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Evaluation of Cilofungin (LY121019) for Treatment of Experimental *Candida albicans* Endocarditis in Rabbits

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The efficacy of cilofungin (LY121019) for aortic valve endocarditis caused by *Candida albicans* in rabbits was studied. Vegetation titers were similar for cilofungin-treated and untreated rabbits. No rabbit survived beyond 5 days in either group. All rabbits given amphotericin B survived, and titers were reduced. Cilofungin was ineffective in this model.

Cilofungin (LY121019) is a semisynthetic antifungal drug that is active in vitro and in vivo against *Candida albicans* (4, 9). Its antifungal activity is due to inhibition of the synthesis of beta-glucan, a component of fungal cell wall. Studies indicate that cilofungin may be fungicidal in vivo (4).

The purposes of these studies were (i) to evaluate the efficacy of cilofungin for treatment of experimental candidal endocarditis in rabbits, (ii) to determine whether cilofungin is fungicidal in an experimental model in which host defenses play little or no role, and (iii) to compare the efficacy of cilofungin with that of amphotericin B.

A clinical isolate of *C. albicans*, M27587, obtained from the clinical microbiology laboratory of San Francisco General Hospital, was used for the endocarditis studies. This strain was identified as *C. albicans* based on biochemical tests (API 20C; Analytab Products, Plainview, N.Y.) and a germ tube test. Three other strains of *C. albicans*, A26 (a laboratory control strain obtained from Eli Lilly & Co., Indianapolis, Ind.), Lieberman (a clinical isolate from sputum), and a control strain (CS) from the clinical microbiology laboratory, were used in susceptibility studies. Microdilution broth susceptibility tests were performed both in Sabouraud dextrose medium (Difco Laboratories, Detroit, Mich.) and in antibiotic medium no. 3 (Difco) according to methods provided by the manufacturer of cilofungin (Eli Lilly). The inoculum was approximately 2×10^4 CFU/ml. The MIC was defined as the lowest concentration of drug that prevented turbidity after 24 h of incubation at 37°C.

Pharmacokinetic studies were performed with groups of three uninfected rabbits to assist in selection of a drug dose for therapeutic studies and to determine the half-lives of cilofungin and amphotericin B. Serum was obtained at 15 and 30 min and 1, 2, 4, and 6 h after a single dose of either cilofungin (50 mg/kg intravenously) or amphotericin B (2 mg/kg intravenously). Serum drug concentrations were assayed by the agar diffusion method with *Aspergillus montevidensis* A35137 as the test organism for cilofungin and *C. albicans* M27587 as the test organism for amphotericin B. The half lives were 1.3 h for cilofungin and 4.9 h for amphotericin B.

Experimental aortic valve endocarditis was established as described previously (8) in 25 2-kg New Zealand White rabbits. After 24 h each rabbit was inoculated with 10^8 CFU of strain M27587 suspended in 1 ml of normal saline. This inoculum was chosen based on preliminary dose-ranging studies comparing inocula, because it was the one that gave a 100% infection rate and produced large numbers of organisms in vegetations within 24 h of infection.

Treatment was initiated 24 h after infection. Rabbits were randomized into one of the following groups: (i) untreated control group (five rabbits were sacrificed after 24 h of infection to determine the number of CFU at the beginning of therapy and three rabbits were allowed to survive to determine the course of untreated infection); (ii) cilofungin group (nine rabbits, 50 mg/kg intravenously every 12 h); (iii) amphotericin B group (eight rabbits, 1 mg/kg intravenously every 24 h). Two rabbits, both in the cilofungin group, died after one dose and were not included in the data analysis. Drug concentrations in the serum of infected rabbits were determined on samples obtained 1 h after dosing, usually on the first or second day of therapy.

After 4 days of treatment, 12 h after the last dose of cilofungin and 24 h after the last dose of amphotericin B, rabbits were sacrificed, and the aortic valve vegetations were aseptically removed. The vegetations were then weighed, homogenized in a tissue grinder (Polytron; Brinkmann Instruments, Inc., Westbury, N.Y.), and quantitatively subcultured. Serial 10-fold dilutions of the homogenate were made in normal saline and plated onto Sabouraud dextrose agar. After 48 h of incubation at 30°C, the number of colonies was counted; the result is expressed as the $\log_{10}$ CFU per gram of tissue.

