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Treatment of Histoplasmosis with MK-991 (L-743,872)

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BALB/c *nu*/+ immunocompetent and athymic (*nu*/*nu*) mice were infected intravenously with yeast cells of *Histoplasma capsulatum*. Mice were either given water (controls) intraperitoneally (i.p.) or given MK-991 i.p. once daily or twice daily. Protection was measured as prolonged survival or reduction in tissue counts. MK-991 was protective in immunocompetent mice, prolonging survival and reducing counts in spleen and livers at a dose as low as 0.05 mg/kg of body weight/day. MK-991 was modestly effective in athymic mice at a higher dose, 5 mg/kg/day. These studies suggest that MK-991 may be appropriate for clinical development in histoplasmosis.

Histoplasma capsulatum is increasingly recognized as a pathogen of immunosuppressed patients, particularly those with AIDS (16). Both amphotericin B and triazoles are potent agents against *H. capsulatum* (17, 18). However, amphotericin B is highly toxic (8), itraconazole must be given orally and is subject to a variety of drug interactions (6), and fluconazole may be less potent than itraconazole (15). Therefore, it is reasonable to explore new classes of drugs for potential use in histoplasmosis. One such group is the lipopeptide echinocandins and their derivatives. These drugs inhibit the synthesis of β1,3-glucan in fungal cell walls (3). They are thought to act rapidly and irreversibly on fungal cell wall synthesis and are at least as potent as the polyenes in vitro (5, 13). The first of these drugs, cilofungin, reached clinical development for treatment of candidemia, but the appearance of vehicle-related nephrotoxicity terminated clinical use (11). Subsequently, water-soluble derivatives of these were developed, including the pneumocandins and papulocandins. These have a much broader antifungal spectrum than cilofungin and are highly potent in vitro and in animal models of aspergillosis and candidemia (1, 2, 4). *Cryptococcus neoformans* is highly resistant, perhaps because of less β1,3-glucan in the cells walls.

We considered that pneumocandins might have value for treatment of histoplasmosis. Therefore, in the present studies we have evaluated the pneumocandin MK-991 in vitro and in a murine model of disseminated histoplasmosis.

MATERIALS AND METHODS

Animals. BALB/c immunocompetent (*nu*/+) and immunodeficient (*nu*/*nu* [athymic]) mice were raised under specific-pathogen-free barrier conditions in our colony. Six-week-old mice were housed in groups of five and throughout the studies were given food and water ad libitum.

Pathogen. *H. capsulatum* clinical isolate 94-255 was obtained from the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio. The fungus was maintained in the yeast phase at 37°C on brain heart infusion agar enriched with 10% sheep blood. Before use in in vivo studies, *H. capsulatum* was harvested by centrifugation at 500 × *g* for 10 min and washed three times in sterile saline. Clumps were disrupted by agitation. Fungal cells were counted with a hemacytometer and adjusted to the desired inoculum in sterile saline.

Infection and treatment. Mice were infected intravenously. The inoculum size was confirmed by serial-dilution colony counts, and viable counts are reported herein. Mice were treated from day 1 through day 7 with either water or MK-991 in various doses, dissolved in water and administered intraperitoneally (i.p.) in 0.2 ml once daily or twice daily.

Protection. Groups of 10 mice were observed for 30 days after infection. Moribund mice were sacrificed, and their deaths were recorded as occurring on the next day. The Wilcoxon and log rank tests were used for comparisons. Because of multiple-dose comparisons in the same study, a P value of <0.0127 was required for differences to be considered significant. For tissue counts, mice were sacrificed on day 8 or 13 after infection. The livers and spleens were harvested by an aseptic technique and homogenized in saline. Serial quantitative dilutions were plated on brain heart infusion agar enriched with 10% sheep blood and held at 37°C for 10 days. Characteristic colonies were counted, and the tissue burden was calculated in CFU per gram of organ. One-way analyses of variance were used to determine if any differences existed between the treatment groups. For pairwise comparisons, Dunnett's one-tailed t test or Sidak's multiple comparison test was used.

