

In Vitro Susceptibilities of Clinical Yeast Isolates to a New Echinocandin Derivative, LY303366, and Other Antifungal Agents

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LY303366 is a new semisynthetic echinocandin derivative with potent, broad-spectrum fungicidal activity. We investigated the in vitro activity of LY303366, amphotericin B, flucytosine (5FC), fluconazole, and itraconazole against 435 clinical yeast isolates (413 *Candida* and 22 *Saccharomyces cerevisiae* isolates) obtained from over 30 different medical centers. MICs for all five antifungal agents were determined by the National Committee for Clinical Laboratory Standards method with RPMI 1640 test medium. LY303366 was also tested in antibiotic medium 3 as specified by the manufacturer. Overall, LY303366 was quite active against all of the yeast isolates when tested in RPMI 1640 (MIC at which 90% of the isolates are inhibited [MIC₉₀], 1.0 µg/ml) but appeared to be considerably more potent when tested in antibiotic medium 3 (MIC₉₀, 0.03 µg/ml). When tested in antibiotic medium 3, LY303366 was 16- to >2,000-fold more active than itraconazole, fluconazole, amphotericin B, or 5FC against all species except *Candida parapsilosis*. When tested in RPMI 1640, LY303366 was comparable to amphotericin B and itraconazole and more active than fluconazole and 5FC. All of the isolates for which fluconazole and itraconazole had elevated MICs (≥128 and ≥2.0 µg/ml, respectively) were inhibited by ≤0.007 µg of LY303366/ml when tested in antibiotic medium 3 and ≤0.5 µg/ml when tested in RPMI 1640. Based on these studies, LY303366 has promising antifungal activity and warrants further in vitro and in vivo investigation.

LY303366 is a new fungicidal agent that is a semisynthetic derivative of a natural product class of antifungal agents called echinocandins. The mechanism of action of LY303366 is thought to involve noncompetitive inhibition of (1,3)-β-D-glucan synthase resulting in cell wall damage and lysis (9). LY303366 has been shown to have potent in vitro and in vivo activity against *Candida* spp., *Aspergillus fumigatus*, and *Pneumocystis carinii* (1, 2, 7, 9, 11-13). Gordee et al. (2) reported in vitro activity against isolates of *Candida albicans*, *Candida krusei*, *Candida glabrata*, and *Candida parapsilosis* and found that LY303366 was active against clinical isolates of *C. albicans* that were resistant to fluconazole and other azoles. Zeckner et al. (11) found that LY303366 was superior to cilofungin, itraconazole, and fluconazole in a murine model of systemic candidiasis.

Because of the lack of comparative data for other antifungal agents and the limited number of clinical isolates of *Candida* spp. included in the previous studies, we compared the in vitro activity of LY303366 with that of other new and established antifungal agents against 435 clinical yeast isolates. The other antifungal agents tested included the triazoles itraconazole and fluconazole, as well as amphotericin B and flucytosine (5FC). The in vitro susceptibility testing method employed was the microdilution adaptation of the macrodilution reference method as described in National Committee for Clinical Laboratory Standards (NCCLS) document M27-T (4). Because LY303366 activity may vary with the in vitro test medium employed, we tested LY303366 in antibiotic medium 3 as well as the standard RPMI 1640 medium.

MATERIALS AND METHODS

Organisms. A total of 435 clinical yeast isolates were selected for testing. The collection included 413 isolates of *Candida* spp. (186 *C. albicans*, 67 *C. glabrata*, 58 *Candida tropicalis*, 28 *C. parapsilosis*, 36 *C. krusei*, 10 *Candida stellatoidea*, 12 *Candida lusitanae*, 9 *Candida guilliermondii*, and 7 *Candida rugosa* isolates) and 22 *Saccharomyces cerevisiae* isolates. The isolates were all recent clinical isolates contributed by more than 30 different medical centers. The majority (387 [89%]) were from blood or normally sterile body fluids. The isolates were identified by standard methods (10) and were stored as water suspensions at ambient temperature until used in the study. Prior to testing, each isolate was passaged at least twice on potato dextrose agar (Remel, Lenexa, Kans.) to ensure optimal growth characteristics.

