ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1989, p. 1391–1392
0066-4804/89/081391-02$02.00/0

Vol. 33, No. 8

Evaluation of Cilofungin, a Lipopeptide Antifungal Agent, In Vitro against Fungi Isolated from Clinical Specimens

LINDA H. HANSON[1]* AND DAVID A. STEVENS[1,2]

Division of Infectious Diseases, Department of Medicine, Santa Clara Valley Medical Center and Institute for Medical Research, San Jose, California 95128,[1] and Division of Infectious Diseases, Department of Medicine, Stanford University School of Medicine, Stanford, California 94305[2]

Received 17 January 1989/Accepted 17 May 1989

Cilofungin (LY121019) is a new lipopeptide antifungal drug. We tested this drug against 141 pathogenic fungal isolates. All fungal species were tested by broth dilution at 35°C. *Malassezia furfur* was tested by agar dilution. The results demonstrate the specificity of cilofungin activity. *Candida albicans*, *Candida tropicalis*, and *Malassezia pachydermatis* were highly susceptible, whereas *Candida parapsilosis*, *Candida pseudotropicalis*, *Candida krusei*, *Torulopsis glabrata*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Aspergillus* species, *M. furfur*, and *Paracoccidioides brasiliensis* were more resistant.

Cilofungin (LY121019) is a new antifungal drug. It is a semisynthetic lipopeptide analog of the polypeptide antibiotic echinocandin B. Echinocandin B reportedly inhibits the incorporation of glucan into the cell wall of *Candida albicans* (1). Cilofungin also inhibits cell wall biosynthesis. One, ten, or one hundred micrograms of the drug per milliliter inhibits up to 95% incorporation of radiolabeled glucose into the glucan cell wall component of actively metabolizing *C. albicans* cells (R. S. Gordee, D. J. Zeckner, and W. E. Alborn, Jr., Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 977, 1987). Using electron microscopy techniques, these same researchers found that cilofungin causes severe damage to log-phase *C. albicans* cells. No effects were seen in stationary-phase cells.

Initial in vitro studies of the susceptibility of fungal organisms to cilofungin suggest that certain *Candida* species are susceptible (4). We screened the in vitro susceptibilities of selected fungi and tested 141 clinical isolates against cilofungin. We report the in vitro susceptibilities of 13 species of pathogenic fungi.

(Part of this research was presented at the 88th Annual Meeting of the American Society for Microbiology, Miami Beach, Fla., 8 to 13 May 1988.)

Most fungal cultures were stored on slants at 4°C. Some specimens were stored underwater at room temperature. After specimens were subcultured to blood agar or Sabouraud dextrose agar plates, the growth was harvested and diluted into appropriate medium. All *Candida* and *Aspergillus* specimens were tested in Sabouraud dextrose broth (pH 5.6). Dimorphic fungi were tested in the yeast form (verified microscopically). *Paracoccidioides brasiliensis* cultures were tested in modified McVeigh-Morton medium (pH 7.0) (6). *Malassezia pachydermatis*, *Blastomyces dermatitidis*, and *Cryptococcus neoformans* isolates were tested in SAAMF medium (pH 7.4) (5), which was modified principally by the elimination of cysteine and Tris buffer and the substitution of 0.165 M MOPS (morpholinepropanesulfonic acid) buffer. The testing of *Malassezia furfur* required an agar dilution method used previously for this organism (3). In brief, Sabouraud dextrose agar plates incorporating a lipid supplement were incubated at 35°C for 10 days. The media and methods used for testing each organism were those which had been shown previously in our laboratory to give satisfactory control growth and sharp endpoints for drug susceptibility testing (2, 3).

All broth cultures were incubated overnight at 35°C on a gyratory shaker (140 rpm) in ambient air. This produced an inoculum in growth phase. The optical density of each of the *Candida* cultures was then measured spectrophotometrically at 540 nm and diluted with Sabouraud dextrose broth so that inoculum tubes contained approximately 2,000 cells per ml (2). All other fungal cultures were counted in a hemacytometer and diluted with medium so that tubes contained 2,000 cells per ml. A 1-ml sample of this culture was added to 1 ml of drug solution so that the final inoculum was 1,000 cells per ml. A dilution of the inoculum was plated on agar to verify absence of contamination, inoculum quantitation, and viability.

To prepare the broth dilution series, cilofungin powder (stored at −20°C) was weighed and combined with equal quantities of ethanol and appropriate medium to make a stock solution of the drug at 1 mg/ml. The stock solution was prepared weekly and stored at 4°C. The stock solution was diluted with medium to produce a drug dilution series of 80 to 0.078 μg/ml. A 1-ml sample of each dilution was combined with 1 ml of inoculum to produce a series of 40 to 0.039 μg/ml. Some control tubes for the series contained cells in medium alone and some contained cells in medium with 2% ethanol, because the solution with highest concentration of the drug, 40 μg/ml, contained 2% ethanol. All tubes were incubated at 35°C in ambient air on a gyratory shaker (140 rpm).

