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Evaluation of the Echinocandin Antifungal MK-0991 (L-743,872): Efficacies in Mouse Models of Disseminated Aspergillosis, Candidiasis, and Cryptococcosis

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The in vivo activity of the Merck antifungal echinocandin drug candidate MK-0991 (L-743,872) was evaluated in mouse models of disseminated candidiasis, aspergillosis, and cryptococcosis. The echinocandins are potent inhibitors of 1,3-β-D-glucan synthase. Two models of disseminated candidiasis were used. In a *Candida albicans* mouse survival model with both DBA/2N and CD-1 mice, estimates of the 50% effective doses (ED_{50}s) of MK-0991 were 0.04 and 0.10 mg/kg of body weight/dose at 21 days after challenge, respectively. In a *C. albicans* target organ assay (TOA) with DBA/2N mice, MK-0991 at levels of ≥0.09 mg/kg/dose significantly reduced the numbers of *C. albicans* CFU/g of kidneys compared to the numbers in the kidneys of control mice from 1 to 28 days after challenge. Even when given as a single intraperitoneal dose either 30 min or 24 h after challenge, MK-0991 was effective and significantly reduced the numbers of *C. albicans* CFU/g of kidney compared to those in the controls. MK-0991 was >300-fold less active when it was administered orally than when it was administered parenterally. MK-0991 was efficacious in mouse TOAs against other *C. albicans* strains and *Candida* species including *Candida tropicalis*, *Candida* (*Torulopsis*) *glabrata*, *Candida lusitaniae*, *Candida parapsilosis*, and *Candida krusei*. MK-0991 was ineffective against disseminated *Cryptococcus neoformans* infections. In the model of disseminated aspergillosis in mice, MK-0991 at doses of ≥0.02 mg/kg/dose significantly prolonged the survival of DBA/2N mice, with estimates of the ED_{50} and ED_{90} of MK-0991 being 0.03 and 0.12 mg/kg/dose, respectively, at 28 days after challenge. MK-0991 is a potent, parenterally administered therapeutic agent against disseminated candidiasis and aspergillosis that warrants further investigation in human clinical trials.

MK-0991 (L-743,872) is a new echinocandin antifungal drug candidate undergoing clinical development by Merck & Co. The echinocandins are cyclic hexapeptides with fatty acyl side chains. MK-0991 is a semisynthetic derivative of the natural product pneumocandin B_O (12). The mechanism of action for the echinocandins is inhibition of 1,3-β-D-glucan synthase, which synthesizes a critical structural cell wall component in certain pathogenic fungi and *Pneumocystis carinii* cysts (4, 11, 12, 26, 27). The activity of MK-0991 is fungicidal in vitro (6, 7). MK-0991 is highly water soluble and has been shown to be generally well tolerated in rodents, rhesus, chimpanzees, and humans (28). The comparative pharmacokinetics of MK-0991 in all these species has shown that it has good bioavailability when it is administered parenterally (22, 23, 28). This report describes the in vivo activity of MK-0991 evaluated in mouse models of disseminated candidiasis, aspergillosis, and cryptococcosis.

MATERIALS AND METHODS

Drugs. MK-0991 was synthesized by the Department of Medicinal Chemistry at Merck Research Laboratories, Rahway, N.J., and was formulated in sterile distilled water. Amphotericin B (AmB) was purchased as Fungizone (Bristol-Myers Squibb, Princeton, N.J.) and was reconstituted according to the manufacturer's instructions and diluted in sterile water.

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Animals. Outbred female CD-1 mice (average weight, 19 to 21 g; Charles River, Wilmington, Mass.) were used in the disseminated candidiasis survival studies. Complement component 5-deficient DBA/2N female mice (average weight, 19 to 21 g; Taconic Farms, Germantown, N.Y.) were used in the disseminated aspergillosis, candidiasis, and cryptococcosis survival studies and in the *Candida* and *Cryptococcus* target organ assays (TOAs).

All procedures were performed in accordance with the highest standards for the humane handling, care, and treatment of research animals and were approved by the Merck Institutional Animal Care and Use Committee. Procedures for the care and use of research animals at Merck meet or exceed all applicable local, national, and international laws and regulations.