Survival of treated rabbits was compared with that of untreated rabbits by the Fisher exact test. Mean vegetation titers of treated rabbits were compared with those in untreated rabbits by the Student t test. Statistical significance was defined as $P < 0.025$ to correct for multiple comparisons.

The MIC of cilofungin for the experimental strain M27587 was 2 μg/ml in Sabouraud dextrose broth and 0.25 μg/ml in antibiotic medium no. 3. The MIC of amphotericin B was <0.06 μg/ml in both media. The MIC of cilofungin for the experimental strain was similar to those for other isolates of *C. albicans* (Table 1).

Cilofungin did not improve survival or increase survival time compared with that of untreated rabbits (85 ± 18 h [range, 60 to 100 h] for treated rabbits versus 63 ± 16 h [range, 48 to 80 h] for untreated rabbits; $P > 0.1$). None of 7 rabbits treated with cilofungin survived to complete 4 days of therapy. No significant difference in vegetation titers between the control group and rabbits treated with cilofungin was found (Table 2). Rabbits treated with amphotericin B

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TABLE 1. MICs of antifungal agents against strains of *C. albicans*

Strain	Medium[a]	MIC (μg/ml)	
		Cilofungin	Amphotericin B
M27587	Sab dex	2	<0.06
	AM3	0.25	<0.06
A26	Sab dex	2	0.25
	AM3	0.25	0.25
CS	Sab dex	0.5	ND[b]
	AM3	0.25	ND
Lieberman	Sab dex	0.5	ND
	AM3	0.5	ND

[a] Sab dex, Sabouraud dextrose broth; AM3, antibiotic medium no. 3.
[b] ND, Not done.

all survived the 4 days of therapy ($P = 0.006$ versus no treatment), and the vegetation titers were significantly lower ($P = 0.001$) than those in untreated rabbits.

Endocarditis is a severe test of drug efficacy because host defenses are impaired. Phagocytic cells are absent in mature vegetations of bacterial (2) and fungal (1) endocarditis. For both bacterial and fungal endocarditis (1, 10), titers in vegetations are several times higher in vivo than is commonly tested in vitro. Antibiotic penetration into the vegetation can be impaired (5, 7). Metabolic activity of the organisms in the vegetation is decreased compared with that in vitro (2). Drugs may be effective only if organisms are metabolically active and dividing. Similar to the beta-lactam antibiotics in terms of bactericidal activity, cilofungin and related agents are fungicidal only against growing fungal organisms (6, 11).

In our studies cilofungin did not reduce the number of organisms in vegetations compared with those in controls. This suggests that cilofungin was not fungicidal in this model.

Other investigators have seen a good correlation between activity of cilofungin in vitro and efficacy in vivo, and cilofungin has been compared favorably to amphotericin B (4, 9). In these other studies, the animal models used either had intact host defenses or modestly impaired defenses with neutrophil counts of 1,000 or more cells per mm^3 (3). The sites of infection were skin, mucosa, or soft tissue.

TABLE 2. Survival and titers of organisms in aortic valve vegetations of rabbits infected with *C. albicans* M27587

Group	Survival (no./total)	Mean titer (log_{10} CFU/ml)	Mean drug concn (μg/ml) at 1 h
Control	0/3	6.3 ± 0.7[a]	
Amphotericin B	8/8	4.1 ± 1.3	2.3 ± 0.2
Cilofungin	0/7	5.6 ± 1.6	13 ± 6

[a] Mean value for eight control rabbits, because vegetation titers for the five rabbits sacrificed at 24 h and the three allowed to survive were similar.

Cilofungin may have been effective for these infections and not endocarditis, because endocarditis is a more severe infection with more impaired host defenses. If drug penetration also was impaired (clearance of cilofungin was relatively rapid in vivo) and fungal cells were relatively inactive metabolically, this could also have contributed to the lack of efficacy of cilofungin in endocarditis.

These studies suggest that cilofungin may not be reliably fungicidal in vivo. Cilofungin may be intrinsically fungistatic. Alternatively, although relatively large doses were used, cilofungin may be fungicidal only at larger doses, producing even higher concentrations in serum than those that were achieved in this model. For serious candidal infections in the severely immunocompromised host, especially the neutropenic patient, cilofungin may not be as effective as amphotericin B.

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