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Notes

A preliminary report concerning the effects of Brolene® on the adherence of *Candida albicans* to human buccal epithelial cells and on hyphal development in vitro

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Abstract

The effects of Brolene™ on adherence of *Candida albicans* blastospores to buccal epithelial cells and on hyphal development were investigated in vitro. Brolene™ significantly reduced the number of adherent blastospores/epithelial cell, increased the number of 'clear' epithelial cells, decreased percentage germination of blastospores and subsequent hyphal development.

Key words: Candida albicans; Adherence; Germination; Hyphal development; Brolene

Candida albicans is a normal resident of the oral cavity, gastrointestinal and female genital tracts of humans which, under certain circumstances, e.g., immunocompromisation, tissue trauma, may become pathogenic (Odds, 1988). In the pathogenesis of candidosis, adherence to the host epithelium is considered to be the initial requisite step as it enables the organism to overcome the flushing mechanism of body secretions (Douglas, 1987). The adherence phenomenon is thought to occur in two phases, termed reversible and irreversible. In the former, the microorganism approaches the epithelial cell due to the predominance of attractive forces between the two cell types, and in the latter, the microorganism adheres to the epithelial cell via interactions between adhesin molecules on the surface of the microorganism and the corresponding receptors on the surface of the epithelial cell (Douglas, 1987). Following adherence to the host epithelium, it has been reported that, *C. albicans* blastospores may undergo germination to the hyphal forms (Martin et al., 1984). These are thought to play pathogenic roles in the initial processes of tissue invasion and epithelial penetration (Taschdjian et al., 1960; Howlett and Squier, 1980). Therefore, any agent which can interfere with or inhibit the adherence interaction and/or germination/hyphal development may be important in the pathogensis of candidosis.

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Brolene® is an non-antibiotic, antimicrobial preparation, containing propamidine isethionate (0.1% w/v) as the active constituent, which is indicated for the prophylaxis and treatment of eye infections (New Ethicals Compendium, 1990). Therefore, in light of previous publications which reported an anti-adherence effect associated with other non-antibiotic, antimicrobial preparations, e.g., Taurolin® (Jones et al., 1986), Anaflex® (Gorman et al., 1988) and Betadine® (Gorman et al., 1987), the aim of this study was to investigate whether Brolene® also reduces the adherence of *C. albicans* to human buccal epithelial cells in vitro. In addition, the effects of Brolene® on germination and hyphal development were examined.

Brolene® eye drops were obtained from Rhone-Poulenc (NZ) Ltd, May and Baker Pharmaceuticals Division, Wellington, New Zealand. When required, the appropriate concentrations were obtained following dilution with sterile deionised water. All other chemicals were of AnalaR or equivalent quality.

One strain (MEN; Cannon, 1986) and one clinical isolate of *C. albicans* from a diagnosed oral infection were employed in this study (Fowler and Jones, 1992). Both were stored on 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 organisms were prepared by transferring a sample of stationary phase *C. albicans* into prewarmed Nutrient broth for growth at 25°C for a further 6 h in a shaking water bath. The absence of hyphal forms was confirmed using light microscopy.

Buccal epithelial cells (BEC) were collected from healthy male and female volunteers by gentle scraping of the buccal mucosa using sterile ampoule files. The ampoule files were placed in phosphate buffered saline (PBS, 0.1 M, pH 7.4), vortexed to dislodge the BEC and the cells washed with PBS.

The assay used to determine the number of adherent *C. albicans* per human buccal epithelial cell has been described previously (Jones et al., 1986). In brief, *C. albicans* or BEC were treated with either Brolene® (100, 10% v/v) or sterile water for 30 min at 37°C. Brolene® was then 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 (*C. albicans*) or 1×10^5 BEC/ml. Equal volumes of

Table 1
The effect of Brolene® (B) on the adherence of *Candida albicans* (MEN and oral clinical isolates, exponential and stationary growth phases) to human buccal epithelial cells (BEC) in vitro

Concentration of B (% w/v)	Cell treatment	*C. albicans* isolate	Number [a] of adherent *C. albicans*/BEC (±SE)		Percentage [b] of BEC free from adherent *C. albicans*	
			Exponential	Stationary	Exponential	Stationary
0 (water)	*C. albicans*	MEN	2.22 ± 0.22	1.40 ± 0.15	25	36
100.0			1.30 ± 0.15	0.65 ± 0.08	37	56
10.0			1.48 ± 0.19	0.55 ± 0.07	44	53
0 (water)	*C. albicans*	Oral	1.94 ± 0.18	2.01 ± 0.20	31	27
100.0			1.11 ± 0.12	1.32 ± 0.13	45	41
10.0			0.95 ± 0.11	1.15 ± 0.14	53	47
0 (water)	BEC	MEN	4.17 ± 0.47	4.05 ± 0.37	33	14
100.0			1.08 ± 0.18	3.46 ± 0.25	47	16 [c]
10.0			3.17 ± 0.30	2.29 ± 0.18	33 [c]	26
0 (water)	BEC	Oral	2.40 ± 0.21	2.94 ± 0.24	25	18
100.0			1.74 ± 0.15	1.61 ± 0.20	33 [c]	43
10.0			1.20 ± 0.13	2.21 ± 0.23	38	25 [c]

Differences between water and antimicrobial agent 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).