Organism and culture conditions. *Aspergillus fumigatus* MF5668 (ATCC 13073), originally isolated from a human pulmonary lesion, was cultured on

TABLE 1. Efficacy of MK-0991 comparing parenteral (q.d. versus b.i.d.), p.o., and delayed therapy against a disseminated *A. fumigatus* MF5668 infection[a]

Treatment	ED_{50} (mg/kg/dose)	ED_{90} (mg/kg/dose)
MK-0991 (i.p. b.i.d.)	0.03 (0.02–0.06)[b]	0.12 (0.07–0.52)
MK-0991 (i.p. q.d.)	0.06 (0.03–0.14)	0.44 (NE[c])
MK-0991 (i.p. b.i.d.), delayed	0.08 (0.05–0.15)	0.25 (0.14–0.97)
MK-0991 (p.o. b.i.d.)	20.53 (NE)	>50.00 (NE)
AmB (i.p. q.d.)	0.05 (0.03–0.09)	0.21 (0.11–0.89)

[a] DBA/2N mice (10 mice/group) were infected i.v. with 1.8×10^6 conidia/mouse. Mice received their first treatment 30 min after challenge; for mice receiving delayed therapy, however, the first treatment was administered 24 h after challenge. All mice were treated for a total of 5 days. ED_{50}s and ED_{90}s were estimated at day 28 after challenge.

[b] Values in parentheses are 95% confidence intervals.

[c] NE, confidence interval could not be estimated.

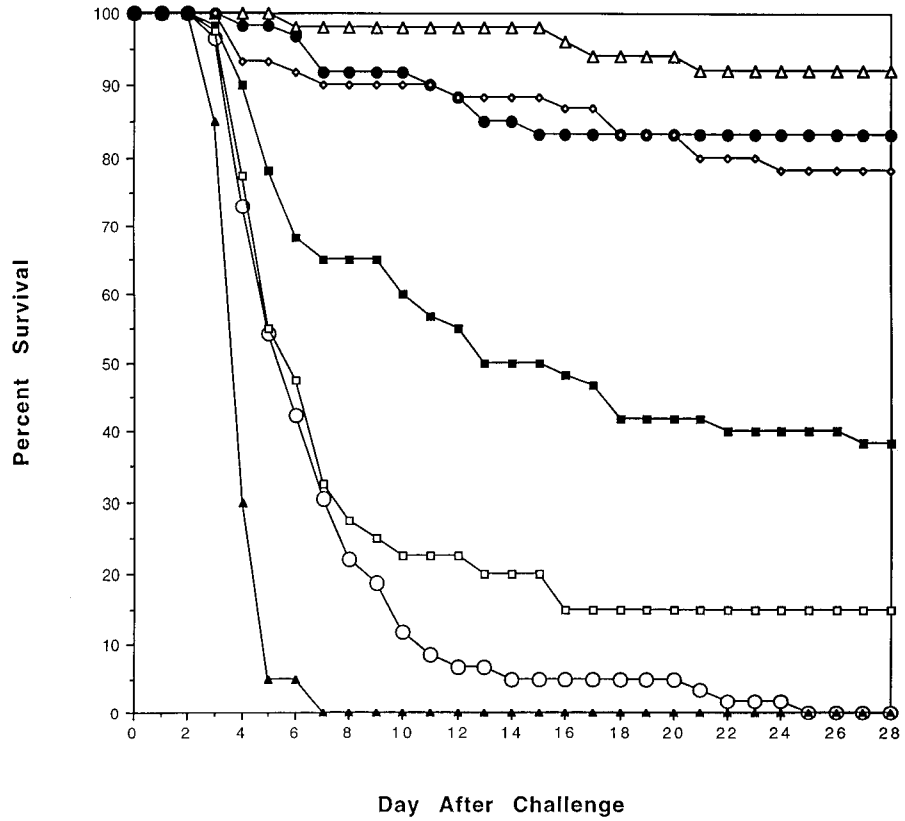

FIG. 1. Efficacy of MK-0991 against a disseminated *A. fumigatus* MY5668 infection (i.v. challenge with 1.8×10^6 conidia/mouse) in DBA/2N mice. Therapy was initiated within 30 min after challenge, and mice were treated i.p. b.i.d. for 5 days (total of 10 doses). △, 1.25 mg/kg; ●, 0.31 mg/kg; ◇, 0.08 mg/kg; ■, 0.02 mg/kg; □, 0.005 mg/kg; ▲, 0.001 mg/kg; ○, sham treatment.

