

## Comparison of Cilofungin and Amphotericin B for Therapy of Murine Candidiasis

KIM R. SMITH,<sup>1</sup> KIM M. LANK,<sup>1</sup> C. GLENN COBBS,<sup>1</sup> GRETCHEN A. CLOUD,<sup>2</sup> AND WILLIAM E. DISMUKES<sup>1\*</sup>

*Division of Infectious Diseases, Department of Medicine,<sup>1</sup> and Division of Biostatistics, Comprehensive Cancer Center,<sup>2</sup> University of Alabama at Birmingham School of Medicine, Birmingham, Alabama 35294*

Received 22 January 1990/Accepted 30 May 1990

**We compared the efficacies of cilofungin and amphotericin B treatment in a murine model of disseminated candidiasis. Three different dosages of each drug plus controls were evaluated. Statistically improved survival was noted only among mice treated with 1 mg of amphotericin B per kg of body weight ( $P < 0.05$ ). While all amphotericin B regimens and the two lower-dosage cilofungin regimens significantly reduced yeast cell counts in kidneys compared with the controls, the amphotericin B-treated mice had a significantly higher percentage of sterile kidneys following therapy compared with those treated with cilofungin ( $P = 0.0001$ ).**

Disseminated (or invasive) candidal disease is becoming increasingly prevalent because of the large reservoir of seriously immunocompromised patients. While amphotericin B is moderately effective against most forms of serious candidiasis, this agent has major toxicities. Consequently, there is a need for new, safe, and effective systemic anticanidial drugs. Cilofungin (LY121019), an analog of echinocandin B, is an investigational lipopeptide agent consisting of a hydrophilic cyclic peptide with a hydrophobic fatty acid side chain (2, 5). The antifungal activity of cilofungin results from inhibition of 1,3- $\beta$ -D-glucan synthase, the enzyme responsible for assembly of 1,3- $\beta$ -D-glucan in the fungal cell wall (2, 12, 14). Cilofungin is highly active in vitro against most *Candida* species.

In a previous study, we examined the in vitro activity of cilofungin against 103 strains of 6 *Candida* species (K. R. Smith, K. M. Lank, W. E. Dismukes, and C. G. Cobbs, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1521, 1988). All isolates of *Candida albicans*, *C. tropicalis*, *C. paratropicalis*, and *C. glabrata* were inhibited by  $\leq 5$   $\mu\text{g/ml}$  at both 24 and 48 h. In contrast, *C. parapsilosis* was more resistant to cilofungin, with MICs ranging from 10 to  $\geq 40$   $\mu\text{g/ml}$ ; similar findings have been reported by others (2-5, 7, 8, 11, 13).

The purpose of our study was to compare the efficacy of cilofungin with that of amphotericin B in a murine model of disseminated candidiasis.

The organism used to infect the mice was *C. albicans* A26, kindly provided by Lilly Research Laboratories, Indianapolis, Ind. Stock cultures were stored at  $-70^\circ\text{C}$  in sterile water. The yeast was plated onto Sabouraud dextrose agar the day before experiments were carried out and incubated overnight at  $35^\circ\text{C}$ . Colonies of yeast were suspended in sterile normal saline to obtain a slightly turbid suspension, and a portion was diluted 1:10 in saline. Yeast cells were enumerated in a hemacytometer, and the suspension was adjusted until the count was  $10^7$  yeast cells per ml. The final inoculum was  $10^6$  yeast cells per mouse, and it was given to each mouse via the lateral tail vein.

White CD-1 male mice (18 to 20 g) (Charles River Breeding Laboratories, Raleigh, N.C.) were used in all experiments. There were four groups of mice per treatment agent, including one control group and three active-treatment groups.