To determine the MIC, the growth in tubes containing cells and drug was compared with the growth in control tubes containing cells in medium only at a time when the latter demonstrated turbidity, i.e., 24 h for all *Candida* spp. and *M. pachydermatis*, 48 h for the *Aspergillus* species and *Cryptococcus neoformans*, and 72 h for *P. brasiliensis* and *B. dermatitidis*. The MIC was designated as the lowest drug concentration with no evident growth. Our assay system did not produce an Eagle effect (growth in tubes with drug concentrations greater than the MIC) (7). Other researchers have reported this problem (E. D. Spitzer, S. J. Travis, and G. S. Kobayashi, 27th ICAAC, abstr. no. 971, 1987; M. G. Rinaldi, D. A. McGough, and J. L. Anderson, 27th ICAAC, abstr. no. 973, 1987; A. Kamyar, M. Crandall, and J.

* Corresponding author.

TABLE 1. Susceptibilities of fungal pathogens to cilofungin

Fungal species (no. of isolates)	No. of isolates for which MIC of cilofungin (μg/ml) was[a]:							
	0.31	0.62	1.25	5.0	10.0	20.0	40.0	>40.0
Candida albicans (58)	1	44	5	7		1		
Candida tropicalis (18)		14	1	3				
Candida parapsilosis (14)			1		1	12		
Torulopsis glabrata (13)				11		1	1	
Malasezzia furfur (12)								12
Paracoccidioides brasiliensis (7)					1	3		3
Blastomyces dermatitidis (5)					1	1	2	1
Aspergillus spp.[b] (5)								5
Candida pseudotropicalis (3)							3	
Malassezia pachydermatis (3)			3					
Candida krusei (1)					1			
Candida lusitaniae (1)			1					
Cryptococcus neoformans (1)								1

[a] For no isolate was the MIC 2.5 μg/ml.

[b] Including *Aspergillus fumigatus* (n = 1), *A. flavus* (n = 1), and *A. niger* (n = 1).

Edwards, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, F85, p. 405). These workers reported that turbidity in tubes with drug concentrations greater than the MIC often cleared after 24 h but sometimes persisted. This phenomenon made it difficult for them to determine the MIC for the assay. In our assays, all tubes were clear at the beginning of our incubation period. The MIC tube and all the tubes containing cilofungin concentrations greater than the MIC remained clear. No evidence of drug precipitate was seen.

Our results are summarized in Table 1. *C. albicans* isolates were highly and consistently susceptible to cilofungin. Of note, MICs for 44 (76%) of 58 isolates were 0.625 μg/ml. *Candida tropicalis* isolates were similarly susceptible. MICs for 14 (78%) of 18 isolates were 0.625 μg/ml. Other *Candida* species, especially *Candida parapsilosis*, were markedly less susceptible. This was true for most other fungal species tested. An exception was *M. pachydermatis* (isolated from premature-infant blood cultures). The MIC for all three *M. pachydermatis* isolates was 1.25 μg/ml. As a control to compare results obtained with the different media and methods we used for different species, we tested a susceptible organism (*C. albicans* A26) and a resistant organism (*Candida pseudotropicalis* SA) in various media. MICs for the *C. albicans* isolate tested in Sabouraud dextrose broth, SAAMF medium, and McVeigh-Morton broth and by the lipid-supplemented agar dilution assay were the same (range of 1 twofold dilution). The *C. pseudotropicalis* isolate was tested in the three broth media and produced the same results. As another control for the agar dilution system, we determined the MICs of itraconazole for 11 *M. furfur* isolates, since the susceptibility of that organism had been reported previously (3) in that system with that drug. All 11 isolates were susceptible to itraconazole.

Initial toxicity studies showed cilofungin to be 20-fold less toxic than amphotericin B (4). Studies of pharmacokinetics and in vivo efficacy as well as further toxicologic studies are needed. Our results suggest pathogens which would be of interest for therapeutic studies in vivo.

LITERATURE CITED

1. **Cassone, A., R. E. Mason, and D. Kerridge.** 1981. Lysis of growing yeast-form cells of *Candida albicans* by echinocandin: a cytological study. Sabouraudia **19**:97–110.
2. **Galgiani, J. N., and D. A. Stevens.** 1976. Antimicrobial susceptibility testing of yeasts: a turbidimetric technique independent of inoculum size. Antimicrob. Agents Chemother. **10**:721–726.
3. **Ganer, A., E. Arathoon, and D. A. Stevens.** 1987. Initial experience in therapy for progressive mycoses with itraconazole, the first clinically studied triazole. Rev. Infect. Dis. **9**(Suppl. 1): S77–S86.
4. **Gordee, R. S., D. Zeckner, L. F. Ellis, A. L. Thakkar, and L. C. Howard.** 1984. *In vitro* and *in vivo* anti-*Candida* activity and toxicology of LY 121019. J. Antibiot. **37**:1054–1065.
5. **Hoeprich, P. D., and P. D. Finn.** 1972. Obfuscation of the activity of antifungal antibiotics by culture media. J. Infect. Dis. **16**:353–361.
6. **Restrepo, A., and B. E. Jiménez.** 1980. Growth of *Paracoccidioides brasiliensis* yeast phase in a chemically defined culture medium. J. Clin. Microbiol. **12**:279–281.
7. **Schoenknecht, F. D., L. D. Sabath, and C. Thornsberry.** 1985. Susceptibility tests: special tests, p. 1000–1008. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.