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In Vitro Activity of a New Echinocandin, LY303366, Compared with Those of Amphotericin B and Fluconazole against Clinical Yeast Isolates

ÖMRÜM UZUN,[1]* SESIN KOCAGÖZ,[1] YEŞIM ÇETINKAYA,[1] SEVTAP ARIKAN,[2] AND SERHAT ÜNAL[1]

Section of Infectious Diseases, Department of Medicine,[1] and Department of Microbiology and Clinical Microbiology,[2] Hacettepe University School of Medicine, Ankara 06100, Turkey

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The in vitro activity of LY303366, a new echinocandin derivative, was evaluated with 191 yeast isolates by a broth microdilution method. The MICs at which 50% of the isolates were inhibited were 0.125 μg/ml for *Candida albicans* and *C. tropicalis*, 0.25 μg/ml for *C. krusei*, *C. kefyr*, and *C. glabrata*, and 2.0 μg/ml for *C. parapsilosis*.

Opportunistic fungal infections have become an important cause of morbidity and mortality in patients with immunosuppression (3, 5). Amphotericin B has been the mainstay of treatment for several decades; however, its use has been limited due to its side effects, particularly nephrotoxicity. Fluconazole has been shown to be effective against candidiasis (4, 10). But its widespread use has been accompanied by concerns for resistance (1, 6, 11). Therefore, newer compounds that target the cell wall of fungi, specifically echinocandins which inhibit 1,3-beta-D-glucan synthase activity, may be promising against a wide variety of pathogens, including *Candida* species and other yeasts. In this study, we evaluated the in vitro activity of LY303366, a new semisynthetic echinocandin B analog, against our yeast isolates.

The test organisms included 10 *Candida albicans* American Type Culture Collection (ATCC) strains (strains ATCC 64544 through ATCC 64552 and ATCC 90028), *Candida parapsilosis* ATCC 90018 and ATCC 22019, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, and 191 isolates from 186 patients, mostly with oropharyngeal candidiasis, who were treated at Hacettepe University Hospital over a 3-year period (1993 to 1995). The germ tube test was performed, and germ tube-negative strains were identified by the API 20C AUX system (bioMérieux API System, Montalieu Vercieu, France). The distribution of yeasts was as follows: 131 *C. albicans* strains, 26 *C. krusei* strains, 14 *Candida kefyr* strains, 8 *C. glabrata* strains, 5 *Candida tropicalis* strains, 3 *C. parapsilosis* strains, 1 *Candida famata* strain, and 3 *Trichosporon beigelii* strains. Two reference strains, *C. albicans* ATCC 64550 and *C. albicans* ATCC 64552, were included in each run of experiments. Isolates were maintained in glycerol stocks at −70°C; they were subsequently subcultured on Sabouraud dextrose agar plates and incubated at 35°C. Twenty-four-hour growth was used in all experiments.

LY303366 was obtained as a powder from Lilly Research Laboratories, Indianapolis, Ind. Amphotericin B and fluconazole were obtained from their respective manufacturers. Fluconazole was dissolved in sterile distilled water to obtain a stock solution with a concentration of 5,120 μg/ml, amphotericin B and LY303366 were dissolved in dimethyl sulfoxide to obtain stock solutions with a concentration of 1,280 μg/ml, and all three solutions were stored at −70°C.

The MICs of all antifungal agents were determined in accordance with the National Committee for Clinical Laboratory Standards M27-T standards by a microdilution method (8). Briefly, RPMI 1640 with L-glutamine and without sodium bicarbonate (Sigma Chemical Co., St. Louis, Mo.), buffered at pH 7 with 0.165 M morpholinepropanesulfonic acid (MOPS), was used. The final drug concentrations ranged from 16 to 0.03 μg/ml for LY303366 and amphotericin B and from 64 to 0.125 μg/ml for fluconazole and were obtained by 10 twofold serial dilutions. The yeast suspensions were spectrophotometrically prepared and diluted to obtain a final inoculum of 0.5×10^3 to 2.5×10^3 CFU/ml. All organisms were tested in duplicate in each run of the experiments.

The microtiter plates were incubated at 35°C, and MICs were assessed at 24 and 48 h visually by three separate investigators. Fluconazole plates were read after agitation as previously described (2). The MICs of LY303366 and amphotericin B were defined as the lowest concentrations at which no growth was observed, and for fluconazole, the MIC was defined as the lowest concentration at which a prominent decrease in turbidity was observed.

LY303366 had the lowest MICs at which 50% of the isolates were inhibited (MIC_{50}s) and MIC_{90}s against the *C. albicans*, *C. krusei*, *C. kefyr*, and *C. tropicalis* strains tested (Table 1). For *C. glabrata* isolates, the MIC_{50} of LY303366 was 0.25 μg/ml, whereas it was 0.5 μg/ml for amphotericin B and 4.0 μg/ml for fluconazole. There were three isolates of *C. parapsilosis*, and MICs of LY303366 for these isolates (2.0 μg/ml for two isolates and 4.0 μg/ml for one isolate) as well as for *C. parapsilosis* ATCC 90018 and ATCC 22019 (2.0 μg/ml) were higher than those for other *Candida* strains. The MICs obtained for the ATCC strains are shown in Table 2. For *C. albicans* ATCC 64550, the MIC range of fluconazole was 16 to 32 μg/ml, the MIC range of amphotericin B was 0.5 to 1.0 μg/ml, and the MIC range of LY303366 was 0.06 to 0.125 μg/ml. For *C. albicans* ATCC 64552 fluconazole had an MIC range of 0.125 to 0.25 μg/ml, amphotericin B had an MIC range of 2.0 to 4.0 μg/ml, and LY303366 had an MIC range of 0.125 to 0.25 μg/ml in all the sets of experiments. LY303366 had no in vitro activity against the *T. beigelii* strains tested; for all three *T. beigelii* isolates, the MICs of LY303366 were >16, with significant turbidity in all wells. The 48-h MIC readings were comparable to the 24-h MIC readings for all the antifungal agents and