Sabouraud dextrose agar (SDA; BBL, Cockeysville, Md.) slants at 30°C for 4 to 5 days. Conidia were washed from the surfaces of several (three to four) agar slants and placed into sterile saline with 0.01% Tween 20 (Fisher Scientific, Fair Lawn, N.J.), and the concentration of conidia was quantitated by counting with a hemacytometer. The viable count was confirmed by serially diluting the conidial suspension 10-fold and plating the inoculum on SDA plates. Merck cultures of *Candida* species (listed in Table 5) were grown on SDA at 35°C for 24 h. *Cryptococcus neoformans* MY2061 (a human isolate obtained from the University of Wisconsin, Madison) was grown on SDA at 35°C for 48 to 72 h. Yeast cells were washed from the surfaces of one to two SDA plates, and cell concentrations were quantitated by counting with a hemacytometer. The viable count was confirmed by serially diluting the yeast suspension 10-fold and plating each inoculum onto SDA plates.

Survival studies. Disseminated aspergillosis was induced in DBA/2N mice by the method described previously (1). Briefly, mice were infected by intravenous (i.v.) inoculation of 0.2 ml of the *A. fumigatus* MF5668 spore suspension (5.0×10^5 to 2.0×10^6 conidia/mouse) into the lateral tail vein. Therapy was initiated either within 30 min after challenge or, in the case of the delayed therapy, 24 h after challenge. The mice were treated for a total of 5 days. MK-0991 was administered intraperitoneally (i.p.) either once daily (q.d.) or twice-daily (b.i.d.). MK-0991 was also tested orally (p.o.) b.i.d. AmB was administered i.p. q.d.

Disseminated candidiasis was induced in DBA/2N and CD-1 mice by the i.v. inoculation of 0.2 ml of a *Candida albicans* MY1055 cell suspension into the lateral tail vein. Each DBA/2N mouse received 10^6 blastoconidia, and each CD-1 mouse received 10^7 blastoconidia. These inocula were previously determined to represent one 14-day 100% lethal dose for each strain of mouse. Therapy was initiated within 30 min after challenge, and the mice were treated for a total of 4 days. MK-0991 was administered either i.p. or p.o. b.i.d.

Disseminated cryptococcosis was induced in DBA/2N mice by the i.v. inoculation of 0.2 ml of a *C. neoformans* MY2061 cell suspension (2×10^6 cells/mouse) into the lateral tail vein. Therapy was initiated within 30 min after challenge, and the mice were treated i.p. for a total of 4 days with MK-0991 (b.i.d.) or AmB (q.d.).

Compounds were tested at titrated concentrations (serial fourfold dilutions), with 10 mice per therapy group. Infected sham-treated mice were administered sterile water. Morbidity and mortality were recorded daily for 21 days in the candidiasis and cryptococcosis models and for 28 days in the aspergillosis model.

TOAs. DBA/2N mice were infected i.v. with approximately one 50% lethal dose of *C. albicans* MY1055 (7.5×10^4 yeast cells per mouse). The inocula for the other *Candida* strains and *Candida* (*Torulopsis*) *glabrata* are listed in Table 5. It should be noted, that strains of *Candida lusitanae*, *Candida parapsilosis*, *Candida krusei*, and *Candida glabrata* were not lethal for mice, and the inoculum used for each strain was previously shown to give detectable kidney colonization at day

TABLE 2. In vivo antifungal efficacy of MK-0991 against a disseminated *C. albicans* survival model[a]

Compound (route)	ED_{50}s (mg/kg/dose)[b] on the following day after challenge					
	Day 7		Day 14		Day 21	
	CD-1	DBA/2N	CD-1	DBA/2N	CD-1	DBA/2N
MK-0991 (i.p.)	0.07 (0.04–0.12)	0.04 (NE[c])	0.08 (0.04–0.17)	0.04 (NE)	0.10 (0.06–0.17)	0.04 (NE)
MK-0991 (p.o.)	42.70 (21.7–84.2)	14.80 (8.5–25.8)	42.70 (21.7–84.2)	14.80 (8.5–25.8)	42.70 (21.7–84.2)	14.80 (8.5–25.8)

[a] ED_{50}s were estimated on the basis of survival at days 7, 14, and 21 after challenge. Each DBA/2N mouse was infected i.v. with 10^6 cells, and each CD-1 mouse was infected i.v. with 10^7 cells MK-0991 was administered b.i.d. either i.p. or p.o. Mice were treated for a total of 4 days (eight total doses).
[b] Values in parentheses are 95% confidence intervals.
[c] NE, confidence interval could not be estimated.