Amphotericin B (E. R. Squibb & Sons, Princeton, N.J.) was formulated by adding 10 ml of sterile water to 50 mg of dehydrated powder, providing a final concentration of 5,000  $\mu\text{g/ml}$ . Dilutions were made with 5% dextrose in water (pH  $> 4.2$ ). Cilofungin (Lilly Research Laboratories) was formulated by suspending the sterile powder in 33% polyethylene glycol 300, providing a stock solution of 20,000  $\mu\text{g/ml}$ .

Treatment with cilofungin and amphotericin B was begun 1 day following infection and given daily for 7 days. All drugs were administered by the intraperitoneal route in a total volume of 0.1 ml. For the amphotericin B and cilofungin control groups, 0.1 ml of 5% dextrose in water once a day or 0.1 ml of 33% polyethylene glycol 300 twice a day, respectively, was administered as a placebo. For the active treatment groups, amphotericin B was administered once daily in a dose of 1, 2, or 4 mg per kg of body weight, and cilofungin was administered every 12 h in a dose of 25, 50, or 100 mg/kg. Mice were observed daily from the initiation of therapy (day 2) to completion of therapy (day 8) and for 5 days following discontinuation of treatment (days 9 through 13). Mice that survived to the fifth day after conclusion of treatment (day 13) were sacrificed, and their kidneys were sterilely harvested.

Quantitative kidney cultures were done by combining and weighing both kidneys from each mouse and homogenizing them in a grinder tube in 1 ml of saline. Homogenized kidneys were then diluted and streaked on blood agar plates. After 48 h of incubation at  $35^\circ\text{C}$ , colonies were enumerated and the numbers of colonies per gram of tissue were determined.

Concentrations of cilofungin and amphotericin B in serum were assayed by the microbiological agar diffusion method, with *Aspergillus montevidensis* A35137 as the test organism for cilofungin and *Paecilomyces varioti* ATCC 36257 as the test organism for amphotericin B (1, 6). Serum samples for assay were obtained on the third day of treatment, at which time randomly picked mice from each treatment group were bled via the axillary artery at 1, 2, 4, and 12 h after cilofungin dosing and at 3, 6, and 24 h after amphotericin B dosing.

Analysis of variance was used to compare the effects within each treatment group on mortality rate and on sterilization of kidneys. The Duncan multiple range test was used to determine differences, if any, between dosages.

Concentrations of cilofungin in serum 1 h after an intraperitoneal dose of 100 mg/kg averaged 262  $\mu\text{g/ml}$  (Fig. 1).

\* Corresponding author.

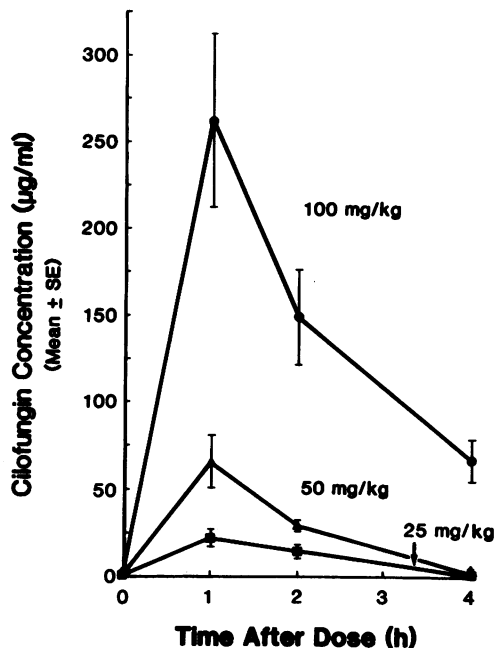


FIG. 1. Concentration of cilofungin in serum after intraperitoneal injection of 25, 50, or 100 mg of cilofungin per kg in mice. SE, Standard error.