* Corresponding author. Mailing address: Section of Infectious Diseases, Department of Medicine, Hacettepe University School of Medicine, Ankara 06100, Turkey. Phone: 90 (312) 311-1271. Fax: 90 (312) 310-4179. E-mail: ou01-k@servis2.net.tr.

TABLE 1. In vitro susceptibilities of yeasts to LY303366, amphotericin B, and fluconazole

Organism (no. of strains)	Antifungal agent	MIC (μg/ml)[a]		
		Range	50%	90%
C. albicans (131)	LY303366	≤0.03–0.5	0.125	0.25
	Amphotericin B	0.25–2.0	0.5	1.0
	Fluconazole	0.125–4.0	0.25	1.0
C. krusei (26)	LY303366	0.125–0.5	0.25	0.25
	Amphotericin B	0.25–2.0	1.0	1.0
	Fluconazole	8.0–32	16	32
C. kefyr (14)	LY303366	0.06–0.5	0.25	0.5
	Amphotericin B	0.5–2.0	1.0	1.0
	Fluconazole	0.25–2.0	1.0	1.0
C. glabrata (8)	LY303366	0.25	0.25	
	Amphotericin B	0.5–1.0	0.5	
	Fluconazole	2.0–32	4.0	
C. tropicalis (5)	LY303366	0.06–0.125	0.125	
	Amphotericin B	0.25–1.0	0.5	
	Fluconazole	0.125–1.0	0.5	
C. parapsilosis (3)	LY303366	2.0–4.0	2.0	
	Amphotericin B	0.25–0.5	0.25	
	Fluconazole	0.25–0.5	0.25	
C. famata (1)	LY303366	4.0		
	Amphotericin B	1.0		
	Fluconazole	4.0		
T. beigelii (3)	LY303366	>16	>16	
	Amphotericin B	0.5–1.0	1.0	
	Fluconazole	0.5–4.0	2.0	

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

strains tested (data not shown); the changes in MICs were within twofold for all strains.

The results of this study show that LY303366 possesses potent in vitro activity against a variety of *Candida* species, including *C. albicans*, *C. krusei*, *C. kefyr*, *C. tropicalis*, and *C. glabrata*. The most exciting observation was the good in vitro activity of this compound against *C. krusei*, which is resistant to fluconazole, and *C. glabrata*, for which fluconazole has higher MICs than for other non-*C. albicans Candida* strains. None of our *C. albicans* isolates were resistant to fluconazole; however, Pfaller et al. reported that LY303366 inhibited all isolates with elevated fluconazole MICs (≥128 μg/ml) at a concentration of ≤0.5 μg/ml when tested in RPMI (9). This excellent in vitro activity against azole-resistant *Candida* strains may have important implications for the management of difficult-to-treat *Candida* infections.

TABLE 2. MICs for ATCC strains of LY303366, amphotericin B, and fluconazole

Strain	MIC (μg/ml) of:		
	Fluconazole	Amphotericin B	LY303366
C. albicans ATCC 64544	1.0	0.5	0.125
C. albicans ATCC 64545	1.0	0.5	0.125
C. albicans ATCC 64546	1.0	0.5	0.125
C. albicans ATCC 64547	1.0	0.5	0.125
C. albicans ATCC 64548	1.0	0.25	0.06
C. albicans ATCC 64549	1.0	0.5	0.125
C. albicans ATCC 64550	16	0.5	0.06
C. albicans ATCC 64551	0.25	0.5	0.125
C. albicans ATCC 64552	0.125	2.0	0.25
C. albicans ATCC 90028	0.25	0.5	0.125
C. parapsilosis ATCC 90018	0.5	0.5	2.0
C. glabrata ATCC 90030	4.0	0.5	0.25
C. krusei ATCC 6258	32	1.0	0.125
C. parapsilosis ATCC 22019	2.0	0.5	2.0

The MICs for the three clinical isolates of *C. parapsilosis* were relatively high. Because of the very small number tested, this could be interpreted as a strain-specific result. However, LY303366 was reported to possess the least activity against *C. parapsilosis* of any antifungal agent tested by other investigators as well (7, 9). On the other hand, LY303366 had no activity against three *T. beigelii* strains. This finding requires further investigation.

In summary, LY303366 is a promising antifungal drug which exhibits good in vitro activity against not only *Candida* species known to be usually susceptible to fluconazole, such as *C. albicans*, but also putatively fluconazole-resistant *C. krusei* and *C. glabrata*. Nevertheless, the true activity of this compound against various fungal pathogens will depend on its pharmacokinetic and pharmacodynamic properties and the toxicity profile in humans.

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