TABLE 3. Log_{10} *C. albicans* MY1055 CFU per gram of kidney at 7 days postchallenge in DBA/2N mice receiving single-dose therapy or a single delayed dose (24 h) with titrated doses of MK-0991 or AmB[a]

Treatment	Mean log_{10} CFU/g of kidney (% sterilization) at the following dose (mg/kg/dose)[b]:										ED_{90} (mg/kg/dose)[c]
	Sham	3.0	1.5	0.75	0.375	0.18	0.09	0.046	0.023	0.01	
MK-0991	6.42 (0)	2.17* (100)	2.13* (100)	2.13* (100)	2.23* (40)	2.89* (40)	3.74* (0)	3.69* (0)	5.51* (0)	4.67* (0)	0.01 (0.003–0.015)[d]
MK-0991, delayed	6.42 (0)	2.10* (100)	2.33* (40)	2.22* (80)	2.59* (40)	3.65* (0)	4.18* (0)	5.45* (0)	5.22* (0)	6.79 (0)	0.03 (0.02–0.04)
AmB	6.42 (0)	3.06* (0)	2.92* (20)	3.27* (0)	2.87* (40)	4.97* (0)	4.06* (0)	5.75 (0)	6.01 (0)	6.13 (0)	0.04 (0.02–0.06)
AmB, Delayed	6.42 (0)	2.77* (20)	2.37* (40)	2.92* (20)	3.19* (0)	4.77* (0)	5.01 (0)	6.09 (0)	6.04 (0)	5.17 (0)	0.03 (0.02–0.06)

[a] DBA/2N mice (five mice/group) were infected i.v. at 3.4×10^4 CFU/mouse. MK-0991 and AmB were administered i.p. Mice received a single treatment 30 min after challenge or 24 h after challenge (delayed).

[b] Mean log_{10} CFU per gram at day 7 after challenge for paired kidneys. Percent sterilization indicates the percentage of mice with no detectable yeast; the limit of detection was 50 yeast cells per pair of kidneys. *, the mean was statistically significantly less than that for the sham-treated control at an α value ≤ 0.05 according to Fisher's least-significant-difference *t* test.

[c] ED_{90}, were estimated by comparison of mean log_{10} CFU per gram at day 7 after challenge for paired kidneys from the treated groups to the values for paired kidneys from the sham-treated controls.

[d] Values in parentheses are 95% confidence intervals.

7 after challenge. The inoculum for *C. neoformans* MY 2061 was 10^6 cells per mouse (approximately one 50% lethal dose).

Therapy was initiated within 30 min after challenge, and mice were treated for a total of 4 days. Compounds were administered as described above. The TOA for *Candida* species monitors the numbers of CFU per gram of paired kidneys at time points following challenge (target organ kidney assay [TOKA]). The TOA for *C. neoformans* monitors the numbers of CFU per gram of brain and spleen at time points following challenge (target organ brain and spleen assay). Organs from euthanized mice (five mice/group/experiment) were removed by aseptic technique, weighed, and placed in sterile Whirl Pak bags (Fisher Scientific) containing 5 ml of sterile saline. The organs were homogenized in the bags and serially diluted in saline, and aliquots were plated onto SDA. The plates were incubated at 35°C, and the organisms were enumerated after 48 h for *Candida* species and 72 h for *Cryptococcus*. The mean numbers of CFU per gram of tissue in the organs of the treated groups were compared with those in the organs of sham-treated mice. Percent clearance indicates the percentage of mice in which there was no detectable yeast; because of the dilution scheme, the limit of detection was 50 yeast cells per brain, spleen, or pair of kidneys.

Statistical analyses. Experiment-to-experiment variability was accounted for in all analyses for data that were pooled across experiments. In the disseminated aspergillosis model, the 50% effective doses (ED_{50}s) and the ED_{90}s were estimated by a robust probit method (29, 35) from survival rates calculated by the Kaplan-Meier (24) technique at days 21 and 28 after challenge. In the disseminated candidiasis model, ED_{50}s were estimated at days 7, 14, and 21 after challenge by the method of Knudson and Curtis (25) and were defined as the concentration of compound in (milligrams per kilogram of body weight per dose) that protected 50% of mice from lethal challenge. In the target organ assays, inverse regression (16) was used to estimate the doses which reduced the CFU/organ counts so that they were 90% lower than those for the sham-treated controls. The mean log_{10} numbers of yeast CFU/organ for mice in the dose groups were compared to those sham-treated control mice by Fisher's least-significant-difference *t*-test procedure (18). Comparisons were determined significant at the level of α equal to 0.05.

