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In Vitro Activity of a New Semisynthetic Echinocandin, LY-303366, against Systemic Isolates of *Candida* Species, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and *Aspergillus* Species

GEORGE G. ZHANEL,[1,2,3]* JAMES A. KARLOWSKY,[1,2,4] GARY A. J. HARDING,[4] TAMARA V. BALKO,[1,4] SHERYL A. ZELENITSKY,[2] MARK FRIESEN,[2] AMIN KABANI,[1,3,4] MICHAEL TURIK,[5] AND DARYL J. HOBAN[1,4]

Department of Medical Microbiology[1] and Faculty of Pharmacy,[2] University of Manitoba, and Departments of Clinical Microbiology[4] and Medicine,[3] Health Sciences Centre, Winnipeg, Manitoba, Canada, and Lilly Research Laboratories, Indianapolis, Indiana[5]

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The in vitro activities of LY-303366, a new semisynthetic echinocandin, and comparators amphotericin B, 5-fluorocytosine, fluconazole, and ketoconazole against 205 systemic isolates of *Candida* species, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and *Aspergillus* species were determined. LY-303366 had MICs of ≤0.32 μg/ml for all *Candida albicans* ($n = 99$), *Candida glabrata* ($n = 18$), and *Candida tropicalis* ($n = 10$) isolates tested. LY-303366 was also active against *Aspergillus* species (minimum effective concentration at which 90% of the isolates are inhibited, 0.02 μg/ml) ($n = 20$), was less active against *Candida parapsilosis* (MIC at which 90% of the isolates are inhibited [MIC_{90}], 5.12 μg/ml) ($n = 10$), and was inactive against *C. neoformans* (MIC_{90}, >10.24 μg/ml) ($n = 15$) and *B. dermatitidis* (MIC_{90}, 16 μg/ml) ($n = 29$).

The incidence of systemic fungal infections has increased dramatically over the past 20 years (1, 5). Only a limited number of antifungal agents are currently available to treat these infections. Polyenes and azoles, although useful, possess certain limitations. Amphotericin B is often effective but is a parenteral agent with significant toxicity (5). Azoles demonstrate a limited spectrum of activity, and isolates resistant to their action are increasingly being reported (5, 15). The development of new antifungal agents, preferably with novel mechanisms of action, that address these deficiencies is therefore imperative.

Echinocandins are lipopeptide antifungal agents that are potent noncompetitive inhibitors of (1,3)-β-D-glucan synthase, an enzyme essential to the structural integrity of the fungal cell wall (3, 6). LY-303366 is a synthetic derivative of echinocandin B, a naturally occurring lipopeptide, that possesses both increased lipophilicity and antifungal activity compared with its parent compound (3, 6). In this report, we assessed the in vitro activity of LY-303366 and comparators amphotericin B, flucytosine (5FC), fluconazole, and ketoconazole against systemic isolates of *Candida* species, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and *Aspergillus* species.

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LY-303366 was supplied by Eli Lilly (Indianapolis, Ind.), amphotericin B was supplied by Bristol-Myers Squibb (Saint-Laurent, Canada), 5FC was supplied by Hoffman-La Roche (Mississauga, Canada), fluconazole was supplied by Pfizer Canada (Kirkland, Canada), and ketoconazole was supplied by Janssen/Ortho (North York, Canada). Stock solutions of LY-303366, amphotericin B, fluconazole, and ketoconazole were prepared in dimethyl sulfoxide, and stock solutions of 5FC were prepared in water. The MIC doubling dilution ranges tested were 0.0006 to 10.24 μg/ml (*Candida* species, *C. neoformans*, and *Aspergillus*) and 1 to 64 μg/ml (*B. dermatitidis*) for LY-303366, 0.0313 to 16 μg/ml for amphotericin B, 0.0156 to 64 μg/ml for 5FC, 0.0625 to 128 μg/ml for fluconazole, and 0.0039 to 16 μg/ml for ketoconazole.

