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Preliminary investigations concerning the anti-adherence properties of polyhexamethylenebiguanide (Vantocil IB™)

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Abstract

The effect of polyhexamethylenebiguanide (PHMB) on adherence of *Candida albicans* blastospores to human buccal epithelial cells (BEC) was examined in vitro. Treatments of either blastospores or BEC with PHMB (50 and 1000 $\mu g\ ml^{-1}$) significantly reduced the number of adherent blastospores per BEC and increased the number of BEC devoid of blastospores.

Keywords: Candida albicans; Reduced adherence; Blastospore; Polyhexamethylenebiguanide

Candida albicans is a dimorphic yeast which is an obligate associate of man where it is commonly found as a commensal of the vaginal and digestive tracts. However, over the last few decades there has been as increase in the incidence of candidosis, both superficial and systemic, due primarily to iatrogenic factors, e.g., immunosuppressive treatment, antibiotic usage and parenteral nutrition (Shepherd et al., 1985). Typical sites of *C. albicans* infection include the oral cavity, fingernails, genitals, respiratory, urinary and gastrointestinal tracts, central nervous and cardiovascular systems (Odds, 1988).

It is now accepted that in the pathogenesis of candidosis, *C. albicans* must first adhere to the epithelial cells of the host. In so doing, this enables the flushing mechanism of the body secrations which cleanse mucous membranes to be successfully overcome (Douglas, 1987). The adherence process is thought to occur in two distinct stages. In the first (reversible) stage, as the yeast cell approaches the host epithelia, it is subjected to both attractive and repulsive forces. If the attractive forces exceed the repulsive forces, the pathogen may approach the epithelial cell at close distances. The second stage then occurs and involves an irreversible interaction between adhesin molecules on the surface of the yeast cell and the corresponding receptors on the surface of the epithelial cell (Douglas, 1987; Fowler and Jones, 1992). The importance of this ability to adhere has been further highlighted by the reported correlation between ability to adhere and virulence potential amongst *Candida* species (Douglas, 1987).

There have been several attempts to reduce or inhibit candidal adherence and thus interfere with

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the infection process. These include the use of isolated adhesin or adhesin analogues (Lee and King, 1983; Collins-Lech et al., 1984), lectins (Sandin et al., 1982) and sub-lethal concentrations of antibiotics (Lee and King, 1983). More recently, it has been reported that non-antibiotic, antimicrobial agents significantly reduced the adherence of *C. albicans* to buccal epithelial cells in vitro. Examples include taurolidine (Jones et al., 1986); chlorhexidine gluconate, cetrimide, cetylpyridinium chloride, dequalinium chloride (Fowler and Jones, 1992); Brolene™ (Jones and Fowler, 1994) and polyvinylpyrrolidone-iodine (Gorman et al., 1987). Therefore, in the light of this activity, this investigation was performed to evaluate whether the biguanide antimicrobial agent polyhexamethylenebiguanide (Vantocil IB) also exhibits this ability to modify the adherence of *C. albicans* to buccal epithelial cells in vitro.

Polyhexamethylenebiguanide hydrochloride (PHMB, Vantocil IB™) was a gift from Zeneca (Manchester, UK). The appropriate concentrations were obtained following dilution with sterile deionised water. All other chemicals used in this investigation were AnalaR, or equivalent, grade.

The strain (MEN) and oral clinical isolate used in this investigation were previously employed in adherence studies (Fowler and Jones, 1992; Jones and Fowler, 1994). Storage was performed on the surface of Nutrient agar (Difco) slopes at 4°C and, when required, stationary phase blastospores were cultivated by inoculating two loopfuls into prewarmed Nutrient broth (Difco) and incubating at 25°C for 18 h in a shaking water bath. Exponential phase blastospores were cultivated by transferring a sample of stationary phase organisms into prewarmed Nutrient broth for growth at 25°C for a further 6 h in a shaking water bath. Confirmation of the absence of hyphal forms was performed using light microscopy.