Antifungal agents. LY303366 was obtained as a standard powder from Eli Lilly and Company (Indianapolis, Ind.). Amphotericin B, fluconazole, itraconazole, and 5FC were obtained from their respective manufacturers. Stock solutions were prepared in dimethyl sulfoxide (amphotericin B and LY303366), polyethylene glycol (itraconazole), or water (fluconazole and 5FC). Serial twofold dilutions of each antifungal agent were prepared exactly as outlined in reference 4. Final dilutions were made in RPMI 1640 medium (Sigma, St. Louis, Mo.) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma). The final concentration of the solvent did not exceed 1% in any well. In addition, a second dilution series of LY303366 was prepared in antibiotic medium 3 (Difco Laboratories, Detroit, Mich.) buffered to pH 7.0 with MOPS buffer. Aliquots (0.1 ml) of each antifungal agent at twice the final concentration were dispensed into the wells of plastic microdilution trays by using a Quick Spense II system (Dynatech Laboratories, Chantilly, Va.). The trays were sealed and frozen at -70°C until used in the study.

Antifungal susceptibility studies. Broth microdilution testing was performed according to NCCLS document M27-T (4) guidelines by the spectrophotometric method of inoculum preparation with an inoculum concentration of (1.5 ± 1.0) × 10³ cells/ml and RPMI 1640 (or antibiotic medium 3) buffered to pH 7.0 with MOPS. Yeast inocula (100 µl) were added to each well of the microdilution trays. The final concentrations of antifungal agents were 0.015 to 8.0 µg/ml for amphotericin B, 0.001 to 2.0 µg/ml for LY303366, 0.12 to 128 µg/ml for fluconazole, 0.007 to 8.0 µg/ml for itraconazole, and 0.06 to 128 µg/ml for 5FC. The trays were incubated in air at 35°C, and MIC endpoints were read after 48 h of incubation. Drug-free and yeast-free controls were included.

Following incubation, the broth microdilution wells were read with the aid of a reading mirror; the growth in each well was compared with that of the growth control (drug-free) well. The MIC of LY303366 and amphotericin B was defined as the lowest concentration of antifungal agent which prevented visible growth, as determined by comparison to the drug-free controls. MICs of itraconazole, fluconazole, and 5FC were defined as the lowest concentrations resulting in 80%

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TABLE 1. In vitro susceptibilities of 435 clinical yeast isolates to LY303366 and other antifungal agents

