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Treatment of Murine *Candida krusei* or *Candida glabrata* Infection with L-743,872

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L-743,872 is a broad-spectrum pneumocandin antifungal drug developed by Merck Research Co., and in the present work it was evaluated in vivo in murine models of *Candida krusei* and *Candida glabrata* infection. Mice were infected intravenously with two isolates of *C. krusei* and treated with fluconazole or L-743,872. Fluconazole was beneficial only in immune-competent mice infected with isolate 94-2696. At >0.5 mg/kg of body weight/day, L-743,872 was effective against both infecting isolates in immune-competent and immune-suppressed mice. Against *C. glabrata*, L-743,872 was effective, at doses ≥0.5 mg/kg, in reducing fungal cell counts in the kidneys but not in the spleen. L-743,872 has significant potential for clinical development.

Disseminated candidiasis is becoming a frequent nosocomial problem (12). Fluconazole is a highly effective well-tolerated treatment for candidiasis (10). However, half or more of disseminated *Candida* infections are caused by *Candida* species other than *Candida albicans* (4, 10). Some of these, such as *Candida krusei* and *Candida glabrata*, are innately resistant to fluconazole, both in vitro and clinically (2, 5, 8, 9, 11, 13). Among the potential alternative drugs under development are the pneumocandins, which act by blocking 1-3-β-glucan synthase, thus inhibiting cell wall synthesis (3, 6). This is a target not affected by fluconazole, so pneumocandins may be effective in fluconazole-resistant *C. krusei* infection. L-743,872 is a potent new pneumocandin found to be effective in *C. albicans* infection of mice (1). In the present work we evaluated the effectiveness of L-743,872 against candidiasis caused by *C. krusei* and *C. glabrata*, two pathogens for which the clinical failure of fluconazole is well known.

MATERIALS AND METHODS

Pathogens. For these studies L-743,872 was obtained from Ken Bartizal of Merck Research Co. (Rahway, N.J.). We utilized *C. krusei* isolates 94-2696 (originally obtained from a vertibral mass) and 94-2501 (urine) and *C. glabrata* isolate 95-1129 (kidney). The MICs of fluconazole and L-743,872 were measured by the National Committee for Clinical Laboratory Standards broth macrodilution method (Table 1) (7). For in vivo studies all isolates of *C. glabrata* or *C. krusei* yeast cells were plated overnight in Sabouraud dextrose agar at 37°C, washed in saline, counted in a hemacytometer, and suspended in saline at the desired inoculum to be given in 0.2-ml volumes intravenously (i.v.).

Infection and treatment. Thirty-gram outbred ICR mice were housed in groups of five per cage. For each study, groups of 7 or 10 mice were used. For infection with *C. krusei* mice were used as immune competent, neutropenic (a 5-fluorouracil dose of 150 mg/kg of body weight was given once, 1 day prior to infection, and a dose of 75 mg/kg was given 5 days after infection), or immune suppressed (hydrocortisone [100 mg/kg] was given subcutaneously from day 1 through day 7 prior to infection, and 5-fluorouracil [150 mg/kg] was given i.v. on day 8, 24 h prior to infection on day 9). For infection with *C. glabrata* mice were rendered neutropenic by treatment one day prior to infection with 5-fluorouracil (150 mg/kg) i.v.

Mice were infected with *C. krusei* at doses approximating 10^8 CFU/mouse. From day 1 after infection through day 7, mice were treated with fluconazole (5 mg/kg) twice daily by mouth or L-743,872 in doses from 0.5 to 5 mg/kg once daily, given intraperitoneally. Fluconazole was diluted in 0.3% Noble agar. L-743,872 was diluted in sterile water prior to use. Both drugs were administered at a volume of 0.2 ml per dose.

Assesment of efficacy. Protection was measured either as reduction of colony counts (*C. krusei* and *C. glabrata*) or as prolongation of survival (*C. krusei*), observed out to 30 days. For tissue colony counts, both kidneys and/or the spleen was homogenized and 0.1-ml aliquots of the homogenate were cultured quantitatively by serial 10-fold dilution. When the initial dilution showed no fungal colonies, the entire organ homogenate was plated. Tukey's studentized range test, with log transformations, was used for comparisons of tissue colony counts ($P < 0.01$ was the criterion for significance). Dunnett's one-tailed t tests for variance were also used. The log rank and Wilcoxon tests were used for comparison of survival times ($P < 0.01$ was the criterion for significance).