A total of 205 fungal isolates were tested. All isolates were obtained from blood cultures, cerebrospinal fluids, or bronchoscopy fluids of patients at the Health Sciences Centre in Winnipeg, Canada, between 1987 and 1996. The collection contained 99 *Candida albicans*, 18 *Candida glabrata*, 10 *Candida tropicalis*, 10 *Candida parapsilosis*, 2 *Candida krusei*, 2 *Candida lusitaniae*, 15 *C. neoformans*, 29 *B. dermatitidis*, 5 *Aspergillus flavus*, 6 *Aspergillus fumigatus*, 3 *Aspergillus glaucus* group, 4 *Aspergillus niger*, and 2 *Aspergillus versicolor* isolates.

Candida species, *C. neoformans*, and *B. dermatitidis* isolates were subcultured onto Sabouraud agar, and *Aspergillus* species were cultured onto Sabouraud dextrose agar (SDA) prior to antifungal susceptibility testing. *Candida* species and *C. neoformans* antifungal MICs were determined by the National Committee for Clinical Laboratory Standards M27-T macrodilution reference method (11). Susceptibility testing of *B. dermatitidis* (yeast phase) and *Aspergillus* species was performed by the same method. *Aspergillus* species suspensions were prepared from mature cultures grown on SDA at 30°C. SDA slants were flooded with 1 ml of sterile water and gently rocked. The homogeneous supernatant containing *Aspergillus* conidia was removed and adjusted spectrophotometrically to 82 to 85% transmission at 530 nm. The suspension was then diluted in RPMI 1640 broth to yield a final inoculum of 0.5×10^3 to 2.5×10^3 CFU/ml. *C. albicans* ATCC 90028, *C. neoformans* ATCC 90112, and *C. glabrata* ATCC 90030 were simultaneously tested with clinical isolates as quality control organisms (11).

MIC endpoints were determined for amphotericin B, 5FC, fluconazole, and ketoconazole as recommended by the National Committee for Clinical Laboratory Standards M27-T reference method (11). LY-303366 MICs were defined as the

* Corresponding author. Mailing address: Health Sciences Centre, MS673-820 Sherbrook St., Winnipeg, Manitoba R3A 1R9, CANADA. Phone: (204) 787-4902. Fax: (204) 787-4699. E-mail: ggzhanel@pcs.mb.ca.

TABLE 1. Activity of LY-303366, amphotericin B, 5FC fluconazole, and ketoconazole against *Candida* species

Candida species (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
C. albicans (99)	LY-303366	≤0.005–0.16	0.02	0.08
	Amphotericin B	≤0.0313–1	0.50	1
	5FC	≤0.0156–4	0.125	0.5
	Fluconazole	≤0.0625–2	0.25	1
	Ketoconazole	≤0.0039–2	≤0.0039	0.0625
C. glabrata (18)	LY-303366	0.04–0.32	0.16	0.32
	Amphotericin B	0.0625–2	0.5	2
	5FC	≤0.0156–0.5	0.0625	0.125
	Fluconazole	2–32	4	32
	Ketoconazole	≤0.0039–2	0.0625	1
C. tropicalis (10)	LY-303366	0.08–0.32	0.16	0.32
	Amphotericin B	0.25–2	1	1
	5FC	0.125–8	0.5	0.5
	Fluconazole	0.125–>128	1	128
	Ketoconazole	≤0.0039–4	≤0.0039	4
C. parapsilosis (10)	LY-303366	1.28–5.12	2.56	5.12
	Amphotericin B	0.25–1	0.5	1
	5FC	0.0313–0.125	0.125	0.125
	Fluconazole	0.25–4	0.5	2
	Ketoconazole	≤0.0039–0.0078	≤0.0039	≤0.0039

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

TABLE 2. Activity of LY-303366, amphotericin B, 5FC, fluconazole, and ketoconazole against *C. neoformans*, *B. dermatitidis*, and *Aspergillus* species