C. albicans and BEC were mixed and incubated at 37°C for 2 h in a shaking water bath. Samples were then removed, stained using crystal violet and the number of *C. albicans* adherent to at least 150 epithelial cells recorded. The differences in the mean number of adherent *C. albicans* per BEC following treatment with sterile water or Brolene® were statistically compared using a two-tailed unpaired *t*-test (Woolfson et al., 1987; Fowler and Jones, 1992) and the differences in the number of BEC free from adherent *C. albicans* following each treatment compared using Chi-squared analysis (Fowler and Jones, 1992).

The effects of Brolene® on the percentage germination and hyphal development of *C. albicans* were examined as previously reported (Gorman et al., 1986, 1988). In brief, stationary phase *C. albicans* blastospores were treated with either Brolene® (10% v/v) or sterile water for 30 min at 37°C in a shaking water bath. The samples were then filtered through 0.45 μm pore-sized filters (Millipore), the filters transferred into prewarmed nutrient broth and vortexed to displace attached blastospores. The media were then incubated at 37°C in an orbital incubator (150 oscillations/min) and, at 1-h intervals, samples removed and the percentage germinated of 300 blastospores and the mean hyphal length of 100 germinated blastospores determined using light microscopy. Chi-squared analysis was used to compare the % germination of blastospores ($p < 0.05$) and a Mann-Whitney U test was used to compare the mean hyphal length, following water and Brolene® treatments with respect to time ($p < 0.05$). Each analysis was performed on the data obtained at each sample period, i.e., at 1-h periods.

The effects of Brolene® treatments on the adherence of *C. albicans* to BEC in vitro is shown in Table 1. At both concentrations examined, Brolene® significantly reduced the adherence of *C. albicans* to BEC when compared to the water control ($p < 0.05$). These effects were observed for both the MEN strain and the oral isolate of *C. albicans* (exponential and stationary phases) whenever either the BEC or blastospores were treated. Reductions in adherence ranged from 25.91 to 76.02% of the water control. In the majority of cases, treatment of either *C. albicans* or BEC resulted in a significant increase in the number of BEC free from adherent blastospores ($p < 0.05$).

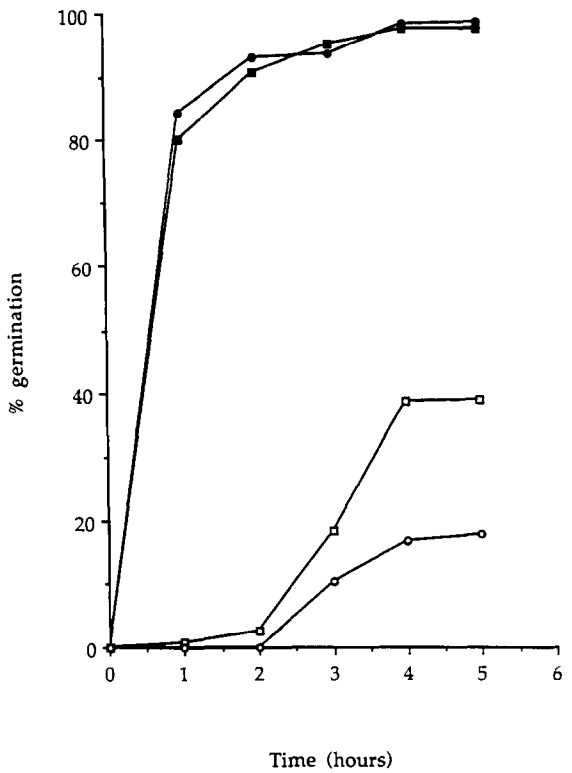

Fig. 1. The effect of Brolene™, 10% v/v (open symbols) and water (closed symbols) treatments (30 min at 37°C) on the percentage germination of *Candida albicans* blastospores in nutrient broth with respect to time: (■) water treated oral isolate; (▲) water treated MEN strain; (□) Brolene™ 10% v/v treated oral isolate; (△) Brolene 10% v/v treated MEN strain.