RESULTS

Efficacy in the disseminated aspergillosis model. The ED_{50}s and ED_{90}s, based on survival against a disseminated *A. fumigatus* MF5668 infection in DBA/2N mice treated with MK-0991 and AmB, are presented in Table 1. Parenteral administration (b.i.d.) of MK-0991 resulted in an ED_{50} and an ED_{90} of 0.03 and 0.12 mg/kg/dose, respectively. When MK-0991 was administered q.d., the ED_{50} and the ED_{90} were 0.06 and 0.44 mg/kg/dose, respectively; these were not that much greater than the values obtained after administration b.i.d. when the total dose per day is considered. Delaying therapy until 24 h after challenge resulted in an ED_{50} and an ED_{90} of 0.08 and 0.25 mg/kg/dose (i.p. b.i.d.), respectively. Efficacy was greatly reduced when MK-0991 was administered p.o. (ED_{50} and ED_{90} of 20.5 and >50.0 mg/kg/dose, respectively).

Percent survival over time for mice treated with MK-0991 is displayed in Fig. 1. MK-0991 administered parenterally (i.p. b.i.d.) at concentrations of ≥ 0.02 mg/kg/dose significantly prolonged the survival of infected mice compared to that of infected, sham-treated animals. Administration of MK-0991 at or above 0.08 mg/kg/dose resulted in $\geq 78\%$ survival at day 28 after challenge.

Efficacy in the mouse model of disseminated candidiasis. MK-0991 administered i.p. and p.o., was tested against an i.v. induced, disseminated *C. albicans* MY1055 infection in both immune-competent CD-1 and complement 5-deficient DBA/2N mice (Table 2). ED_{50}s at day 21 after challenge were 0.10 and 0.04 mg/kg/dose (i.p. b.i.d.) for CD-1 and DBA/2N mice, respectively. Orally administered MK-0991 (b.i.d.) was much less active, with ED_{50}s of 42.7 and 14.8 mg/kg/dose for CD-1 and DBA/2N mice, respectively.

Efficacy in the TOA of disseminated candidiasis. MK-0991 administered as a single i.p. dose given either immediately after challenge or 24 h after challenge (delayed) was tested for efficacy in reducing recoverable yeast from the kidneys of mice challenged i.v. with *C. albicans* MY 1055. ED_{90}s were estimated on day 7 after challenge and were based on the mean log_{10} numbers of CFU per gram of paired kidneys from treated

TABLE 4. Log_{10} *C. albicans* MY1055 CFU per gram of kidney at 7 days postchallenge in DBA/2N mice receiving p.o. therapy with titrated doses of MK-0991[a]

Treatment	Mean log_{10} CFU/g of kidney (% sterilization) at the following Dose (mg/kg/dose)[b]:						ED_{90} (mg/kg/dose)[c]
	Sham	50.0	25.0	12.5	6.25	3.13	
MK-0991 (p.o.)	6.40 (0) ($n = 14$)	2.29* (90) ($n = 10$)	2.51* (60) ($n = 10$)	3.74* (13.3) ($n = 15$)	5.06 (10) ($n = 10$)	6.54 (0) ($n = 10$)	4.40 (2.8–7.0)[d]

[a] DBA/2N mice were infected i.v. at 7.5×10^4 CFU/mouse. MK-0991 was administered p.o. Mice were treated for 4 days (eight total doses).

[b] Mean log_{10} CFU per gram at day 7 after challenge for paired kidneys. Percent sterilization indicates the percentage of mice with no detectable yeast. *n*, number of mice per group; *, the mean was statistically significantly less than that for the sham-treated control at an α level of ≤ 0.05 according to Fisher's least-significant-difference *t* test.

[c] ED_{90}s were estimated by comparison of mean log_{10} CFU per gram at day 7 after challenge for paired kidneys from the treated groups to the values for paired kidneys from sham-treated controls.

[d] Values in parentheses are 95% confidence intervals.

groups compared to those for paired kidneys from sham-treated controls. For comparison, AmB was also tested. The data are presented in Table 3. The ED_{90} of single-dose therapy with MK-0991 was 0.01 mg/kg, and the value for AmB was 0.04 mg/kg. The ability of MK-0991 to sterilize the kidneys of mice was superior to that of AmB. Even when therapy was delayed until 24 h after challenge, MK-0991 at doses of ≥0.02 mg/kg significantly reduced the load of *C. albicans* in the kidneys up to day 7 after challenge.

