MYCOLOGY

Evaluation of phospholipase activity of *Candida albicans* and its correlation with pathogenicity in mice

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Sixty-one isolates of *Candida albicans* were tested for their phospholipase activity and this parameter was correlated with pathogenicity in albino mice. Of the isolates tested, 57 (93%) showed appreciable phospholipase activity and of these, 55 (90%) were pathogenic to mice. A significant correlation was found between phospholipase activity and involvement of kidneys in animal pathogenicity studies. The isolates of *C. albicans*, that exhibited higher phospholipase activity were found to be pathogenic for mice.

Introduction

The dimorphic fungus *Candida albicans* is an opportunist pathogen capable of causing superficial as well as systemic infections. Its ability to produce phospholipase is considered to be an important pathogenic feature [1]. It has been reported that the ability of *C. albicans* to produce cytolytic enzymes such as proteinase [2, 3] and phospholipases [4, 5] may be associated with pathogenicity of this dimorphic fungus. Phospholipase activity of *C. albicans* was first detected by growing the fungus on media supplemented with egg yolk [6, 7] and lecithin [8]. Previous studies have shown 30–70% of *C. albicans* isolates to be phospholipase positive with varying degrees of phospholipase activity [9, 10]. Samaranayake *et al.* showed that the secreted phospholipase activity of *C. albicans* can be a potential determinant of virulence [10]. Hence the present investigation examined the phospholipase activity of 61 clinical isolates of *C. albicans* with Sabouraud's dextrose agar supplemented with egg yolk, and correlated this parameter with pathogenicity *in vivo* in albino mice.

Materials and methods

Sixty-one isolates of *C. albicans* were obtained from patients who were attending the dermatology clinic of Sir J. J. Group of hospitals and Grant Medical

College, Bombay-8, during the period March–November 1995.

Skin scrapings were taken from infected parts of the body, e.g., nail fold (paronychia), glans of penis (candidal balanitis in diabetic patients), peri-anal area (perianal candidosis), groin (inguinal candidosis), interdigital spaces (interdigital candidosis) and oral cavity (oral thrush/oral candidosis). Skin scrapings, subjected to a KOH 10% wet mount, were cultured on Sabouraud's dextrose agar (SDA) supplemented with chloramphenicol and incubated at room temperature for 7 days. Colonies on SDA were identified by standard methods [11], i.e., the germ tube test, ability to form chlamydospores on cornmeal agar and sugar fermentation and assimilation tests.

Of the 61 clinical isolates of *C. albicans*, the greatest number, 19 (31%), were from the oral cavity, and the least came from groin and interdigital spaces – 5 (8%) each.

Phospholipase activity

Phospholipase activity was determined by the method of Price *et al.* [9]. An 18-h culture of each isolate on SDA was harvested in 5 ml of sterile saline and the yeast numbers were then adjusted to yield a suspension of 10^6 organisms/ml by haemocytometric counts. This suspension ($10\ \mu\text{l}$) was spot inoculated on to SDA plates supplemented with egg yolk (8% w/v) and mineral salts (0.05 M calcium chloride and 1 M NaCl). After incubation of the inoculated plates at 37°C for up to 4 days in a humid chamber, the diameter of the resultant colonies and that of the precipitation zone

around them were measured with a measuring scale. The experiment was performed in triplicate. Phospholipase activity (Pz value) was calculated as the ratio of the diameter of the colony to the diameter of the colony plus that of the precipitation zone [9].

Animal pathogenicity

Swiss albino mice weighing 18–20 g were used (Haffkine Research Institute, Parel, Bombay 400012, India). A suspension of 5×10^3 yeast cells/ml was prepared for inoculation with a Levy haemocytometer chamber slide (Weber). Two sets of six mice were used for each isolate of *C. albicans*. Each of the six mice was inoculated intravenously *via* the tail vein with 1 ml of a *C. albicans* suspension prepared as above. Control mice were inoculated with 1 ml of yeast-free saline. After 15 days, the mice were killed, dissected and inspected for macroscopic lesions of the kidneys. Both kidneys of each mouse were weighed. Small portions of infected kidneys were crushed between two slides and smears were stained by Gram’s method and examined microscopically for yeast and hyphal elements. Both kidneys were also cut into two equal parts – half of the infected organ was put into sterile normal saline for culture and the remainder into formalin 10% solution for histopathological studies with the periodic acid-Schiff (PAS) staining method.