The 1-h concentrations after doses of 50 and 25 mg of cilofungin per kg were considerably lower: 62 and 20  $\mu\text{g/ml}$ , respectively. At 12 h after administration of cilofungin, no drug was detectable in serum. Amphotericin B was detectable in concentrations of approximately 0.1  $\mu\text{g/ml}$  in serum only at 3 and 6 h after administration of the highest dose (4 mg/kg). The range of the assay for amphotericin B was 0.08 to 5.0  $\mu\text{g/ml}$ .

Table 1 provides mortality rates for the mice which died prior to sacrifice (day 13). Of mice given the cilofungin control vehicle, 39% died, and 26% of mice given the

TABLE 1. Effect of cilofungin and amphotericin B treatment in disseminated candidiasis

Treatment and dosage <sup>a</sup>	Presacrifice mortality rate (no. dead/no. tested [%])	No. of mice with sterile kidneys at sacrifice/no. tested (%)
Placebo control		
0.1 ml of 33% PEG q12h	12/31 (39) <sup>b</sup>	1/19 (5) <sup>c</sup>
Cilofungin		
25 mg/kg q12h	6/24 (25) <sup>b</sup>	8/18 (44) <sup>c</sup>
50 mg/kg q12h	4/22 (18) <sup>b</sup>	6/18 (33) <sup>c</sup>
100 mg/kg q12h	7/25 (28) <sup>b</sup>	4/18 (22) <sup>c</sup>
Placebo control		
0.1 ml of 5% D5W QD	8/31 (26) <sup>d</sup>	2/23 (9) <sup>e</sup>
Amphotericin B		
1 mg/kg QD	1/28 (4) <sup>d</sup>	21/27 (78) <sup>e</sup>
2 mg/kg QD	2/20 (10) <sup>d</sup>	16/18 (89) <sup>e</sup>
4 mg/kg QD	3/28 (11) <sup>d</sup>	22/25 (88) <sup>e</sup>

<sup>a</sup> PEG, Polyethylene glycol; q12h, every 12 h; 5% D5W, 5% dextrose in water; QD, every day.

<sup>b</sup>  $P = 0.42$ .

<sup>c</sup>  $P = 0.04$ .

<sup>d</sup>  $P = 0.07$ .

<sup>e</sup>  $P = 0.0001$ .

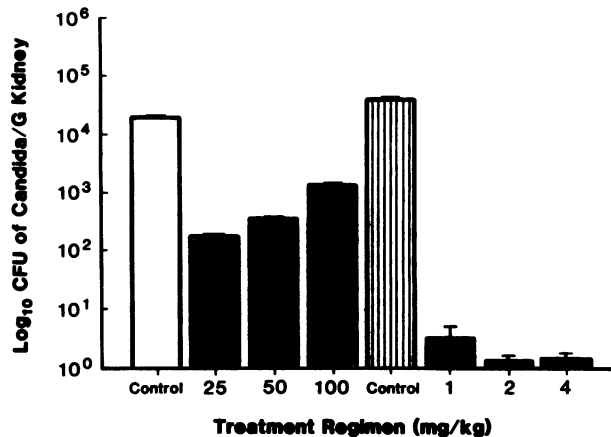


FIG. 2. Geometric mean yeast titers of kidneys from mice sacrificed 5 days after therapy with cilofungin (▨), cilofungin control (□), amphotericin B (■), or amphotericin B control (▩). Values are means  $\pm$  standard errors.

amphotericin B control vehicle died. For all treatment regimens, a mortality rate lower than that of the control was observed only among mice treated with 1 mg of amphotericin B per kg: 26 versus 4%, respectively ( $P < 0.05$ ). No treatment regimen with cilofungin exerted a beneficial effect on survival. Also shown in Table 1 are the percentages of mice with sterile kidneys at the time of sacrifice on day 13. While only 5% of the mice in the cilofungin control group and 9% in the amphotericin B control group had sterile kidneys, both treatment regimens resulted in significantly more mice with sterile kidneys than the controls (cilofungin [ $P = 0.04$ ] and amphotericin B [ $P = 0.0001$ ]). In addition, a statistically greater percentage of kidneys was sterile after amphotericin B treatment than after cilofungin treatment ( $P = 0.0001$ ).

