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Comparison of Fluconazole and Amphotericin B for Treatment of Experimental *Candida* Endocarditis Caused by Non-*C. albicans* Strains

MALLORY D. WITT,[1,2] TRACY IMHOFF,[1] CONG LI,[1] AND ARNOLD S. BAYER[1,2]*

Division of Adult Infectious Diseases, Harbor-UCLA Medical Center, Torrance, California 90509,[1] and UCLA School of Medicine, Los Angeles, California 90024[2]*

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Amphotericin B and fluconazole were compared for the treatment of experimental *Candida* endocarditis caused by *Candida tropicalis* and *C. parapsilosis*. Rabbits received no therapy, amphotericin B (1 mg/kg of body weight per day intravenously), or fluconazole (100 mg/kg/day intraperitoneally) for either 11 or 21 days. Against both species, amphotericin B and fluconazole were equally effective overall; however, amphotericin B was more rapidly fungicidal than fluconazole in vivo against *C. tropicalis*.

Endovascular infections caused by *Candida* species, including endocarditis, prosthetic graft infections, and septic thrombophlebitis, are being increasingly recognized (4, 11, 13, 14, 17). This increase relates to the common use of indwelling intravascular catheters for antibiotic, chemotherapy, or hyperalimentation administrations, as well as prosthetic valve and graft placements for cardiovascular indications, highlighting the propensity of *Candida* strains to adhere to such prosthetic materials (10). Although *Candida* endovascular infections are difficult to eradicate with antifungal therapy alone (3, 11), such treatment is an integral part of the combined medical-surgical approaches to these infections (16).

To date, amphotericin B has been the preeminent antifungal agent for the treatment of invasive *Candida* infections, since this agent is active in vitro against most *Candida* strains and is fungicidal (6). However, enthusiasm for the therapeutic use of this drug has been counterbalanced by its well-known acute and chronic toxicities, such as fevers, rigors, hypotension, and renal insufficiency (7). Fluconazole is a new antifungal triazole with a limited toxicity profile, favorable human pharmocokinetics, and activity in vitro against the majority of *Candida* species (2, 12).

We previously showed, using the rabbit model of *Candida albicans* endocarditis (19), that both fluconazole and amphotericin B were effective in the treatment of an established valvular infection, although amphotericin B was more rapidly fungicidal in vivo. The current study was designed to compare the therapeutic efficacies of amphotericin B and fluconazole in the rabbit model of *Candida* endocarditis caused by the non-*C. albicans* species *C. parapsilosis* and *C. tropicalis*. This study was prompted by the recent emergence of these latter species in a variety of human infections, especially endocarditis (*C. parapsilosis* [10]) and fungemia in immunocompromised hosts (*C. tropicalis* [8, 18]). Because of the large challenge inoculum used in the induction of experimental *Candida* endocarditis and the high density of fungal organisms achieved within experimental lesions, this model serves as a rigorous test of antifungal therapeutic efficacies.

The two *Candida* species used in this study (*C. parapsilosis* and *C. tropicalis*) were clinical isolates from the Clinical Microbiology Laboratory of Harbor-UCLA Medical Center. These *Candida* species were maintained as stock cultures at 4°C on agar slants (yeast extract; Difco Laboratories, Detroit, Mich.). For preparation of the infecting fungal inoculum, organisms were inoculated into yeast extract-peptone broth (Difco) supplemented with 1% glucose and grown overnight at 25°C on a rotating platform. These stationary-phase organisms were sonicated with a model 350 device (Branson Sonic Power Co., Danbury, Conn.) for 3 s to ensure the development of single-blastospore suspensions as previously described (10). The organisms were pelleted by centrifugation, washed twice, and resuspended in 0.85% NaCl. After resonication, the organisms were counted and adjusted with a hemocytometer to achieve the final appropriate infecting fungal inoculum (ca. 2×10^7 CFU/ml). Confirmation of the infecting inoculum was obtained by making standard serial dilution plate counts on yeast nitrogen base agar (Difco).

Fluconazole was supplied as a powder (Diflucan; Pfizer Inc., Groton, Conn.). The drug was dissolved in 10% (vol/vol) Cremophor EL (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 12.5 mg/ml for intraperitoneal (i.p.) administrations. We previously showed that equivalent serum fluconazole levels are achieved when this agent is administered to rabbits by either the intravenous (i.v.) or the i.p. route (19). Amphotericin B (Fungizone; E. R. Squibb & Sons, Princeton, N.J.) was purchased and prepared in accordance with the manufacturer's directions for i.v. administrations.