As seen in the survival assay, MK-0991 had activity when it was administered p.o., but the ED_{90} (4.40 mg/kg/dose) was considerably higher than that of parenterally administered drug (Table 4). Even MK-0991 administered orally at 50 and 25 mg/kg showed high percentages of kidney sterilization (90 and 60%, respectively).

The efficacy of MK-0991 against several *Candida* species is shown in Table 5. MK-0991 was highly effective against the four other *C. albicans* strains tested, with ED_{90}s ranging from 0.003 to 0.02 mg/kg/dose. Against three *C. tropicalis* strains the ED_{90}s ranged from 0.03 to 0.055 mg/kg/dose. The efficacy of MK-0991 against two *C. glabrata* strains and one strain each of *C. lusitaniae*, *C. parapsilosis*, and *C. krusei* was also tested. It should be noted that these organisms are not lethal for normal mice and very high inocula were required to establish acceptable kidney colonization. The ED_{90}s for the *C. glabrata* strains were 0.03 and 0.06 mg/kg/dose, and for *C. lusitaniae* and *C. parapsilosis* the ED_{90}s were 0.16 and 1.0 mg/kg/dose, respectively. An effective dose for the *C. krusei* strain could not be calculated due to the lack of a significant dose-response relationship over the range of doses tested in this assay. However, a significant reduction from the effective dose for the sham-treated control was found at a level of 0.375 mg/kg/dose.

The efficacy of MK-0991 against *C. albicans* MY 1055 over time, as determined by the TOKA, is indicated in Fig. 2. MK-0991 at 0.09 and 0.375 mg/kg rapidly clears the kidneys of recoverable yeast, and the counts remained greater than 2 $\log_{10}$ CFU/g of kidney lower than those for the sham-treated control group for 28 days after challenge.

Efficacy in the TOA for disseminated cryptococcosis. MK-0991 was tested for its activity both in protecting mice from lethal challenge and in reducing the numbers of yeast recoverable from both the brains and spleens (day 7 after challenge) of mice infected i.v. with *C. neoformans*. MK-0991 at concentrations up to 20 mg/kg/dose (i.p. b.i.d.) was ineffective at protecting mice from lethal challenge and reducing the counts in the organs. AmB fully protected mice from the lethal challenge and cleared 100% of the yeast from the organs when it was used at 0.31 mg/kg (data not shown).

DISCUSSION

MK-0991 has been reported to have in vitro activity against most of the clinically relevant species of *Candida* (6, 7, 17, 20, 39) and *C. glabrata* (6, 7, 17, 39) including AmB- and azole-resistant isolates (6, 7, 32, 40). Studies of the growth inhibition kinetics of MK-0991 against *C. albicans* and *C. tropicalis* demonstrated that the drug has fungicidal activity, with a 99.9% reduction in growth by 5 to 7 h after administration (6, 7). A good correlation between in vitro activity and efficacy has also been shown for MK-0991 in our models of disseminated candidiasis. Potent in vivo efficacy in other models of disseminated candidiasis in neutropenic mice (30, 38) and against a fluconazole-resistant *Candida* isolate (30) has been reported for MK-0991. MK-0991 has also been shown to have in vivo efficacy in a mouse model of oropharyngeal and gastrointestinal candidiasis (19).

TABLE 5. In vivo antifungal efficacy of MK-0991 against disseminated *Candida* infections (TOKA) in DBA/2N mice[a]