In vitro susceptibilities. Mould-phase conidial cells were tested. The National Committee for Clinical Laboratory Standards macrobroth method was used, modified for testing molds (14). For these tests MK-991 was diluted in water. The modifications used included standardizing the inoculum to 10^4 CFU/ml and incubation at 30°C. The MICs for molds were determined at the first 24-h interval where growth could be measured in the drug-free control, and observations were repeated 24 h later. This is in the range of MICs reported by others (7). In the Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio, the mean MK-991 MIC at 72 h and the MIC at 96 h for 10 blood isolates of *H. capsulatum* were 0.25 and 0.27 μg/ml, with a range of 0.06 to 0.5 μg/ml. The duration of the effective concentration of the drug in serum (defined by Merck in-house data as the time above 0.5 μg/ml, the concentration at which 90% of *Candida albicans* isolates are inhibited) is 6 to 8 hours in rats, and kinetics are similar in mice (10). The terminal half-life is 77 to 89 h in the rat following doses of ≥2 mg/kg (13a).

RESULTS

The MIC of MK-991 for *H. capsulatum* isolate 94-255 was 0.25 μg/ml at 72 and 96 h.

Survival of immunocompetent mice infected with *H. capsulatum* and treated with MK-991 at various doses is shown in Table 1. With an inoculum of 10^7 CFU/mouse, MK-991 at 5 mg/kg of body weight minimally prolonged survival. When the inoculum was reduced by a little more than 50%, the 5- and 10-mg/kg doses significantly prolonged survival.

When the inoculum was further reduced by 50%, MK-991 was protective all the way down to 0.05 mg/kg. However, when athymic mice were used, at an even smaller inoculum, protection was irregular and minimal, as measured by extension of survival. This apparent dose dependency of protection was further explored in a study in which mice were infected with various doses of *H. capsulatum* and treated with either water (control) or MK-991 at 5 mg/kg/day i.p. Results are shown in Table 2. At an inoculum of 10^6 CFU/mouse, MK-991 did not

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TABLE 1. Survival of mice infected with various doses of *H. capsulatum* and treated with MK-991

Inoculum (mouse)	Group	Dose (mg/kg/day)	Survival time (days)[a]
1.9×10^7 (*nu*/+)	Control	0	8.0 ± 2.6
	MK-991	10	6.6 ± 0.2
		5 BID[b]	7.0 ± 0.9
		5	8.8 ± 2.5
8×10^6 (*nu*/+)	Control	0	14.4 ± 1.9
	MK-991	10	30.0 ± 0.0*
		5 BID	30.0 ± 0.0*
		5	30.0 ± 0.0*
4.8×10^6 (*nu*/+)	Control	0	7.5 ± 0.2
	MK-991	0.5	13.8 ± 1.9*
		0.1	13.7 ± 2.2*
		0.05	10.1 ± 0.9*
		0.01	8.0 ± 0.5
1.1×10^6 (*nu*/*nu*)	Control	0	12.5 ± 3.3
	MK-991	10	10.8 ± 2.0
		5 BID	10.6 ± 2.5
		5	16.4 ± 2.9
		1	9.3 ± 1.5
6×10^5 (*nu*/*nu*)	Control	0	8.7 ± 1.2
	MK-991	10	9.5 ± 1.0
		5	12.7 ± 2.6*
		1	11.1 ± 2.2
		0.1	7.7 ± 0.2

[a] Values are means ± standard errors of the means. *, significantly different from control value.
[b] BID, twice daily.

TABLE 3. Yeast cell counts in spleens and livers of mice infected with *H. capsulatum* intravenously, treated from day 1 through day 7, and sacrificed on day 8 after infection[a]

Mouse	Inoculum	Group	Dose (mg/kg)	Median count (10^6 CFU/g) in[a]:	
				Spleen	Liver
nu/+	8×10^6	Control	0	88.2	20.6
		MK-991	10	52.7	14.0
			5 BID[b]	10.9*	3.4*
			5	10.9*	2.8*
			0.5	5.8*	3.5*
nu/+	1×10^6	Control	0	30.3	11.4
		MK-991	10	2.8*	1.6*
			0.25	2.4*	0.8*
			0.1	3.6*	1.6*
			0.05	5.6*	2.0*
nu/*nu*	2×10^6	Control	0	781	377
		MK-991	5	131*	104*
	8×10^5	Control	0	123	127
		MK-991	5	25.6**	28.0**
	6×10^4	Control	0	6.3	1.3
		MK-991	5	0.9*	0.6
	3×10^4	Control	0	11.9	2.2
		MK-991	5	3.5*	1.2*

[a] *, significantly different from control value. **, not significant because of two outlying low-count values.
[b] BID, twice daily.

prolong survival. As the inoculum size decreased, survival of controls lengthened, and MK-991 prolonged survival. At the lowest inoculum size, survival of controls was 40+ days, and MK-991 did not extend it.