RESULTS

Studies of tissue colony counts of mice infected with *C. krusei* are presented in Table 2. In immunocompetent mice (nonlethal infection) fluconazole had a modest effect in reducing kidney colony counts but at 5 mg/kg twice daily was less effective than a single 10-fold-lower dose of L-743,872. Also, the benefit conferred by fluconazole can be overwhelmed at a higher inoculum (study 2), but this was not the case for L-743,872, which was still effective. Neutropenic mice were protected by L-743,872 but not by fluconazole. Mice rendered neutropenic and also given steroids for immune suppression (study 4) were protected by L-743,872. Mice given steroids for immunosuppression (study 5) were protected by L-743,872, but fluconazole had no effect. In immune-competent mice (study 6), fluconazole did not reduce either spleen or kidney colony counts; L-743,872 was effective in reducing kidney colony counts at a dose of 5 mg/kg. The results of survival study using the same immune suppression as study 4 are presented in Fig. 1. Mice treated with fluconazole did not survive significantly

TABLE 1. In vitro susceptibilities to fluconazole and L-743,872

Species or isolate	MIC (μg/ml) of:			
	Fluconazole at:		L-743,872 at:	
	24 h	48 h	24 h	48 h
C. krusei				
94-2696	16	32	0.25	0.25
94-2501	16	64	0.25	0.25
C. glabrata	8	16	0.25	0.25

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TABLE 2. Tissue colony counts of *C. krusei*

Study	Isolate (tissue)	Immune suppression	Inoculum (no. of cells)	No. of mice	Drug (dose [mg/kg])[a]	Log mean CFU	SEM
1	94-2696 (kidney)	None	1.2×10^8	7	None	11.34	0.45
					Flu[b] (5)	9.80[c]	0.29
					L-734,872 (5)	8.13[c]	0.36
					L-743,872 (0.5)	8.07[c]	0.25
2	94-2501 (kidney)	None	9.0×10^8	7	None	8.73	0.63
					Flu (5)	9.79	0.49
					L-743,872 (5)	5.73[c]	0.44
3	94-2696 (kidney)	5FU[d] (150 mg/kg) 24 h prior to infection and 5FU (75 mg/kg) on day 5 postinfection	1.4×10^8	7	None	11.41	1.26
					Flu (5)	9.00	1.41
					L-743,872 (5)	7.52[c]	0.98
					L-743,872 (2)	5.35[c]	0.78
					L-743,872 (0.5)	8.49	0.77
4	94-2696 (kidney)	Cortisone (125 mg/kg) 3 consecutive days prior to infection and cyclophosphamide (125 mg/kg) 24 h prior to infection	8.0×10^7	10	None	13.51	0.52
					L-743,872 (5)	10.56[c]	0.49
					L-743,872 (2)	11.36[c]	0.39
					L-743,872 (0.5)	12.75[c]	0.24
5	94-2501 (kidney)	Cortisone (100 mg/kg) 3 consecutive days prior to infection	1.8×10^8	7	None	15.98	1.02
					Flu (5)	15.91	0.69
					L-743,872 (10)	4.95[c]	1.05
					L-743,872 (5)	6.71[c]	0.46
					L-743,872 (2)	10.11[c]	0.74
					L-743,872 (0.5)	15.86	0.76
6	94-2501 (kidney)	None	1.0×10^8	7			
					None	8.92	0.72
					Flu (5)	7.37	0.61
					L-743,872 (5)	5.87[c]	0.37
					L-743,872 (0.5)	5.94	0.49
	94-2501 (spleen)				None	8.81	0.20
					Flu (5)	9.70	0.52
					L-743,872 (5)	10.33	0.63
					L-743,872 (0.5)	9.11	0.26

[a] All fluconazole doses were given twice daily; all L-743,872 doses were given once daily.
[b] Flu, fluconazole.
[c] Significantly different ($P < 0.01$) from control.
[d] 5FU, 5-fluorouracil.

longer than controls, but mice given L-743,872 at dosages of 0.5, 2, or 5 mg/kg/day (the results for 5 mg/kg/day are not shown but are similar to those for 2 mg/kg/day) were protected. Protection at 0.5 mg/kg/day was significantly less than that at higher dosages.

C. glabrata is a pathogen of relatively low virulence, and preliminary studies with mice indicated that 5-fluorouracil-treated mice develop a nonlethal infection after i.v. injection. The results of two identical studies are combined in Table 3. Neutropenic mice were infected i.v. with 1.4×10^8 *C. glabrata* cells, treated from day 1 through 7 after infection, and sacrificed on day 8. Fluconazole at 5 mg/kg twice daily was minimally (but significantly) effective in reducing kidney tissue colony counts. L-743,872 was highly effective in kidneys, showing a dose-dependent reduction of between 3 and >4 logs units. In contrast, there was no effect of fluconazole or L-743,872 on spleen tissue colony counts.