Buccal epithelial cells (BEC) were collected as previously described (Jones et al., 1986; Gorman et al., 1987; Fowler and Jones, 1992; Jones and Fowler, 1994). In brief, the buccal mucosae of healthy male and female volunteers were gently scraped using sterile ampoule files, the ampoule files subsequently placed in phosphate-buffered saline (PBS, 0.03 M, pH 7.4), vortexed to dislodge the BEC and the cells washed with PBS. Volunteers were selected on the basis that they were not taking any oral medication or antibiotics.

The assay used to examine the effects of PHMB

Table 1
The effect of polyhexamethylenebiguanide (Vantocil IB™) on the adherence of *Candida albicans* (MEN strain and a clinical oral isolate, exponential and stationary growth phases) to human buccal epithelial cells (BEC) in vitro

Concentration of PHMB (% v/v)	Cell treatment	*C. albicans* strain or isolate	Number [a] of adherent *C. albicans* per buccal epithelial cell (mean ± S.E.)		Percentage [b] of buccal epithelial cells free from adherent *C. albicans*	
			Exponential	Stationary	Exponential	Stationary
0 (water)	*C. albicans*	MEN strain	2.38 ± 0.24	1.44 ± 0.16	23.8	34.6
0.005			0.51 ± 0.08	0.54 ± 0.08	69.3	61.9
0.1			1.23 ± 0.12	0.50 ± 0.09	44.7	68.1
0 (water)	*C. albicans*	oral isolate	1.93 ± 0.18	2.18 ± 0.24	30.0	26.7
0.005			1.13 ± 0.15	0.71 ± 0.1	52.0	60.0
0.1			0.56 ± 0.08	1.69 ± 0.15	67.3	39.0
0 (water)	BEC	MEN strain	3.41 ± 0.30	4.64 ± 0.43	19.0	13.5
0.005			2.55 ± 0.24	2.25 ± 0.19	23.0 [c]	38.6
0.1			2.39 ± 0.17	2.30 ± 0.15	27.5 [c]	24.3
0 (water)	BEC	oral isolate	2.40 ± 0.21	4.41 ± 0.34	24.6	8.13
0.005			1.83 ± 0.18	2.24 ± 0.22	31.3 [c]	23.3
0.1			0.70 ± 0.1	1.61 ± 0.15	61.3	32.6

Differences between water and Vantocil™ treatments analysed using either a two-tailed unpaired *t*-test [a] ($p < 0.05$), or a χ^2 analysis [b] ($p < 0.05$), [c] $p > 0.05$ (non-significant).

on the adherence of *C. albicans* to human BEC in vitro has been described previously (Jones et al., 1986; Gorman et al., 1987; Fowler and Jones, 1992; Jones and Fowler, 1994). In this method either blastospores or BEC were treated for 30 min at 37°C with either PHMB (50 and 1000 μg ml^{-1}) or sterile water. Following this PHMB was removed by decantation following centrifugation (3000 $\times g$ for 10 min), the cells washed with PBS and resuspended to approx. 1×10^7 cfu ml^{-1} (*C. albicans*) or 1×10^5 BEC ml^{-1}. Equal volumes of blastospores and BEC were mixed and incubated at 37°C for 2 h in a shaking water bath (150 oscillations min^{-1}). Following this samples were removed, stained using crystal violet and the number of adherent blastospores to at least 150 BEC counted.

Statistical evaluation of the mean number of adherent *C. albicans* per BEC following treatment with either PHMB or sterile water was performed using a two-tailed unpaired *t*-test (Woolfson et al., 1987; Fowler and Jones, 1992; Jones and Fowler, 1994). The differences in the number of BEC free from adherent *C. albicans* following each treatment were compared using Chi-squared analysis (Fowler and Jones, 1992; Jones and Fowler, 1994).

