtz-Jørgensen *et al.* (1975) found that yeasts other than *C. albicans* were isolated in pure culture from only 6.5% of patients with CAC. Furthermore, using *C. tropicalis* and *C. glabrata*, instead of *C. albicans*, Shakir *et al.* (1983) were unable to induce palatal candidosis in rats fitted with acrylic plates. *C. albicans* also adheres to epithelial cells to a significantly greater degree than other species tested (King *et al.*, 1980). It is interesting, from a treatment point of view, that the strains most sensitive to chlorhexidine were those closely related to infection, i.e., I strains of *C. albicans*, while those least sensitive were those rarely involved in disease, i.e., species other than *C. albicans*.

The results reported here for *C. albicans* GDH 2346 suggest that soaking dentures in 2.0% chlorhexidine for 30 min would be optimal for reducing yeast adherence and for killing any attached yeasts. The effect of length of exposure to chlorhexidine on the subsequent adherence of six other *Candida* isolates was investigated (*C. albicans* MRL 3153, GDH 2023, GDH 3212 and GRI 681; *C. tropicalis* GDH 1160 and *C. glabrata* GDH 820) and similar results were obtained. Even if the length of exposure was reduced to 10 min, the killing and inhibition of adherence would still be high, e.g., about 100% and 70% respectively with *C. albicans* GDH 2346. Because preliminary work indicates that the effect of chlorhexidine on yeasts is not substantially inactivated by the presence of saliva and serum (unpublished data), regular soaking of dentures in a 2.0% solution may be a useful adjunct to treatment of CAC and help to prevent recurrence of the infection.

This work was supported by a grant from ICI Pharmaceuticals Ltd, Macclesfield.

REFERENCES

- Bonesvoll P, Olsen I 1974 Influence of teeth, plaque and dentures on the retention of chlorhexidine in the human oral cavity. *Journal of Clinical Periodontology* **1**:214-221.
- Budtz-Jørgensen E, Løe H 1972 Chlorhexidine as a denture disinfectant in the treatment of denture stomatitis. *Scandinavian Journal of Dental Research* **80**:457-464.
- Budtz-Jørgensen E, Stenderup A, Brabowski M 1975 An epidemiologic study of yeasts in elderly denture wearers. *Community Dentistry and Oral Epidemiology* **3**:115-119.
- Davies A 1973 The mode of action of chlorhexidine. *Journal of Periodontal Research* **8**: Supplement 12, 68-75.
- Douglas L J, Houston J G, McCourtie J 1981 Adherence of *Candida albicans* to human buccal epithelial cells after growth on different carbon sources. *FEMS Microbiology Letters* **12**:241-243.
- King R D, Lee J C, Morris A L 1980 Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. *Infection and Immunity* **27**:667-674.
- Lamb D J, Martin M V 1983 An *in vitro* and *in vivo* study of the effect of incorporation of chlorhexidine into autopolymerizing acrylic resin plates upon the growth of *Candida albicans*. *Biomaterials* **4**:205-209.

- Langslet A, Olsen I, Lie S O, Løkken P 1974 Chlorhexidine treatment of oral candidiasis in seriously diseased children. *Acta Paediatrica Scandinavica* **63**:809-811.
- Lodder J 1970 The yeasts, a taxonomic study, 2nd edn. North Holland, Amsterdam, p 1385.
- McCourtie J, Douglas L J 1981 Relationship between cell surface composition of *Candida albicans* and adherence to acrylic after growth on different carbon sources. *Infection and Immunity* **32**:1234-1241.
- McCourtie J, Douglas L J 1984 Relationship between cell surface composition, adherence and virulence of *Candida albicans*. *Infection and Immunity* **45**:6-12.
- Odds F C 1979 Candida and Candidosis. Leicester University Press. Leicester.
- Olsen I 1975a Denture stomatitis—Effects of chlorhexidine and amphotericin B on the mycotic flora. *Acta Odontologica Scandinavica*, **33**:41-46.
- Olsen I 1975b Denture stomatitis—The clinical effects of chlorhexidine and amphotericin B. *Acta Odontologica Scandinavica* **33**:47-52.
- Samaranayake L P, MacFarlane T W 1980 An *in vitro* study of the adherence of *Candida albicans* to acrylic surfaces. *Archives of Oral Biology* **25**:603-609.
- Shakir B S, Martin M V, Smith C J 1983 Relative effectiveness of various yeasts, *Candida* spp. and *Torulopsis glabrata* for inducing palatal infection in the Wistar rat. *Archives of Oral Biology*, **28**:1069-1071.