Results

Of the 61 clinical isolates of *C. albicans*, 25 (41%) showed Pz values of 0.40–0.45 (Table 1).

Three oral isolates, two from nail-fold and two from interdigital spaces showed the highest phospholipase activity ($P_z = 0.35\text{--}0.40$) and also induced lesions in both kidneys in all 42 mice inoculated with these strains ($r = 0.90$). A total of 25 isolates exhibited Pz values of 0.40–0.45 and also elicited lesions in both kidneys of the 150 mice inoculated with these strains ($r = 0.83$) (Table 1, Fig. 1).

A total of 23 isolates with Pz values of 0.45–0.60 elicited unilateral right kidney infection in all 138 mice inoculated ($r = 0.82$) while the remaining six isolates with Pz values >0.65 failed to infect the kidneys of any of the 36 test animals (Table 1, Fig. 1).

The results of this study indicate that the strains of *C. albicans* with the highest phospholipase activity showed appreciable involvement of both the kidneys in experimental infections. However, the strains with lower phospholipase activity showed unilateral involvement of right kidneys. Overall, the correlation between these two parameters was high ($r = 0.85$). The isolates with low phospholipase activity (in the range 0.65–0.80) and negative strains ($P_z = 95\text{--}100$) failed to show any infection in either kidney.

Table 1. The number of clinical isolates of *C. albicans*, their sources and measurement of phospholipase activity on SDA medium supplemented with egg yolk and mineral salts

Source	Total number of isolates tested	Number of isolates with phospholipase activity (Pz values)																	
		0.35–0.40	0.40–0.45	0.45–0.50	0.50–0.55	0.55–0.60	0.60–0.65	0.65–0.70	0.70–0.75	0.75–0.80	0.80–0.85	0.85–0.90	0.90–0.95	0.95–1.00					
Oral cavity	19	3	8	2	1	3	0	0	0	0	0	0	0	2					
Nail-fold	15	2	7	3	2	0	0	0	0	0	0	0	0	1					
Glans of penis	10	0	6	2	2	0	0	0	0	0	0	0	0	0					
Peri-anal area	7	0	2	2	0	1	0	1	0	1	0	0	0	0					
Groin	5	0	2	0	2	0	0	0	0	0	0	0	0	1					
Interdigital space	5	2	0	1	0	2	0	0	0	0	0	0	0	0

Fig. 1. *C. albicans* (cfu) recovered from kidneys of mice inoculated with phospholipase positive and negative isolates (5×10^3 yeast cell/ml); ■ and ■ Bilateral involvement of kidneys; ▩ and ▩ unilateral involvement of kidneys. *Number of isolates.

Discussion

These results suggest that clinical isolates of *C. albicans* exhibit varying degrees of phospholipase activity and are consistent with previous observations by Samaranayake *et al.* [10]. The percentage of phospholipase-negative *C. albicans* isolates in the present study was very low (6.55%) compared with previous studies, which demonstrated that 23% (6 of 28) of oral isolates, 10.45% (5 of 28) of blood isolates, 50% (14 of 28) of wound isolates and 70% (9 of 13) of urine isolates were phospholipase-negative [9]. The phospholipase-negative isolates in the present study were obtained from the oral cavity (2; 10%), from the nail fold (1; 1.64%) and from the peri-anal area (1; 1.64%).

The rationale behind selecting the kidneys for evaluating the pathogenicity of *C. albicans* was that when isolates of *C. albicans* are injected *via* the tail vein, the kidneys are more severely infected than any other organ [12, 13]. As a result, the kidneys have been used commonly for monitoring pathogenicity of *C. albicans* *in vivo* [14].

