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Treatment of Murine Disseminated Candidiasis with L-743,872

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L-743,872 (M991), which is a pneumocandin derivative, was evaluated in a mouse model of disseminated candidiasis caused by a fluconazole-resistant isolate of *Candida albicans*. In immunocompetent mice M991 prolonged survival at doses as low as 0.0125 mg/kg of body weight per day. In neutropenic mice 0.05 mg/kg was the lowest effective dose. M991 is a very potent drug for treatment of disseminated candidiasis.

Candida species are causing increasing problems with disseminated disease in neutropenic patients and mucosal disease in patients with human immunodeficiency virus (HIV) infection. *Candida albicans* causes about one-half to two-thirds of episodes of candidemia and generally is susceptible to fluconazole. To treat these infections there has been increasing reliance on fluconazole, an antifungal azole with potent activity against *Candida* (15). However, the non-*C. albicans* species, particularly *C. krusei*, *C. glabrata*, and to a lesser degree *C. tropicalis*, tend to be less sensitive or entirely resistant to fluconazole (4, 13, 16). Patients with HIV infection may have multiple organisms associated with thrush or esophagitis. Nevertheless, *C. albicans* is almost invariably the pathogen (6). Fluconazole resistance in these patients is almost entirely due to *C. albicans* and now occurs in upwards of 5% of patients, particularly the severely immune depressed who receive chronic or repeated fluconazole treatment (7, 12, 14, 18, 21).

While amphotericin B has been effective for many patients resistant to fluconazole, the toxicities of this drug are formidable and not all patients respond. Accordingly, there has been an increasing effort to develop new antifungals with high efficacy and less toxicity. The lipopeptides are among these. They act by inhibiting synthesis of β-1,3-glucans (1, 3, 8, 9). The target is an enzyme involved in the synthesis of the fungal cell wall and is found in most pathogenic fungi. This is a completely different site of action from that of the azole antifungals, which act by impairing ergosterol synthesis in the fungal cell membrane. Therefore, the echinocandin derivatives (including pneumocandins and papulocandins) offer a site independent of azole activity, with potential both for additive effects with azoles and also for activity against azole-resistant fungi. Cilofungin was initially developed as a drug with activity against *C. albicans* and *C. tropicalis*, a rather limited spectrum (6). Pneumocandins are more recently developed derivatives which are water soluble (permitting parenteral administration), act rapidly, have a broader spectrum than cilofungin, and are extremely potent in vitro (2, 3). Preliminary studies with animals have shown these drugs to be well tolerated and effective at low doses in mice infected with *C. albicans* (3). However, few isolates were studied. In the present studies we have sought to determine whether L-743,872 (M991) is effective in immunocompetent and neutropenic mice infected with a fluconazole-resistant clinical isolate of *C. albicans*. Our hypothesis is that M991 activity is unrelated to fluconazole activity and that in vivo response should be similar to that reported elsewhere from limited studies of this infection in mice (3).

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MATERIALS AND METHODS

Animals. Outbred ICR male mice were purchased from Harlan Sprague Dawley Laboratories. They were housed in cages of five mice each and had access to food and water ad libitum. Mice to be rendered neutropenic were given a single dose of 5-fluorouracil at 150 mg/kg of body weight (Solak Laboratories, Inc.) intravenously (i.v.) 1 day before infection. This dose has been found in earlier studies to depress peripheral blood neutrophils to less than 100/μl for >10 days.

Infection. *C. albicans* isolate 93-1226 was grown overnight in brain heart infusion broth, washed three times, suspended in normal saline, and counted in a hemacytometer. The dose was adjusted to 10^6 CFU/mouse in 0.2 ml for studies of survival, and to 10^5 CFU/mouse for studies of tissue burden. Mice were inoculated i.v. in a 0.2-ml volume. Quantitative cultures were used to confirm the inoculum size.

Treatment. Beginning 1 day after infection, mice were treated either with water (controls) intraperitoneally (i.p.), with fluconazole (Pfizer) at 5 mg/kg twice daily in 0.2 ml by gavage, or with M991 (Merck and Co., Inc.) given in 0.2 ml of water i.p. in doses ranging from 5 mg/kg down to 0.0125 mg/kg once daily. Treatment was continued through day 7. For survival studies, groups of 10 mice were observed through day 30. Moribund mice were terminated, and their deaths were recorded as occurring on the next day. For studies of tissue burden, groups of seven mice were sacrificed on day 8 after infection. Both kidneys were removed aseptically, homogenized in 2 ml of saline, and cultured in serial 10-fold dilutions. The entire organ was plated when we achieved very low counts with serial dilutions.