The antimicrobial susceptibilities of *C. parapsilosis* and *C. tropicalis* to amphotericin B and fluconazole were determined by Michael Rinaldi (Fungus Testing Laboratory, University of Texas Health Sciences Center, San Antonio) using the broth macrodilution method and synthetic amino acid medium for fungi. The 24-h in vitro antifungal susceptibilities of the two species were determined in parallel at final inocula of ca. 10^4 and 10^6 CFU/ml to encompass the range of fungal densities ordinarily achieved within experimental vegetations in the rabbit model (19).

We used the same therapeutic antifungal strategies as those previously published (19). Since this latter study had already characterized the pharmacokinetic profiles of both amphotericin B and fluconazole in the rabbit endocarditis

* Corresponding author.

model, no antifungal drug levels were measured in the current investigation.

Female New Zealand White rabbits weighing ca. 2.5 kg were used. Animals were caged separately and given food and water ad libitum. Rabbits were anesthetized by intramuscular injections of ketamine hydrochloride (Ketaset; Aveco Co., Inc., Fort Dodge, Iowa) at 35 mg/kg of body weight and xylazine (Rompun; Mobay Corp., Shawnee, Kans.) at 1.5 mg/kg. Sterile vegetations were induced on the aortic valve following transcarotid-to-left ventricular catheterization (5) with a sterile polyethylene catheter (internal diameter, 0.86 mm; Becton Dickinson and Co., Parsippany, N.J.) at 48 h prior to i.v. fungal challenge; the catheter remained in place for the duration of the study. At 48 h postcatheterization, *Candida* endocarditis was induced by the i.v. injection of ca. 2×10^7 CFU via the marginal ear vein, a procedure that reliably induced *C. parapsilosis* and *C. tropicalis* valvular infections in pilot studies of this model.

At 24 h after the i.v. *Candida* challenge to induce aortic endocarditis in catheterized rabbits, the rabbits were randomized to receive no therapy (untreated controls), fluconazole (100 mg/kg of body weight per day i.p.), or amphotericin B (1 mg/kg/day i.v.); on the basis of the results of our prior study (19), antifungal treatments were continued for either 11 or 21 days. Rabbits were sacrificed 24 h after the last antifungal dose.

At the assigned sacrifice date, rabbits were euthanized with 200 mg of pentobarbital sodium (Abbott Laboratories, North Chicago, Ill.) administered by rapid i.v. injection. Only animals with catheters in the proper transaortic valve position and macroscopic aortic valve vegetations were analyzed in this study. Cardiac vegetations from each animal were removed, pooled, weighed, homogenized in 1 ml of sterile normal saline, serially diluted, and quantitatively cultured on yeast potassium dextrose agar at 35°C for 48 h, and the mean $\log_{10}$ CFU per gram of vegetation (± the standard deviation) was calculated. For statistical comparisons, culture-negative vegetations were considered to contain ≤ 2 $\log_{10}$ CFU/g on the basis of the average vegetation weight in this model.

Differences in intravegetation fungal densities among the three treatment groups were compared by use of the Kruskal-Wallis test. If a statistically significant treatment effect was found, pairwise comparisons were made by use of a two-tailed Wilcoxon rank sum test. A maximum P value of 0.05 was considered statistically significant.

Table 1 lists the minimal fungistatic and fungicidal concentrations of amphotericin B and fluconazole against the *C. parapsilosis* and *C. tropicalis* strains subsequently used in the in vivo aspects of this investigation. Both species were susceptible in vitro to the fungistatic and fungicidal actions of amphotericin B, with no definable inoculum effect being observed (ca. 10^4 versus 10^6 CFU). Similarly, there was little change in the fluconazole minimal fungistatic concentration for either species at the higher in vitro inoculum; however, in contrast to amphotericin B, there was a significant disparity between the minimal fungistatic and fungicidal concentrations of fluconazole for both *Candida* species.

For the treatment of established endocarditis caused by *C. parapsilosis*, both fluconazole and amphotericin B were equally and significantly active in this model. By 11 days of therapy, both treatment regimens had significantly reduced intravegetation fungal densities (CFU per gram), compared with those in untreated controls (Table 2).

Similarly, both fluconazole and amphotericin B were effective in the treatment of established *C. tropicalis* endocarditis. However, with this organism, as opposed to *C. parapsilosis*, the rates of clearance of intravegetation fungi were significantly more rapid with amphotericin B than with fluconazole (Table 3). For example, after 11 days of therapy, only amphotericin B had significantly reduced intravegetation fungal densities, compared with those in untreated controls, while both agents were equally effective in this regard after 21 days of therapy.