Species and strain (MIC [μg/ml])[b]	Mean $\log_{10}$ CFU/g of kidney (% sterilization) at the following dose (mg/kg/dose)[c]:						ED_{90} (mg/kg/dose)[d]
	Sham	1.5	0.375	0.09	0.02	0.005	
C. albicans MY1055 (0.25)	6.24 (0) (n = 31)	NT[e]	2.22* (100) (n = 15)	2.26* (89) (n = 35)	5.08 (0) (n = 34)	5.91 (0) (n = 25)	0.013 (0.009–0.018)
C. albicans B-311 (0.125)	6.82 (0)	2.13* (100)	2.18* (100)	3.81* (20)	6.63 (0)	6.79 (0) (n = 3)	0.02 (0.01–0.03)
C. albicans MY1585 (0.25)	5.88 (0) (n = 1)	2.22 (100)	2.17 (80)	2.21 (100)	5.75 (0)	4.67 (0) (n = 1)	NE[f]
C. albicans CLY538 (0.25)	7.27 (0)	NT	2.17* (100)	2.19* (100)	4.35* (0)	5.33* (0)	0.003 (0.001–0.004)
C. tropicalis MY1124 (0.25)	6.16 (0)	2.19* (60)	2.26* (60)	5.51* (0)	5.97 (0)	6.56 (0)	0.055 (0.04–0.08)
C. tropicalis MY1163 (0.25)	5.80 (0)	2.29* (80)	2.25* (100)	4.42* (0)	5.68 (0)	5.99 (0)	0.03 (0.02–0.05)
C. tropicalis CLY545, FCZ[r] (0.125)	6.29 (0)	NT	2.19* (100)	5.02* (0)	5.73* (0)	6.66 (0)	0.05 (0.03–0.10)
C. glabrata MY1381 (0.25)	5.65 (0)	3.39* (0)	3.64* (0)	4.83 (0)	5.39 (0)	5.24 (0)	0.06 (0.01–0.14)
C. glabrata MY1382 (0.5)	5.48 (0)	3.29* (0)	2.51* (0)	3.70* (0)	5.15 (0)	5.66 (0)	0.03 (0.025–0.04)
C. lusitaniae MY1396 (0.5)	5.19 (0)	2.39* (20)	3.85* (0)	4.78 (0)	5.35 (0)	5.27 (0)	0.16 (0.10–0.24)
C. parapsilosis MY1943 (1.0)	5.17 (0)	3.90* (0)	4.79 (0)	5.09 (0)	5.25 (0)	5.11 (0)	1.0 (0.5–4.8)
C. krusei CK4935, FCZ[r] (1.0)	4.93 (0)	NT	3.98* (0)	4.96 (0)	4.41 (0)	4.83 (0)	NE

[a] DBA/2N mice (five mice/group unless noted otherwise) were infected i.v. Infectious challenges for each strain are as follows; MY1055, 7.5×10^4 CFU/mouse; B-311, 4.0×10^4 CFU/mouse; MY1585, 1.7×10^4 CFU/mouse; CLY538, 1.0×10^5 CFU/mouse; MY1124, 5.2×10^5 CFU/mouse; MY1163, 1.3×10^5 CFU/mouse; CLY545, 3.6×10^5 CFU/mouse; MY1381, 1.4×10^6 CFU/mouse; MY1382, 1.5×10^6 CFU/mouse; MY1396, 1.3×10^7 CFU/mouse; MY1943, 1.2×10^7 CFU/mouse; and CK4395, 8.6×10^7 CFU/mouse. MK-0991 was administered i.p. b.i.d. Mice were treated for a total of 4 days (eight doses).

[b] MICs were determined by a broth microdilution method described by Bartizal et al. (6). FCZ[r], fluconazole resistant.

[c] Mean $\log_{10}$ CFU per gram at day 7 after challenge for paired kidneys. Percent sterilization indicates the percentage of mice with no detectable yeast; the limit of detection was 50 yeast cells per pair of kidneys. n, number of mice per group; *, the mean was statistically significantly less than that for the sham-treated control at $P \leq 0.05$ according to Fisher's least-significant-difference t test.

[d] ED_{90}s were estimated by comparison of mean $\log_{10}$ CFU per gram at day 7 after challenge for paired kidneys from the treated groups to the values for paired kidneys from sham-treated controls. Values in parentheses are 95% confidence intervals.

[e] NT, not tested.

[f] NE, effective doses were not estimated due to the lack of a significant dose-response relationship over the range of doses tested.