With regard to the virulence of *C. albicans*, all 42 mice in the group that received phospholipase-positive yeast cells (Pz values 0.35–0.45) showed macroscopic lesions (i.e., grey to white lesions) in both kidneys after 15 days. However, five of six isolates yielded more yeast cells (cfu) from left kidneys than from

right kidneys. Although MacDonald and Odds [13] found no significant difference in cfu between right and left kidneys when the inoculum size was 1×10^6 yeast cells/ml/mouse, Hurley and Winner [12] observed unilateral kidney involvement in experimental candidosis in mice, particularly with a low inoculum. As in the present study, Kwong-Chung and Tomm [14] also reported that the frequency of unilateral involvement of kidneys decreased with an increase in inoculum and the phenomenon of unilateral kidney enlargement or atrophy was also reported in mice that were inoculated with conidial suspensions of *Aspergillus bisporus* [15]. When the conidial suspension was injected *via* the tail vein, this atrophy or enlargement was three times more frequent in right kidneys. It is reported that right kidneys are slightly heavier and higher in position [16], and this was the case in the present study (0.02 g *versus* 0.5 g). Whether this weight difference was related to infection rate is a question which needs to be resolved.

References

1. Odds FC. *Candida* and candidiasis. Leicester, Leicester University Press. 1979.
2. Macdonald F, Odds FC. Inducible proteinase of *Candida albicans* in diagnostic serology and in the pathogenesis of systemic candidosis. *J Med Microbiol* 1980; 13: 423–425.
3. Ruchel R, Tegeler R, Trost M. A comparison of secretory proteinases from different strains of *Candida albicans*. *Sabouraudia* 1982; 20: 233–244.
4. Costa AL, Costa AC, Misefari A, Amato A. Atti Sociata

- peloritana di scienze fisiche matematiche naturali 1968; **XIV**: 93–101.
5. Louria DB, Brayton RG, Finkel G. Studies on the pathogenesis in experimental *Candida albicans* infections in mice. *Sabouraudia* 1963; **2**: 271–283.
 6. Costa AL, Misefari A, Amato A. Enzymatic activities of mycetes. I. Enzymatic activity of *Candida albicans* in egg yolk containing media. Atti XIV Congresso Nazionale di Microbiologia, Messina Taormina. 1969.
 7. Werner H. [Studies on the lipase activity in yeasts and yeast-like fungi.] Untersuchungen über die lipase-aktivität bei hefen und hefeähnlichen pilzen. *Zentralbl Bakteriol Orig* 1966; **200**: 113–124.
 8. Costa AL, Misefari A, Amato A. Enzymatic activities of mycetes. II. Phosphatidase activity in *C. albicans* in media containing lecithin. Atti XIV Congresso Nazionale di Microbiologia, Messina Taormina. 1969.
 9. Price MF, Wilkinson ID, Gentry LO. Plate method for detection of phospholipase activity of *Candida albicans*. *Sabouraudia* 1982; **20**: 7–14.
 10. Samaranayake LP, Raeside JM, MacFarlane TW. Factors affecting the phospholipase activity of *Candida* species in vitro. *Sabouraudia* 1984; **22**: 201–207.
 11. Lodder J. The yeasts: taxonomic study, 2nd edn. Amsterdam, Elsevier. 1984.
 12. Hurley R, Winner HI. Experimental renal moniliasis in the mouse. *J Pathol Bacteriol* 1963; **86**: 75–82.
 13. Macdonald F, Odds FC. Virulence for mice of a proteinase-secreting strain of *Candida albicans* and proteinase-deficient mutant. *J Gen Microbiol* 1983; **129**: 431–438.
 14. Kwon-Chung KJ, Tom WK. Unilateral involvement of kidneys in mice infected with *Candida albicans*. *Sabouraudia* 1985; **23**: 81–83.
 15. Kwon-Chung KJ, Fennell DI. A new pathogenic species of *Aspergillus*. *Mycologia* 1971; **63**: 478–489.
 16. Dunn TB. Renal disease of the mouse. In: Cotchin E, Roe FJC (eds) Pathology of laboratory rats and mice. Oxford, Blackwell Scientific Publications. 1967: 149–179.