TABLE 1. In vitro susceptibilities of *C. tropicalis* and *C. parapsilosis* to the fungistatic and fungicidal actions of amphotericin B and fluconazole

Species and agent	Minimal drug concn (μg/ml) at the following final in vitro inoculum:			
	~10^4 CFU/ml		~10^6 CFU/ml	
	Fungistatic	Fungicidal	Fungistatic	Fungicidal
C. parapsilosis				
Amphotericin B	<0.14	0.58	<0.14	1.16
Fluconazole	2.5	10	2.5	>80
C. tropicalis				
Amphotericin B	<0.14	0.58	<0.14	0.58
Fluconazole	<1.25	>80	<1.25	>80

The current study was prompted by the recent emergence of non-*C. albicans Candida* species as important causes of fungemia and disseminated infections in both immunocompetent (6, 8) and immunocompromised (1, 9, 18) hosts. Several interesting observations were forthcoming from this study. We found that both amphotericin B and fluconazole were very efficacious agents in the treatment of established endocarditis caused by the non-*C. albicans Candida* species studied. For *C. parapsilosis*, both amphotericin B and fluconazole were equally effective and rapidly fungicidal in vivo, with significant reductions in intravegetation fungal densities after 11 days of therapy. However, against established *C. tropicalis* endocarditis, amphotericin B was more rapidly fungicidal in vivo after 11 days of therapy, although both regimens were equally effective when therapy was extended to 21 days. This latter experience with *C. tropicalis* parallels our previous observations in comparing amphotericin B and fluconazole for the treatment of experimental *C. albicans* endocarditis (19). Thus, our previous and current studies both confirm that fluconazole and amphotericin B are effective in treating established endovascular infections with various *Candida* species, although the treatment response is

TABLE 2. Fluconazole versus amphotericin B in the treatment of *C. parapsilosis* endocarditis

Regimen	Vegetation fungal density, mean $\log_{10}$ CFU/g ± SD (no. of animals sacrificed), after the following no. of days of therapy:	
	11	21
Amphotericin B (1 mg/kg/day i.v.)	2.37 ± 0.76 (9)[a]	2.61 ± 0.39 (9)[b]
Fluconazole (100 mg/kg/day i.p.)	2.27 ± 0.37 (7)[c]	2.48 ± 0.64 (7)[d]
Controls (untreated)	4.63 ± 1.0 (7)	5.96 ± 1.09 (8)

[a] $P = 0.001$ for amphotericin B versus controls.
[b] $P = 0.00016$ for amphotericin B versus controls.
[c] $P = 0.0006$ for fluconazole versus controls.
[d] $P = 0.0003$ for fluconazole versus controls.

TABLE 3. Fluconazole versus amphotericin B in the treatment of *C. tropicalis* endocarditis

Regimen	Vegetation fungal density, mean $\log_{10}$ CFU/g ± SD (no. of animals sacrificed), after the following no. of days of therapy:	
	11	21
Amphotericin B (1 mg/kg/day i.v.)	4.06 ± 1.4 (16)[a]	3.38 ± 1.6 (9)[b,c]
Fluconazole (100 mg/kg/day i.p.)	6.71 ± 0.8 (8)	4.7 ± 1.1 (7)[b,c]
Controls (untreated)	7.27 ± 1.0 (6)	7.6 ± 1.1 (9)

[a] $P < 0.01$ for amphotericin B versus controls or fluconazole.
[b] $P < 0.01$ for amphotericin B or fluconazole versus controls.
[c] $P > 0.5$ for amphotericin B versus fluconazole.

generally slower with fluconazole. The reason(s) for the more rapid in vivo killing of *C. parapsilosis* than of *C. tropicalis* by fluconazole in this endocarditis model is undoubtedly complex and may relate in part to the lower minimal fungicidal concentration for *C. parapsilosis* (10 μg/ml) than for *C. tropicalis* (>80 μg/ml) and the lower in vivo rate of growth of *C. parapsilosis*, yielding substantially lower final intravegetation fungal densities in untreated *C. parapsilosis* controls than in untreated *C. tropicalis* controls.

The dose of fluconazole used in this study (100 mg/kg i.p.) yielded peak levels in serum (approximately 100 μg/ml [19]) at least 40 times the MICs for both *Candida* species. The pharmacokinetics of fluconazole at standard clinical dosages in humans (50 to 100 mg/day) appear to be linear, with achievable levels in serum ranging from 2 to 6 μg/ml (15). On the basis of these data, it is possible that the current high-dose fluconazole strategies now being studied for invasive human mycoses (1,200 to 1,800 mg/day) may well result in peak levels in serum approaching those achieved in our animal model.