The effect of PHMB on the adherence of *C. albicans* to BEC in vitro is shown in Table 1. As may be observed PHMB (50 and 1000 μg ml^{-1}) significantly reduced the adherence of *C. albicans* to human BEC in vitro in comparison with the water control ($p < 0.05$). Noticeably, these effects were observed with the stationary and exponential growth phases of both the MEN strain and the oral clinical isolate whenever either the blastospores or BEC were treated with PHMB. In this course of experiments the range in mean number ($\pm$S.E.) of adherent blastospores per BEC was 1.44 ± 0.16–4.64 ± 0.34. In the experimental design, sets of experiments were performed on different days and accordingly BEC were collected for use on the same day. Therefore, the variation in the mean number ($\pm$S.E.) of adherent blastospores per BEC is a reflection of the variation in the pool of BEC used for the adherence experiments. It has previously been reported that there are distinct sub-populations of epithelial cells with high and low affinity for attachment by *C. albicans* in vitro (Sandin et al., 1987). Reductions in adherence following treatment with PHMB 50 and 1000 μg ml^{-1}, respectively, ranged from 23.7 to 78.6% and 22.5 to 70.98% of the water control. In the majority of cases, PHMB treatments of either *C. albicans* blastospores or BEC produced a significant increase in the number of clear BEC, i.e., BEC free from adherent blastospores ($p < 0.05$).

Vantocil IB is an aqueous solution containing polyhexamethylenebiguanide hydrochloride, which primarily exhibits inhibitory and cidal activity against bacteria, however, it is less active against fungi (Hugo and Russell, 1982). Polyhexamethylenebiguanide is a mixture of polydispersed oligomers with a molecular weight average of approx. 3000 and is one of the few biologically active synthetic polymers in commercial use (Anon, 1988). Whilst the antimicrobial spectrum of activity and mode of antibacterial action have been reported (Davies et al., 1968; Hugo and Russell, 1982), to this author's knowledge this is the first report of the ability of PHMB to reduce the adherence of *C. albicans* to BEC and to increase the number of BEC free from adherent *C. albicans*. Given the importance of microbial adherence in the pathogenesis of infection (Beachey, 1981; Douglas, 1987) it is conceivable that PHMB may be clinically useful in the prophylaxis of superficial candidosis, e.g., as a mouthwash for oral candidosis. A similar role has been suggested for another biguanide chlorhexidine (Tobgi et al., 1987; Darwazeh et al., 1994; Jones and Fowler, 1994). Interestingly, it has been reported that PHMB interacts strongly with the negatively charged bacterial surface due to its cationic nature (Anon, 1988). Therefore, following instillation of a solution of PHMB into the oral cavity, it is likely that the active molecule will persist within the oral cavity either attached to the negatively charged mucosa or in the saliva (which subsequently bathes the oral mucosa) for several hours. Similarly, chlorhexidine has been reported to be retained for several hours within the oral cavity and indeed, when instilled twice daily, provides a plaque-inhibiting effect (Bonesvoll and Gjermo, 1978). It is difficult to extrapo-

late the findings of this current study to the in vivo situation as there have been few in vitro-in vivo correlations of adherence reported. However, studies have been performed in which the oral mucosa has been rinsed with chlorhexidine gluconate, the BEC have been removed and have been used in an in vitro adherence assay (Tobgi et al., 1987; Darwazeh et al., 1994). In these studies chlorhexidine was effective in reducing candidal adherence. Therefore, it is likely that PHMB, given the findings of this current study, will exhibit similar anti-adherence effects.

Interestingly, treatment of either *C. albicans* blastospores or BEC with PHMB resulted in an increase in the number of epithelial cells devoid of adherent *C. albicans* blastospores, in the majority of treatments. This property has been associated with other cationic, non-antibiotic, antimicrobial agents, e.g., chlorhexidine, cetylpyridinium chloride, cetrimide, dequalinium chloride (Fowler and Jones, 1992) and may further contribute to the potential efficacy of PHMB in the prophylaxis of oral candidosis.

In conclusion, this study has shown that PHMB treatments of either *C. albicans* blastospores or buccal epithelial cells significantly reduced the subsequent adherence of *C. albicans* to BEC and, in the majority of cases, significantly increased the number of BEC free from adherent *C. albicans*. These observations would indicate that PHMB may have a potential role in the prophylaxis of superficial candidosis, e.g., oral candidosis. However, investigations are ongoing to further evaluate these findings.

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