Figure 2 illustrates the geometric mean yeast titers of homogenized kidneys from animals that survived to day 13 (5 days posttreatment) in control and treatment groups. A significant reduction in yeast cell counts was observed for the three amphotericin B regimens ( $P = 0.0001$ ) and the two lower-dosage regimens of cilofungin ( $P = 0.01$ ). Higher dosages of either drug were no more effective than lower dosages. In fact, a paradoxical effect was observed with cilofungin regimens; higher titers of *C. albicans* were observed in kidneys of mice receiving higher dosages of drug. Results of several in vitro studies with cilofungin have also shown a paradoxical response, namely, higher titers of yeasts in the presence of higher concentrations of drug (5, 11, 13). Amphotericin B was more effective than cilofungin, as determined by the fact that kidneys of amphotericin B-treated mice had lower yeast titers than kidneys from cilofungin-treated mice.

No difference was noted in the microscopic appearance of yeast cells seen in kidney homogenates from animals receiving active treatment regimens compared with the appearance of the yeast cells in the pretreatment inocula. Similarly, there was no difference in the pre- and posttreatment susceptibilities of the infecting organism. In both instances, *C. albicans* A26 was inhibited and killed by 0.625  $\mu\text{g}$  of cilofungin per ml and 0.625  $\mu\text{g}$  of amphotericin B per ml.

Studies of the efficacy of cilofungin in in vitro animal models have been limited. Gordeev et al. showed that the 50% effective dose for cilofungin was threefold higher than that for amphotericin B in an experimental model of candidiasis

with irradiated CD-1 mice (1). The same investigators evaluated recovery of *C. albicans* from kidneys in a study similar in design to our study, except that mice were sacrificed 24 h after the conclusion of treatment, rather than at 5 days posttherapy, and the cilofungin dosages were lower (1). There were no differences in yeast cell titers in the cilofungin- and amphotericin B-treated mice. In a third murine study which assessed survival in animals treated for 30 days, Khardori and co-investigators noted cilofungin to be superior to amphotericin B for treatment of disseminated *Candida* disease (N. Khardori, L. Kalvakuntla, E. Wong, B. Rosenbaum, and G. P. Bodey, 29th ICAAC, abstr. no. 817, 1989). Using a rat model of systemic candidiasis, McIntyre and Galgiani showed that cilofungin significantly prolonged survival compared with a placebo (7).

Less encouraging results have been obtained when rabbits have been used as the experimental animal. For example, in their rabbit model of disseminated candidiasis, Perfect and co-workers demonstrated that cilofungin significantly reduced yeast cell counts in the renal cortex and the vitreous humor of the eye but not in endocarditic vegetations (10). Other investigators using a *Candida* endocarditis model also observed that cilofungin did not improve survival and did not reduce yeast titers in cardiac vegetations (9). In both of these studies in which cilofungin was administered on a twice-a-day schedule, frequency of administration may have played a role since cilofungin is rapidly eliminated in rabbits. In a third study with rabbits, Chapman and coauthors observed that cilofungin was not fungicidal in a subcutaneously implanted semipermeable chamber and postulated that protein binding of cilofungin to rabbit serum accounted for the lessened activity (R. Chapman, J. Moody, C. Fasching, L. Sinn, D. Gerding, and L. Peterson, 29th ICAAC, abstr. no. 819, 1989). This issue of the protein binding of cilofungin may be important clinically and raises the question whether higher dosages or more frequent dosing of cilofungin, especially in rabbits, will be necessary.