In vitro sensitivity tests. For in vitro sensitivity tests, MICs were determined by the method outlined in National Committee for Clinical Laboratory Standards document M27-T (11). This macrobroth method involves an inoculum of 0.5×10^3 to 2.5×10^3 CFU/ml; RPMI-1640 with L-glutamine, without bicarbonate, buffered with morpholinepropanesulfonic acid (MOPS), adjusted to pH 7; and incubation at 35°C for 48 h. The only variation from the method was that the test was read at 24 h in addition to the 48-h reading. Due to prior studies of cryptococcosis, in which we had found that the 24-h MIC correlated best with in vivo results (20), we relied on the 24-h fluconazole MIC for assessment of fluconazole resistance. The 24- and 48-h MICs of M991 were both ≤0.125 μg/ml, while the 24- and 48-h MICs for fluconazole were 32 and >64 μg/ml, respectively. Two quality control (QC) strains, *Candida parapsilosis* (CP) ATCC 22019 and *Candida krusei* (CK) ATCC 2658, were set up in conjunction with the test isolate. These two strains were chosen for the mid-range (CP, with 24- and 48-h MICs of 1.0 and 2.0 μg/ml, respectively) and high-range (CK, with 24- and 48-h MICs of 16 and 32 μg/ml, respectively) fluconazole MIC values. Although QC ranges for M991 have not been established, the QC strains gave 24- and 48-h results of 0.25 and 0.5 μg/ml, respectively, for CP and 0.25 μg/ml at both time points for CK. We considered a strain resistant when the MIC was >16 μg/ml at 24 h, in part based on the data with *Cryptococcus neoformans* and in part based on earlier studies with *C. albicans* (5, 20).

Statistics. For survival the log rank and Wilcoxon tests were used. The *P* values varied because of correction for multiple comparisons. For tissue burden studies, Dunnett's one-tailed *t* test or the rank sums test (Wilcoxon scores) was used.

TABLE 1. Survival of mice after infection with *C. albicans* and treatment with fluconazole or M991

Study no.[a] and group	Dose (mg/kg)	Survival (mean no. of days ± SEM)	*P* (compared with control)
1			
Control	None	7.3 ± 0.8	
Fluconazole	5 (BID)[b]	8.4 ± 1.1	0.3347
M991	5	31.0 ± 0	0.0001
	2	23.7 ± 3.7	0.0008
	0.5	28.2 ± 2.4	0.0001
2			
Control	None	8.0 ± 0.8	
M991	0.5	28.7 ± 2.3	0.0001
	0.1	22.9 ± 2.5	0.0001
	0.05	22.5 ± 2.0	0.0001
3A			
Control	None	7.5 ± 0.7	
M991	0.05	24.6 ± 2.2	0.0001
	0.025	19.2 ± 2.3	0.0001
	0.0125	16.7 ± 2.4	0.0003
3B			
Control	None	6.5 ± 0.2	
M991	0.05	11.4 ± 2.4	0.0058
	0.025	6.5 ± 0.5	0.1744
	0.0125	9.2 ± 2.4	0.0920

[a] Studies 1, 2, and 3B were performed with neutropenic mice, while study 3A was performed with immunocompetent mice.
[b] BID, twice a day.

RESULTS

In study 1 we wished to evaluate M991 in a model using neutropenic mice. Survival data are presented in Table 1. The mean survival time of control mice was 7.3 days, and that of fluconazole recipients was 8.4 days. Thus, this *C. albicans* isolate was resistant to fluconazole in vivo. Significant prolongation of survival ($P \leq 0.0001$) was noted with once-daily dosing of M991 at 5 and 0.5 mg/kg, and it was of borderline significance at 2 mg/kg. In study 2 neutropenic mice were treated at 0.5, 0.1, and 0.05 mg/kg once daily with M991. Due to the activity of M991 down to 0.05 mg/kg, in study 3 we compared dosing at 0.05, 0.025, and 0.0125 mg/kg once daily with both immunocompetent and neutropenic mice. In this study, with immunocompetent mice all three doses continued to show significant prolongation of survival over controls ($P < 0.001$). However, for neutropenic mice M991 at 0.05 mg/kg showed marginal significance over controls; therefore, a repeat study was done (data not shown) which showed 0.05 mg/kg to be not significant. Therefore, while M991 is extremely potent, neutropenia reduces some of the efficacy of this compound.