Yeast cell counts in the liver and spleen also showed that MK-991 was protective in *nu*/+ and athymic mice, though counts ran higher in athymic mice (Table 3). Significant reduction of tissue counts occurred in both liver and spleen, though it was more consistent in the spleen.

DISCUSSION

Histoplasmosis follows sharply contrasting courses in immunocompetent and in athymic mice. Immunocompetent BALB/c mice are rather resistant to histoplasmosis. Small inocula ($<10^5$ CFU) often do not cause mortality. In those infected with larger doses, infection generally progresses through the second week, by which time mice have either succumbed or begun a recovery course that is essentially complete by 1 month. In contrast, athymic mice develop progressive lethal infection with much lower doses of organisms (19). Antifungal therapy delays mortality at higher doses, but eventually the mice succumb, sometimes even while treatment is continuing.

TABLE 2. Effect of inoculum size on response to MK-991 at 5 mg/kg/day in groups of seven athymic (*nu*/*nu*) mice[a]

Inoculum size (CFU/mouse)	Group	Survival time (days)[b]
10^6	Control	21.7 ± 1.9
	MK-991	23 ± 1.0
5×10^5	Control	19.7 ± 0.7
	MK-991	32.7 ± 4.4*
5×10^4	Control	25.6 ± 0.8
	MK-991	38.0 ± 2.5*
10^4	Control	41.1 ± 2.9
	MK-991	40.4 ± 3.1

[a] Mice were observed to day 56 for survival.
[b] Values are means ± standard errors of the means. *, significantly different from control values.

MK-991 is highly active against histoplasmosis, but the benefit can be overcome by very high infecting doses ($\geq 10^7$ CFU) and can be partially overcome by immunosuppression. MK-991 shows great potency against *H. capsulatum*. For immunocompetent mice, in which all treated groups (down to doses of 5 mg/kg) survived the full month of observation, the minimal dose which significantly extended survival was 0.05 mg/kg. This is quite effective in comparison with fluconazole and amphotericin B. Other studies showed >80% survival with 0.3 mg of amphotericin B and 60 mg of fluconazole per kg twice daily (9). The same dose of fluconazole reduced bronchoalveolar lavage fluid counts of *H. capsulatum* by about 1 log. In another study the 50% protective dose (dose preventing 50% of deaths with a lethal inoculum) was 6 mg/kg/day for fluconazole and 1.8 mg/kg/day with amphotericin B (12). Although we did not calculate the 50% protective dose for MK-991 in our studies, it was much less than 5 mg/kg/day. Thus, MK-991 appears more potent milligram for milligram than fluconazole and is similar or superior to amphotericin B.

MK-991 was much less effective in athymic mice. At a 10^6-CFU infecting dose, MK-991 did not prolong survival of athymic mice, but with smaller inocula MK-991 was protective at 5 mg/kg. Despite the lack of a survival benefit at a high infecting dose, MK-991 did reduce spleen and liver counts by 80 to 90%

in mice infected with 8×10^6 CFU. With an inoculum of 10^6 CFU, MK-991 reduced counts by about 1 log at the lowest tested dose, 0.05 mg/kg.

MK-991 is cleared relatively rapidly in mice, with an effective half-life (time above the concentration at which 90% of the *Candida* isolates are inhibited) of 6 h. One concern was that the drug might clear too rapidly to allow a dose-dependent response upon once-daily dosing. For this reason we explored doses of 5 mg/kg twice daily versus 10 mg/kg once daily. MK-991 was, in some studies, more useful at 5 mg/kg twice daily than at 10 mg/kg once daily.

The present studies suggest that MK-991 may have a role in treatment of histoplasmosis. Although the drug was more potent in immunocompetent animals, MK-991 may be of some value even in the severely immunodepressed. MK-991 targets a process (fungal cell wall synthesis) that has no mammalian counterpart. Thus, it is possible that this drug has much lower toxicity for humans than other antifungals. These results suggest that this new class of drugs may be effective in histoplasmosis as well as other mycoses.

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