In summary, the results of our study suggest that cilofungin may not be as effective in killing yeasts in murine kidneys as amphotericin B is, at least as assayed in mice sacrificed 5 days after discontinuation of therapy. These data, together with those of other investigators (9, 10), argue that cilofungin may not be reliably fungicidal *in vivo*. Further studies employing various dosages and frequencies in mouse, rabbit, and other animal models and humans are needed in order to evaluate the importance of pharmacokinetic parameters on efficacy. Study methods must also be considered in assessing our results. For example, the relatively low mortality rates in our control groups, the sample sizes employed, and the delay in sacrifice of the survivors may have obscured differ-

ences between treatment agents and within treatment groups.

This work was supported by a grant from Lilly Research Laboratories.

#### LITERATURE CITED

1. Gordee, R. S., D. J. 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.
2. Gordee, R. S., D. J. Zeckner, L. C. Howard, W. E. Alborn, Jr., and M. Debono. 1988. Anti-*Candida* activity and toxicology of LY 121019, a novel semisynthetic polypeptide antifungal antibiotic. *Ann. N.Y. Acad. Sci.* 544:294-309.
3. Hall, G. S., C. Myles, K. J. Pratt, and J. A. Washington. 1988. Cilofungin (LY121019), an antifungal agent with specific activity against *Candida albicans* and *Candida tropicalis*. *Antimicrob. Agents Chemother.* 32:1331-1335.
4. Hanson, L. H., and D. A. Stevens. 1989. Evaluation of cilofungin, a lipopeptide antifungal agent, *in vitro* against fungi isolated from clinical specimens. *Antimicrob. Agents Chemother.* 33:1391-1392.
5. Hobbs, M., J. Perfect, and D. Durack. 1988. Evaluation of *in vitro* antifungal activity of LY 121019. *Eur. J. Clin. Microbiol. Infect. Dis.* 7:77-80.
6. McGinnis, M. R., and M. G. Rinaldi. 1986. Antifungal drugs: mechanisms of action, drug resistance, susceptibility testing, and assays of activity in biological fluids, p. 233-281. *In V. Lorian* (ed.), *Antibiotics in laboratory medicine*, 2nd ed. The Williams & Wilkins Co., Baltimore.
7. McIntyre, K. A., and J. N. Galgiani. 1989. pH and other effects on the antifungal activity of cilofungin (LY121019). *Antimicrob. Agents Chemother.* 33:731-735.
8. Odds, F. C. 1988. Activity of cilofungin (LY 121019) against *Candida* species *in vitro*. *J. Antimicrob. Chemother.* 22:891-897.
9. Padula, A., and H. F. Chambers. 1989. Evaluation of cilofungin (LY121019) for treatment of experimental *Candida albicans* endocarditis in rabbits. *Antimicrob. Agents Chemother.* 33:1822-1823.
10. Perfect, J. R., M. M. Hobbs, K. A. Wright, and D. T. Durack. 1989. Treatment of experimental disseminated candidiasis with cilofungin. *Antimicrob. Agents Chemother.* 33:1811-1812.
11. Pfaller, M. A., S. Wey, T. Gerarden, A. Houston, and R. P. Wenzel. 1989. Susceptibility of nosocomial isolates of *Candida* species to LY 121019 and other antifungal agents. *Diagn. Microbiol. Infect. Dis.* 12:1-4.
12. Sawistowska-Schröder, E. T., D. Kerridge, and H. Perry. 1984. Echinocandin inhibition of 1,3- $\beta$ -D-glucan synthase from *Candida albicans*. *FEBS Lett.* 173:134-138.
13. Spitzer, E. D., S. J. Travis, and G. S. Kobayashi. 1988. Comparative *in vitro* activity of LY 121019 and amphotericin B against clinical isolates of *Candida* species. *Eur. J. Clin. Microbiol. Infect. Dis.* 7:80-81.
14. Taft, C. S., T. Stark, and C. P. Selitrennikoff. 1988. Cilofungin (LY121019) inhibits *Candida albicans* (1-3)- $\beta$ -D-glucan synthase activity. *Antimicrob. Agents Chemother.* 32:1901-1903.