The effect of Brolene (10% v/v) on the percentage germination and hyphal development of *C. albicans* is graphically illustrated in Fig. 1 and 2. The percentage germinations of both the water treated MEN strain and the oral isolate with respect to time were similar, with 84.30 and 80.00% of blastospores germinated, respectively, 1 h after inoculation into nutrient broth. Conversely, the extent of germination of Brolene® treated blastospores was significantly lower at each time interval for both *C. albicans* MEN

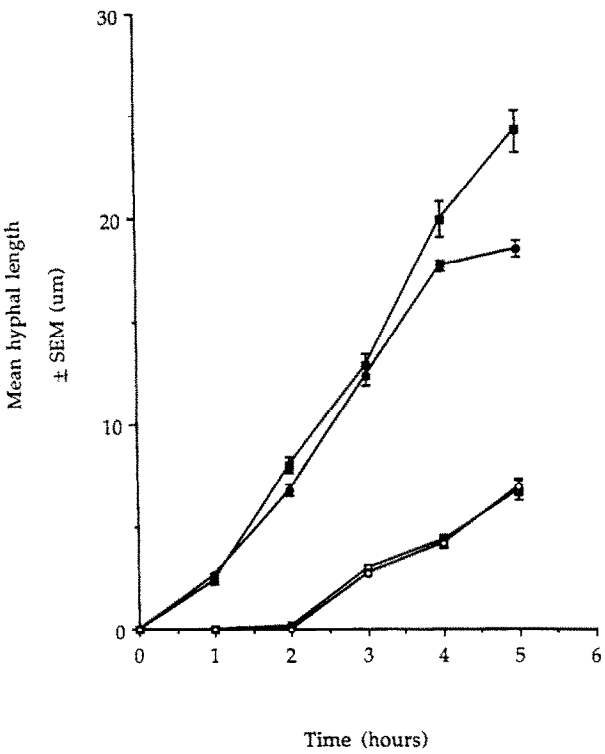

Fig. 2. The effect of Brolene™, 10% v/v (open symbols) and water (closed symbols) treatments (30 min at 37°C) on the mean hyphal length (±SE) of *Candida albicans* blastospores in nutrient broth with respect to time: (■) water treated oral isolate; (▲) water treated MEN strain; (□) Brolene™ treated oral isolate; (△) Brolene treated MEN strain.

strain and the oral isolate when compared to the water treated cells. The rate of hyphal development of water treated *C. albicans* was statistically similar for both the MEN strain and oral clinical isolate, and in addition, the rate of development of Brolene® treated *C. albicans* was statistically similar for the MEN strain and oral isolate for the entire investigation. However, whenever the rates of hyphal development of water and Brolene® treated *C. albicans* are compared, it may be observed that the rates of hyphal development of Brolene® treated blastospores were significantly less than that of water treated blastospores. In addition, a marked lag time before germination commenced was observed for both the Brolene® treated *C. albicans* MEN strain and the oral isolate.

The observed abilities of Brolene® to reduce the adherence of *C. albicans* to BEC and to increase the number of BEC free from adherent *C. albicans* are interesting phenomena. As adherence to the host epithelium is accepted to be the initial stage of candidosis (Douglas, 1987), then it is conceivable that Brolene® may be clinically useful if formulated and employed as a rinse for local application to the oral mucosa in the prophylaxis of candidosis. Brolene® contains propamidine isethionate as the antimicrobially active agent, however, also contains benzalkonium chloride as a preservative, both of which are cationic agents. Although benzalkonium chloride is present at a lower concentration than propamidine isethionate (0.10 compared with 0.005% w/v), the possible effects of benzalkonium chloride on the observed anti-adherent activity can not be overlooked as other quaternary ammonium compounds (cetrimide, cetylpyridinium chloride) have been reported to possess anti-adherent activities, at both super and sub-minimal inhibitory concentrations, against the same strain and isolate of *C. albicans* used in this present study (Fowler and Jones, 1992). The exact contributions of both propamidine isethionate and benzalkonium chloride to these anti-adherent effects is the subject of current investigations. Extrapolation of the findings of this study to the in vivo situation is difficult and indeed there are a limited number of reports concerned with the in vivo anti-adherence properties of antimicrobial agents. However, in one study (Tobgi et al., 1987) it was shown that chlorhexidine, a compound reported to reduce the adherence of *C. albicans* to epithelial cells in vitro (Gorman et al., 1987, Fowler and Jones, 1992), significantly reduced the adherence of *C. albicans* to the oral mucosa in vivo, 30 min after rinsing.

The ability of Brolene® to significantly reduce the % germination and hyphal development may further suggest the applicability for the use of this preparation for the prophylaxis of oral candidosis. As the hyphae are thought to be responsible for the penetration of host epithelia (Howlett and Squier, 1980), then it is conceivable that agents, e.g., Brolene®, which inhibit this process may reduce both the incidence of tissue invasion and

the spread of and severity of candidosis. Other non-antibiotic, antimicrobial preparations, e.g., Noxyflex® (Gorman et al., 1986) and Anaflex® (Gorman et al., 1988) have been reported to exhibit similar abilities to inhibit the germination process.

In conclusion, the abilities of Brolene® to reduce the adherence of *C. albicans* to BEC, to increase the number of BEC free from adherent *C. albicans*, to reduce both the germination of blastospores of *C. albicans* and the subsequent rate of hyphal development may indicate potential applications in the prophylaxis of, or reduction in the spread of candidosis of localised epithelial surfaces, e.g., the oral cavity.

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