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The effect of Betadine on *Candida albicans* virulence factors

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Adherence of *Candida albicans* blastospores to buccal epithelial cells (BEC) and the formation of hyphae are virulence factors involved in the pathogenesis of Candida infections. These factors, together with the hyphal penetration of the oral tissues when the host's immune system is compromised, precipitate superficial and/or systemic candidosis (Cannon et al 1995). Clinically, it would be advantageous to identify agents that modify these virulence factors. Therefore, this study reports the effects of povidone-iodine (PVP-I, Betadine®) on both the *ex vivo* adherence, and the *in vitro* morphogenesis of blastospores of *C. albicans* .

In adherence experiments, BEC were removed from the left cheek of volunteers, standardised with respect to cell number in PBS($1x10^5 mL^{-1}$) and challenged with blastospores of *C. albicans* NCYC 1467 (1×10^7 cfu mL^{-1}), that had been previously stained with acridine orange. These formed the control adherent population. Volunteers then gargled PVP-I according to the manufacturers instructions and discarded the mouthwash. BEC were subsequently removed from the right cheek at a range of times post-treatment (15 min, 30 min, 1 hour, 2 hours, 4 hours and 24 hours), standardised in PBS as previously described and challenged with *C. albicans* blastospores. The number of viable (orange staining) or non-viable (green-staining) *C. albicans* blastospores adherent to at least 150 BEC was determined using fluorescence microscopy (Jones et al 1997). The effects of PVP-I on the adherence of blastospores to BEC at each time period examined were statistically evaluated using a Wilcoxon Signed Rank test ($p<0.05$, denoting significance).

In morphogenesis experiments, a known number of yeast cells of *C. albicans* ($1x10^7$ cfu/mL^{-1}) were suspended in PBS, 10% PVP-I 1% PVP-I and 0.1% PVP-I at 37°C for predetermined times (10s, 30s, 60s). Following this, yeast cells were removed by filtration, washed and inoculated into nutrient broth at 37°C. Samples were removed at hourly intervals and the % germination and hyphal lengths determined using light microscopy. The effects of treatment with PVP-I on % germination and hyphal lengths were statistically analysed using Chi-square analysis and Mann-Whitney U test ($p<0.05$),respectively.

Treatment of BEC with PVP-I *in vivo* significantly decreased *ex vivo* adherence of *C.albicans* . These significant reductions in adherence of both viable and non-viable blastospores were observed at each time interval up to and including 4 hours (Table 1). There was no significant difference in adherence of *C.albicans to* control BEC and BEC 24 hours post-treatment. Significant percentage reduction in adherence ranged from 28.31% to 83.47%. Treatment of yeast cells of *C. albicans in vitro* for short periods (10, 30s), equivalent to the recommended period of clinical use, significantly decreased the resultant % germination and rate of hyphal extension. In addition, the higher concentration of PVP-I (10%) arrested germination and hence hyphal development for at least 5h.

Table 1: % reduction in adherence of *C.albicans* to BEC following 15 min, 30 min and 1 hour post-treatment time with PVP-I

	Non-viable ±sd	Viable±sd
15 min	76.54±5.29	83.47±6.84
30 min	73.82±5.88	76.04±6.07
1 hour	56.82±7.82	61.51±16

In conclusion, treatment of BEC with PVP -I *in vivo* resulted in significant reductions in the subsequent adherence of yeast cells of *C. albicans* to BEC *ex vivo*. The *in vitro* studies likewise indicated a reduction/inhibition of *Candidal* morphogenesis following a similar period of treatment with PVP-I .

Given, the clinical relevance of both the adherence and morphogenesis assays, these observations indicate a potential clinical role for topical administration of PVP-I for the prophylaxis of superficial, and perhaps, systemic candidosis .

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