Organism (<i>n</i>)	Antifungal agent	Test medium	MIC ($\mu\text{g/ml}$)		
			Range	50%	90%
<i>C. albicans</i> (186)	LY303366	Antibiotic medium 3	0.001->2.0	0.001	0.003
	LY303366	RPMI 1640	0.015->2.0	0.12	0.5
	Itraconazole	RPMI 1640	0.015->8.0	0.03	0.25
	Fluconazole	RPMI 1640	0.12->128	0.25	2.0
	Amphotericin B	RPMI 1640	0.25-1.0	0.5	1.0
	5FC	RPMI 1640	0.06->128	0.25	4.0
<i>C. glabrata</i> (67)	LY303366	Antibiotic medium 3	0.001-0.5	0.007	0.007
	LY303366	RPMI 1640	0.12->2.0	0.25	0.5
	Itraconazole	RPMI 1640	0.015->8.0	0.5	2.0
	Fluconazole	RPMI 1640	0.25->128	8.0	128
	Amphotericin B	RPMI 1640	0.5-1.0	1.0	1.0
	5FC	RPMI 1640	0.06-1.0	0.06	0.12
<i>C. tropicalis</i> (58)	LY303366	Antibiotic medium 3	0.001-0.25	0.003	0.003
	LY303366	RPMI 1640	0.06-2.0	0.25	0.5
	Itraconazole	RPMI 1640	0.015->8.0	0.06	0.12
	Fluconazole	RPMI 1640	0.12->128	0.5	1.0
	Amphotericin B	RPMI 1640	0.25-1.0	1.0	1.0
	5FC	RPMI 1640	0.06-1.0	0.25	1.0
<i>C. parapsilosis</i> (28)	LY303366	Antibiotic medium 3	0.001-2.0	0.25	2.0
	LY303366	RPMI 1640	0.12->2.0	2.0	4.0
	Itraconazole	RPMI 1640	0.015-0.25	0.12	0.12
	Fluconazole	RPMI 1640	0.25-2.0	0.5	1.0
	Amphotericin B	RPMI 1640	0.5-1.0	1.0	1.0
	5FC	RPMI 1640	0.06-2.0	0.12	0.25
<i>C. krusei</i> (36)	LY303366	Antibiotic medium 3	0.001-0.015	0.007	0.015
	LY303366	RPMI 1640	0.12-1.0	0.25	0.5
	Itraconazole	RPMI 1640	0.03-1.0	0.5	0.5
	Fluconazole	RPMI 1640	0.25-128	32	64
	Amphotericin B	RPMI 1640	0.5-1.0	1.0	1.0
	5FC	RPMI 1640	1.0-32	16	16
<i>C. stellatoidea</i> (10)	LY303366	Antibiotic medium 3	0.001-0.004	0.001	0.003
	LY303366	RPMI 1640	0.12-0.5	0.12	0.5
	Itraconazole	RPMI 1640	0.015-0.12	0.015	0.03
	Fluconazole	RPMI 1640	0.25-0.5	0.25	0.5
	Amphotericin B	RPMI 1640	0.25-1.0	0.5	0.5
	5FC	RPMI 1640	0.06-0.5	0.25	0.5
<i>C. lusitanae</i> (12)	LY303366	Antibiotic medium 3	0.001-0.007	0.004	0.007
	LY303366	RPMI 1640	0.03-2.0	0.5	2.0
	Itraconazole	RPMI 1640	0.007-0.25	0.12	0.25
	Fluconazole	RPMI 1640	0.12-4.0	1.0	4.0
	Amphotericin B	RPMI 1640	0.5-2.0	1.0	1.0
	5FC	RPMI 1640	0.06-128	0.06	128
<i>C. guilliermondii</i> (9)	LY303366	Antibiotic medium 3	0.12-4.0	0.5	
	LY303366	RPMI 1640	1.0-4.0	4.0	
	Itraconazole	RPMI 1640	0.12-1.0	0.5	
	Fluconazole	RPMI 1640	2.0-64	4.0	
	Amphotericin B	RPMI 1640	0.25-1.0	0.5	
	5FC	RPMI 1640	0.06-0.25	0.12	
<i>C. rugosa</i> (7)	LY303366	Antibiotic medium 3	0.007-0.15	0.007	
	LY303366	RPMI 1640	1.0-4.0	4.0	
	Itraconazole	RPMI 1640	0.03-0.12	0.03	
	Fluconazole	RPMI 1640	1.0-8.0	1.0	
	Amphotericin B	RPMI 1640	0.5-1.0	1.0	
	5FC	RPMI 1640	0.12-1.0	0.5	
<i>S. cerevisiae</i> (22)	LY303366	Antibiotic medium 3	0.007-0.06	0.03	0.03
	LY303366	RPMI 1640	0.25-1.0	0.5	1.0
	Itraconazole	RPMI 1640	0.03-0.5	0.5	0.5
	Fluconazole	RPMI 1640	0.5-16	2.0	16
	Amphotericin B	RPMI 1640	0.5-1.0	1.0	1.0
	5FC	RPMI 1640	0.06-0.12	0.06	0.12
All organisms (435)	LY303366	Antibiotic medium 3	0.001->2.0	0.003	0.03
	LY303366	RPMI 1640	0.015->2.0	0.25	1.0
	Itraconazole	RPMI 1640	0.007->8.0	0.12	0.5
	Fluconazole	RPMI 1640	0.12->128	0.5	32
	Amphotericin B	RPMI 1640	0.25-2.0	1.0	1.0
	5FC	RPMI 1640	0.06->128	0.12	16

inhibition of growth compared to that of untreated controls (4). The data are reported as the MICs of each antifungal agent necessary to inhibit 50% (MIC₅₀) and 90% (MIC₉₀) of the isolates tested.