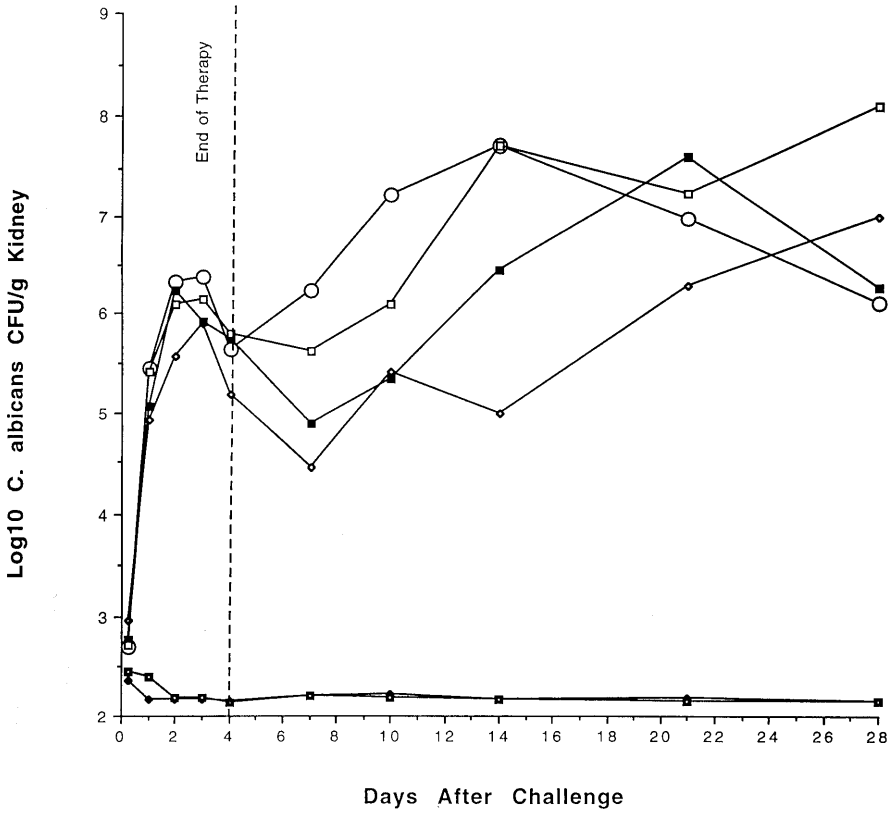

FIG. 2. Efficacy of MK-0991 against a disseminated *C. albicans* MY1055 infection in DBA/2N mice (full TOKA). Mice were infected with 7.4×10^4 CFU/mouse. Therapy was initiated within 30 min after challenge, and mice were treated i.p. b.i.d. for 4 days (total of eight doses). Data are for five mice per time point. □, 0.001 mg/kg; ◇, 0.02 mg/kg; ■, 0.005 mg/kg; ○, sham treatment; ◘, 0.09 mg/kg; ◆, 0.375 mg/kg.

Although the pneumocandins do not give MICs for *Aspergillus* species when tested by the classic broth microdilution assays (8, 33), this class of compounds has been shown to have profound morphological effects, in vitro; these are attributed to inhibition of 1,3-β-D-glucan synthesis (26, 27). These measurable morphological effects, termed the minimal effective concentration, appear to correlate well with the potent activity of L-733560 in animal models of *A. fumigatus* infection (1, 3, 10). MK-0991 was highly effective in our model of disseminated aspergillosis in mice (2) and in another model of pulmonary aspergillosis in rats (9).

It has been reported (17, 20) that MK-0991 has good in vitro activity against *Histoplasma capsulatum*, and it has also been reported that MK-0991 has in vivo efficacy against *H. capsulatum* in a murine model of histoplasmosis (31). The reported in vitro activity of MK-0991 against other dimorphic fungi and the opportunistic molds showed considerable species and strain variability (13, 15, 17).

Although MK-0991 exhibited measurable in vitro activity (MICs, 16 to 32 μg/ml) against clinical isolates of *C. neoformans* (6, 7, 17, 20), this activity did not yield efficacy in our in vivo models. However, recent in vitro studies indicate that MK-0991 may enhance the efficacies of fluconazole and AmB against *C. neoformans* (21).

MK-0991 administered both parenterally and p.o. has been shown to exhibit potent in vivo activity against *P. carinii* cysts in an immunocompromised rat model (34). Other echinocandins have also been shown to have anti-*Pneumocystis* activity (5, 14, 36, 37).

Comparative pharmacokinetics of MK-0991 in mice, rats, rhesus monkeys, and chimpanzees have shown that it has good bioavailability when it is administered parenterally (22, 23). MK-0991 had half-lives in plasma of 5.2 and 7.6 h and a high level of distribution in tissue (22, 23). MK-0991 has been shown to have a low bioavailability when it is administered p.o. (22), which correlates with the greatly reduced efficacy when it was administered p.o. in our murine models.

The potent activity of MK-0991 against clinically relevant fungal species combined with its good aqueous solubility, favorable bioavailability when administered parenterally, and acceptable therapeutic index has led Merck & Co. to initiate safety and clinical efficacy studies in humans.

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