Two studies measured fungal burden after treatment with M991, and in one of these fluconazole was also included. One study is presented in Fig. 1, and data are not given for the other, whose results were similar to those of the first study. M991 is effective in reducing *Candida* burden in spleen and kidney tissues.

DISCUSSION

The pneumocandins are potent antifungals which act to impede cell wall synthesis of a variety of fungi. Of the major fungal pathogens, *C. neoformans* is resistant, possibly because there is little β-1,3-glucan in the cell wall. Since the target of these agents is unique, it is not surprising that M991 is effective against fluconazole-resistant *C. albicans*. What is remarkable is the great potency of these agents. In the present study we could show efficacy in immunocompetent mice at a dose of ≤0.0125 μg/ml. Although we did not utilize amphotericin B as a control, this dose is more than 10 times less than the anticipated minimal effective dose of amphotericin B. Indeed, drugs in this class may be the most potent antifungals available today.

Earlier studies claimed that pneumocandins could sterilize kidneys of *Candida* (3). However, data for these studies were generated from serial dilution cultures of aliquots of renal tissue homogenates, so there was generally a minimal threshold of 20 to 50 organisms below which there were no data. In our studies we performed serial dilutions but also plated the entire organ tissue homogenate of a mouse if cultures showed less than our minimum threshold on the 0.2-ml aliquots first cultured. Although we achieved some very low counts, fewer than 10 organisms per pair of kidneys for a few animals, we did not achieve true sterilization of tissues.

Further development of these antifungal drugs depends on tolerance, clearance, and ease of administration. Unfortunately, for maximal effect M991 must be given parenterally. In mice cilofungin, a related compound, is cleared as unchanged drug by the biliary route, with a short half-life of 30 min. In rabbits the half-life is 13 min. This can be significantly extended in a rabbit model by continuous infusion, perhaps by saturating the degrading mechanism and increasing tissue distribution (10, 17). There are no data available for optimizing the dosing of M991. There are also no clinical data available for M991; however, the potent activity, suitability for i.v. administration, and, as we have shown, excellent activity against fluconazole-resistant *Candida* make this an attractive candidate for development for treatment of patients with disseminated candidiasis. Although there is some loss of activity in neutropenic mice (two studies showed protection at a dose of 0.05 mg/kg and one did not), M991 retains great potency in these animals as well. In this regard, M991 is more similar to amphotericin B, which shows decreased efficacy in neutropenic mice, than to fluconazole, which is equally efficacious in neutropenic and immunocompetent mice (19). Therefore, the loss of potency is only modest, and this agent may be of particular value in the more severely immune depressed patient, for whom there is great urgency for potent, rapidly acting agents.

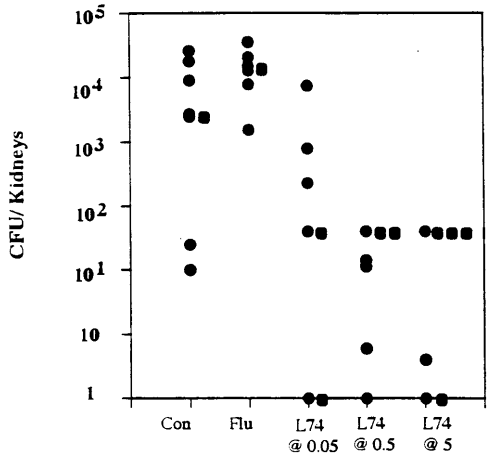

FIG. 1. Whole-organ tissue burden of both kidneys in mice sacrificed 8 days after infection. Con, control (dosed with water); Flu, fluconazole (5 mg/kg twice daily); L-74, M991 (0.05, 0.5, and 5.0 mg/kg daily).

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