Quality control. Quality control was ensured by testing the following strains recommended by NCCLS document M27-T (4): *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 (6, 8).

RESULTS AND DISCUSSION

Table 1 summarizes the in vitro susceptibilities of the 435 yeast isolates to LY303366, itraconazole, fluconazole, amphotericin B, and 5FC as determined by broth microdilution testing performed according to NCCLS guidelines. A broad range of MICs was observed with each antifungal agent for the various species tested.

Overall, LY303366 was active against clinical isolates of *Candida* spp. and *S. cerevisiae* when tested in RPMI 1640 (MIC₉₀, 1.0 µg/ml) but appeared to be considerably more potent when tested in antibiotic medium 3 (MIC₉₀, 0.03 µg/ml). LY303366 was most active in antibiotic medium 3 against *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. stellatoidea*, and *C. lusitanae* (MIC₉₀, ≤0.007 µg/ml) and least active against *C. parapsilosis* (MIC₉₀, 2.0 µg/ml). When tested in antibiotic medium 3, LY303366 was 16- to >2,000-fold more active than itraconazole, fluconazole, amphotericin B, and 5FC against all species except *C. parapsilosis*. LY303366 was more active in RPMI 1640 than fluconazole and 5FC against *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. lusitanae* but was less active than these two agents against *C. parapsilosis* and *C. rugosa*. LY303366 was less active than itraconazole against *C. parapsilosis*, *C. lusitanae*, *C. guilliermondii*, and *C. rugosa* and was also less active than 5FC against *C. glabrata*, *C. guilliermondii*, and *S. cerevisiae* when tested in RPMI 1640. All 23 of the isolates for which fluconazole and itraconazole had elevated MICs (≥128 and ≥2.0 µg/ml, respectively) were inhibited by ≤0.007 µg of LY303366/ml when tested in antibiotic medium 3 and ≤0.5 µg/ml when tested in RPMI 1640 (data not shown).

These results support the findings reported previously by Gordee et al. (2). We have shown that susceptibility testing performed according to NCCLS guidelines is an acceptable means of evaluating the in vitro activity of LY303366; however, it is notable that this agent appears to be considerably more potent when tested in antibiotic medium 3. A similar effect was observed with an earlier echinocandin derivative, cilofungin (3). As with cilofungin, it is assumed that the higher salt content of antibiotic medium 3 promotes lysis of LY303366-treated yeast cells that are depleted of (1,3)-β-D-glucan. Whether the lower MIC observed in antibiotic medium 3 is more, or less, representative of activity in vivo remains to be seen and will require additional in vivo and clinical studies.

In agreement with Gordee et al. (2), we found LY303366 to be very active against most species of *Candida* and against *S. cerevisiae*. Although LY303366 was least active against *C. parapsilosis*, the MICs observed in either antibiotic medium 3 (MIC₉₀, 2.0 µg/ml) or RPMI 1640 (MIC₉₀, 4.0 µg/ml) were considerably lower than those observed earlier for cilofungin (MIC₉₀, ≥40 µg/ml) (5). Again, determination of the clinical relevance of these values awaits further in vivo and pharmacokinetic studies (14).

Like Gordee et al. (2), we observed that LY303366 had very potent activity against isolates of *C. krusei* and *C. glabrata*, species usually considered refractory to azoles. The difference in potency between LY303366 and the triazoles is underscored by the extremely low MICs of LY303366 (≤0.007 µg/ml) ob-

served with isolates of *Candida* spp. for which fluconazole and itraconazole have elevated MICs.

The translation of this in vitro data into clinical efficacy is uncertain; however, LY303366 was shown by Zeckner et al. (11) to be more potent than fluconazole and itraconazole in a murine model of systemic candidiasis. Further assessment of the clinical significance of the relative potency of LY303366 and other antifungal agents awaits the generation of human pharmacokinetic data and the results of comparative clinical trials. Based on the available in vitro data, LY303366 is a promising new broad-spectrum antifungal agent that merits further investigation.