Fungus species (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
C. neoformans (15)	LY-303366	>10.24	>10.24	>10.24
	Amphotericin B	≤0.0313–0.25	0.125	0.25
	5FC	≤0.0156–16	2	8
	Fluconazole	1–4	4	4
	Ketoconazole	≤0.0039–0.5	0.0156	0.25
B. dermatitidis (29)	LY-303366	4–64	8	16
	Amphotericin B	≤0.0313–0.25	0.0625	0.25
	5FC	64	>64	>64
	Fluconazole	2–32	8	16
	Ketoconazole	≤0.0039–0.25	0.0156	0.125
Aspergillus species (20)[b]	LY-303366	0.00125–10.24	0.005/5.12[c]	0.02/10.24[c]
	Amphotericin B	0.5–4	1	2
	5FC	1–>64	4	16
	Fluconazole	16–>128	>128	>128
	Ketoconazole	≤0.0156–4	1	2

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[b] Five *A. flavus*, six *A. fumigatus*, three *A. glaucus* group, four *A. niger*, and *A. versicolor* isolates.
[c] MEC/MIC.

lowest concentration of LY-303366 that inhibited 100% of visible growth. MICs for *Candida* species were read after 48 h of incubation at 35°C, MICs for *C. neoformans* were read after 72 h of incubation at 35°C, MICs for *B. dermatitidis* were read after 96 to 120 h of incubation at 30°C, and MICs and minimum effective concentrations (MECs) for *Aspergillus* species were read after 48 h of incubation at 30°C. *Aspergillus* species demonstrate two distinct endpoints when their echinocandin or pneumocandin susceptibilities are tested (9). The first endpoint, the MEC, represents an abrupt transition from test tubes containing a hyphal mass to test tubes containing small, distinct spherical colonies (9). The second endpoint, the MIC, is found at higher echinocandin and pneumocandin concentrations and is the concentration that prevents visible colony growth (9). The MEC has been demonstrated to most closely correlate with the efficacy of lipopeptides such as LY-303366 in animal models of disseminated aspergillosis (9). Colony counts to confirm initial inocula were performed for each MIC determination except for those for *Aspergillus* species.

The MICs of LY-303366 were ≤0.32 μg/ml for all *C. albicans*, *C. glabrata*, and *C. tropicalis* isolates tested (Table 1). LY-303366 was as active as ketoconazole and was more active than amphotericin B, 5FC, and fluconazole against *C. albicans* (Table 1). LY-303366 was approximately as active as 5FC and was more active than amphotericin B, fluconazole, and ketoconazole against *C. glabrata* and *C. tropicalis* and less active than its four comparators against *C. parapsilosis* (Table 1).

Two strains each of *C. krusei* and *C. lusitaniae* were also tested. The MICs for the two strains of *C. krusei*, respectively, were as follows: LY-303366, 0.25 and 1.28 μg/ml; amphotericin B, 2 μg/ml; 5FC, 32 and 16 μg/ml; fluconazole, 16 and 32 μg/ml; and ketoconazole, 0.0625 and 0.5 μg/ml. The MICs for the two strains of *C. lusitaniae* were as follows: LY-303366, 0.64 μg/ml; amphotericin B, 0.25 and 0.5 μg/ml, respectively; 5FC, ≤0.0156 μg/ml; fluconazole, 0.25 and 2 μg/ml, respectively; and ketoconazole, ≤0.0039.

The MICs of fluconazole for three strains of *C. tropicalis*, seven strains of *C. glabrata*, and two strains of *Candida krusei* were ≥16 μg/ml. The MICs of LY-303366 for 11 of these 12 strains ranged from 0.08 to 0.32 μg/ml. The twelfth strain was a *C. krusei* strain (fluconazole MIC, 32 μg/ml) for which the LY-303366 MIC was 1.28 μg/ml. The MIC at which 90% of the isolates are inhibited (MIC_{90}) for these 12 strains was 0.32 μg/ml. The LY-303366 MIC_{90} for the remaining 18 *C. glabrata* isolates and *C. tropicalis* isolates for which the fluconazole MICs were ≤8 μg/ml was also 0.32 μg/ml. LY-303366 appeared equally active against *Candida* species for which the fluconazole MICs were ≥16 μg/ml and against those for which the fluconazole MICs were ≤8 μg/ml.