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REFERENCES

1. **Blinkhorn, R. J., D. Adelstein, and P. J. Spagnuolo.** 1989. Emergence of a new opportunistic pathogen, *Candida lusitaniae*. J. Clin. Microbiol. **27:**236–240.
2. **Bodey, G. P.** 1992. Azole antifungal agents. Clin. Infect. Dis. **14(5):**161–169.
3. **Crislip, M. A., and J. E. Edwards, Jr.** 1989. Candidiasis. Infect. Dis. Clin. N. Am. **3:**103–133.
4. **Doscher, W., K. V. Krishnasastry, and S. L. Deckoff.** 1987. Fungal graft infections: case report and review of the literature. J. Vasc. Surg. **6:**398–402.
5. **Durack, D. T., P. B. Beeson, and R. G. Petersdorf.** 1973. Experimental bacterial endocarditis. III. Production and progress of the disease in rabbits. Br. J. Exp. Pathol. **54:**142–151.
6. **Filler, S. G., and J. E. Edwards, Jr.** 1992. Current strategies for treating invasive candidiasis: emphasis on infections in non-neutropenic patients. Clin. Infect. Dis. **14(51):**S106–S113.
7. **Gallis, H. A., R. H. Drew, and W. W. Pickard.** 1990. Amphotericin B: 30 years of clinical experience. Rev. Infect. Dis. **12:**308–329.
8. **Komshian, S. V., A. K. Uwaydah, J. D. Sobel, and L. R. Crane.** 1989. Fungemia caused by *Candida* species and *Torulopsis glabrata* in the hospitalized patient: frequency, characteristics and evaluation of factors influencing outcome. Rev. Infect. Dis. **11:**379–390.
9. **Powderly, W. G., G. S. Kobayashi, G. P. Herzig, and G. Medoff.** 1988. Amphotericin B-resistant yeast infection in severely immunocompromised patients. Am. J. Med. **84:**826–832.
10. **Rotrosen, D., R. Calderone, and J. E. Edwards, Jr.** 1986. Adherence of *Candida* species to host tissues and plastic surfaces. Rev. Infect. Dis. **8:**73–85.
11. **Rubinstein, E., E. R. Noriega, M. A. Simberkoff, R. Holzman, and J. J. Rahal.** 1975. Fungal endocarditis: analysis of 24 cases and review of the literature. Medicine (Baltimore) **54:**331–344.
12. **Saag, M. S., and W. E. Dismukes.** 1988. Azole antifungal agents: emphasis on new triazoles. Antimicrob. Agents Chemother. **32:**1–8.
13. **Seelig, M. S., P. J. Kozinn, P. Goldberg, and A. R. Berger.** 1979. Fungal endocarditis: patients at risk and their treatment. Postgrad. Med. J. **55:**632–641.
14. **Seelig, M. S., C. P. Speth, P. J. Kozinn, C. L. Taschdjian, E. F. Toni, and P. Goldberg.** 1974. Patterns of *Candida* endocarditis following cardiac surgery: importance of early diagnosis and therapy analysis of 91 cases. Prog. Cardiovasc. Dis. **18:**125–160.
15. **Tucker, R. M., P. L. Williams, E. G. Arathoon, E. B. Levine, A. I. Hartstein, L. H. Hanson, and D. A. Stevens.** 1988. Pharmacokinetics of fluconazole in cerebrospinal fluid and serum in human coccidioidal meningitis. Antimicrob. Agents Chemother. **32:**369–373.
16. **Turnier, E., J. H. Kay, S. Bernstein, A. M. Mendez, and P. Zubiate.** 1975. Surgical treatment of *Candida* endocarditis. Chest **67:**262–268.
17. **Wiley, E. L., and G. M. Hutchins.** 1977. Superior vena cava syndrome secondary to *Candida* thrombophlebitis complicating parenteral alimentation. J. Pediatr. **91:**977–999.
18. **Wingard, J. R., W. G. Merz, and R. Saral.** 1979. *Candida tropicalis*: a major pathogen in immunocompromised patients. **91:**539–543.
19. **Witt, M. D., and A. S. Bayer.** 1991. Comparison of fluconazole and amphotericin B for prevention and treatment of experimental *Candida* endocarditis. Antimicrob. Agents Chemother. **35:** 2481–2485.