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REFERENCES

1. Current, W. L., C. J. Boylan, and P. P. Raab. 1993. Anti-*Pneumocystis* activity of LY303366 and other echinocandin B analogs, abstr. 368, p. 186. In Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
2. Gordee, R., J. Farmer, J. Flokowitsch, and P. Haeber. 1993. In vitro antifungal activity of LY303366, a new antifungal agent derived from echinocandin B, abstr. 360, p. 185. In Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
3. Gordee, R. S., D. J. Zeckner, L. F. Ellis, A. L. Thakker, and L. C. Howard. 1984. In vitro and in vivo anti-*Candida* activity and toxicology of LY121019. *J. Antibiot.* 37:1054-1065.
4. National Committee for Clinical Laboratory Standards. 1995. Reference method for broth dilution antifungal susceptibility testing of yeasts; tentative standard. M27-T. National Committee for Clinical Laboratory Standards, Wayne, Pa.
5. Pfaller, M. A., T. Gerarden, M. Yu, and R. P. Wenzel. 1989. Influence of in vitro susceptibility testing conditions on the anti-candidal activity of LY121019. *Diagn. Microbiol. Infect. Dis.* 11:1-9.
6. Pfaller, M. A., M. Bale, B. Buschelman, M. Lancaster, A. Espinel-Ingroff, J. H. Rex, M. G. Rinaldi, C. R. Cooper, and M. R. McGinnis. 1995. Quality control guidelines for National Committee for Clinical Laboratory Standards recommended broth microdilution testing of amphotericin B, fluconazole, and flucytosine. *J. Clin. Microbiol.* 33:1104-1107.
7. Raab, P., D. Zeckner, B. Boyll, C. Boylan, and W. Current. 1994. LY303366; comparison of activity in murine models of *Aspergillus fumigatus* infection, abstr. F167, p. 252. In Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
8. Rex, J. H., M. A. Pfaller, M. Lancaster, F. C. Odds, A. Bolmström, and M. G. Rinaldi. 1996. Quality control guidelines for National Committee for Clinical Laboratory Standards-recommended broth microdilution testing of ketoconazole and itraconazole. *J. Clin. Microbiol.* 34:816-817.
9. Tang, J., T. R. Parr, Jr., W. Turner, M. DeBono, L. LaGrandeur, F. Burkhardt, M. Rodriguez, M. Zweifel, J. Nissen, and K. Clingerman. 1993. LY303366: a noncompetitive inhibitor of (1,3)-β-D-glucan synthases from *Candida albicans* and *Aspergillus fumigatus*, abstr. 367, p. 186. In Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
10. Warren, N. G., and K. C. Hazen. 1995. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 723-737. In P. R. Murray, E. J. Baron, M. A. Tenover, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
11. Zeckner, D., T. Butler, C. Boylan, B. Boyll, Y. Lin, P. Raab, J. Schmidtke, and W. Current. 1993. LY303366, activity in a murine systemic candidiasis model, abstr. 365, p. 186. In Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
12. Zeckner, D., T. Butler, C. Boylan, B. Boyll, Y. Lin, P. Raab, J. Schmidtke, and W. Current. 1993. LY303366, activity against systemic aspergillosis and histoplasmosis in murine models, abstr. 364, p. 186. In Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

13. **Zeckner, D., T. Butler, C. Boylan, B. Boyll, P. Raab, and W. Current.** 1994. LY303366, activity in a rat mucocutaneous vaginal candidiasis model, abstr. F169, p. 252. *In* Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
14. **Zornes, L., R. Stratford, M. Novilla, D. Turner, C. Boylan, B. Boyll, T. Butler, Y. Lin, D. Zeckner, W. Turner, and W. Current.** 1993. Single dose IV bolus and oral administration pharmacokinetics of LY303366, a new lipopeptide antifungal agent related to echinocandin B, in female Lewis rats and beagle dogs, abstr. 370, p. 187. *In* Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.