LY-303366 had significantly less activity against *C. neoformans* and *B. dermatitidis* than against *C. albicans*, *C. glabrata*, and *C. tropicalis* (Table 2). Ketoconazole and amphotericin B were the most active antifungal agents tested for both *C. neoformans* and *B. dermatitidis* (Table 2). LY-303366 demonstrated potent in vitro activity against *Aspergillus* species with a MEC_{90} of 0.02 μg/ml (Table 2). Ranges for each *Aspergillus* species are presented in Table 3. MICs of LY-303366 for the control strain yeast isolates were 0.02 μg/ml for *C. albicans*

TABLE 3. MIC ranges of LY-303366, amphotericin B, 5FC, fluconazole, and ketoconazole for individual *Aspergillus* species

Aspergillus species (no. of isolates)	MIC/MEC (μg/ml) of LY-303366	MIC (μg/ml) range of:			
		Amphotericin B	5FC	Fluconazole	Ketoconazole
A. fumigatus (6)	0.00125/10.24	1–2	2–>64	64–>128	1–4
A. flavus (5)	0.005/10.24	0.5–4	4–>64	16–>128	0.125–2
A. niger (4)	NP[a]/0.01	1–2	1–32	16–>128	≤0.0156–2
A. glaucus group (3)	0.0025/10.24	1–2	1–8	64–>128	0.5–2
A. versicolor (2)	NP/0.005	1	8–16	>128	1

[a] NP, not present.

ATCC 90028, 0.16 μg/ml for *C. glabrata* ATCC 90030, and >10.24 μg/ml for *C. neoformans* ATCC 90112.

LY-303366 has demonstrated potent in vitro (12–14) and in vivo (2, 16–18) activity against *Candida* species, *Aspergillus* species, and *Pneumocystis carinii*. In vitro studies have demonstrated that LY-303366 is as active as amphotericin B and is more active than 5FC and fluconazole against most *Candida* species, with less activity against *C. parapsilosis* and *C. lusitaniae* (12–14). Our work demonstrated similar results (Table 1). We found that LY-303366 possessed activity against *C. albicans*, *C. glabrata*, and *C. tropicalis*, including strains for which the fluconazole MICs were ≥16 μg/ml, at concentrations lower than those achievable in human serum (10). A recent publication reported that a 500-mg dose of LY-303366 taken orally was safe and produced maximum peak concentrations in serum of approximately 0.75 μg/ml with a serum half-life of 30 h (10). Others have also demonstrated potent in vitro LY-303366 activity against *Candida* species for which there are elevated fluconazole (≥128 μg/ml) and itraconazole (≥2 μg/ml) MICs (12). Similarly, two strains of *C. albicans*, one for which the fluconazole MIC was ≥128 μg/ml and the other for which the fluconazole MIC was 0.5 μg/ml, were demonstrated to possess similar LY-303366 killing kinetics and susceptibilities (4). LY-303366 was less active against *C. parapsilosis*, *C. lusitaniae*, and one of two strains of *C. krusei*. Previously, cilofungin (LY-121019), another echinocandin B derivative, was shown to be active against several *Candida* species but inactive against *C. parapsilosis* (8).

LY-303366 was not active in vitro against *C. neoformans* or *B. dermatitidis* (Table 2). The lack of activity against *C. neoformans* (7, 13, 14) and *B. dermatitidis* (7) has been reported previously and may arise because of greater cellular reliance upon α-(1-3)-D-glucan linkages and not β-(1,3)-D-glucan linkages in cell wall glucan polymers of these fungi (3).

Echinocandin and pneumocandin MECs have been demonstrated to correlate well with in vivo activity (9). LY-303366 demonstrated potent activity against *Aspergillus* species, for which the MEC_{90} was 0.02 μg/ml. This result is in agreement with previously published work reporting a MEC of 0.04 μg/ml for a collection of *Aspergillus* species (13).

We conclude that LY-303366 has promising antifungal activity for *C. albicans*, *C. glabrata*, *C. tropicalis*, and *Aspergillus* species. LY-303366 was less active against *C. parapsilosis*, *C. lusitaniae*, and *C. krusei* and was inactive against *B. dermatitidis* and *C. neoformans*. Further in vitro and in vivo investigation of LY-303366 is warranted.

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