DISCUSSION

These studies raise several issues about animal models of *Candida* infections other than those by *C. albicans* and also about chemotherapy. *C. krusei* and *C. glabrata* are low-virulence pathogens in humans and also low-virulence pathogens

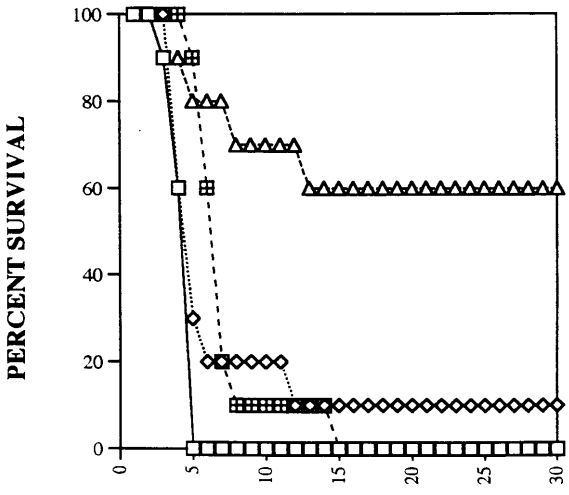

FIG. 1. Survival of groups of 10 mice after infection with *C. krusei* and treatment with L-743,872 or fluconazole. L-743,872 at 5 mg/kg (data not shown) gave similar results. □, control; ◇, fluconazole at 5 mg/kg twice daily; △, L-743,872 at 2 mg/kg; ⊞, L-743,872 at 0.5 mg/kg.

TABLE 3. Tissue colony counts after i.v. infection with 1.4×10^8 *C. glabrata* cells

Tissue	Drug	Dose (mg/kg)[a]	Log mean CFU	SEM
Kidney	None		14.84	0.7
	Flu[b]	5	12.71[c]	0.58
	L-743,872	5	4.93[d]	0.31
	L-743,872	2	7.24[d]	0.29
	L-743,872	0.5	8.27[d]	0.20
Spleen	None		11.36	0.24
	Flu	5	11.75	0.36
	L-743,872	5	10.80	0.68
	L-743,872	2	11.98	0.18
	L-743,872	0.5	11.24	0.25

[a] All fluconazole doses were given twice daily; all L-743,872 doses were given once daily.
[b] Flu, fluconazole.
[c] $P < 0.05$ compared with control.
[d] $P < 0.001$ compared with either fluconazole or control.

in mice. Both produce nonlethal infections when given intravenously to immune-competent mice at very large inocula. Further, the virulence is not consistent from strain to strain. One isolate of *C. krusei* given to neutropenic mice produced a lethal infection, while the other, under similar circumstances, did not. We examined three isolates of *C. glabrata* (data not shown), and none produced a lethal outcome when given in large inocula to neutropenic mice. Thus, the best comparators for drug efficacy may not be survival studies but studies of the reduction of tissue colony counts.

Efficacy in reducing tissue fungal burden depends in part on the innate susceptibility of the fungus to the drug and in part on the access of the drug to the relevant tissue. Both of these fungal species are relatively resistant to fluconazole in vitro and clinically. The correlation of clinical failure with rising MIC is not precise, and a number of patients with thrush or with fungemia may respond to fluconazole therapy even though they have "resistant" isolates (10, 11).

The isolates we used also display decreased susceptibility to fluconazole in vitro. The dosage of 5 mg/kg twice daily that we used was chosen because of its efficacy in mice infected with *C. albicans* and *Candida tropicalis* isolates highly susceptible to fluconazole and its reduced efficacy in mice infected with isolates for which the MIC was ≥ 8 μg/ml. Our studies somewhat mirror clinical experience in that fluconazole was effective in reducing kidney colony counts in one of two studies with immune competent mice but not in studies with immune-suppressed mice. This may reflect the contributions of both fluconazole and the host immune defense to efficacy for immune-competent mice but a lack of efficacy when fluconazole is used in immune-suppressed mice.

In addition to drug susceptibility, tissue access is important. Fluconazole accesses virtually all tissues well, but is excreted primarily by the kidneys as unchanged drug. Thus, renal concentrations are high. This may also have contributed to the modest efficacy of fluconazole in reducing renal tissue colony counts in study 1 and in the study with *C. glabrata*. Whichever of these factors contributed to efficacy, the fluconazole benefit was modest at best. In marked contrast, L-743,872 was highly effective in prolonging the survival of mice infected with one isolate of *C. krusei* and in reducing kidney tissue colony counts in mice infected with each isolate, at dosages down to 2 mg/kg once daily. However, L-743,872 was not effective in reducing spleen tissue colony counts. The kinetics of excretion of active drug have not yet been worked out for L-743,872, but these studies suggest that the drug may also be concentrated in renal tissue.

Fluconazole failed under conditions of high infecting doses and immune system suppression, but L-743,872 did not lose efficacy under these conditions. Although the L-743,872 potency against *C. krusei* infection is about 20-fold less than that against *C. albicans* infection (4a), its efficacy at 2 mg/kg/day in mice suggests that L-743,872 is a very promising candidate for clinical development, with in vitro and in vivo spectra that are broader than those of fluconazole.

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