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

ARNOLD S. BAYER,[1,2,3]* MALLORY D. WITT,[1,2,3] ERIC KIM,[1,2] AND MAHMOUD A. GHANNOUM[1,2,3]

Division of Adult Infectious Diseases, Harbor-UCLA Medical Center,[1] and St. John's Cardiovascular Research Center,[2] Torrance, California 90509, and UCLA School of Medicine, Los Angeles, California 90024[3]

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Amphotericin B (1 mg/kg of body weight, intravenous) and fluconazole (100 mg/kg, intraperitoneal) were compared in the prophylaxis of experimental *Candida* endocarditis caused by drug-susceptible, non-*C. albicans* strains *C. tropicalis* and *C. parapsilosis*. Neither antifungal agent was effective at preventing endocarditis due to either *Candida* strain when either agent was administered in a single-dose regimen (1 h prior to fungal challenge); the prophylactic efficacy of both agents increased substantially when a second prophylactic dose was given (24 h postchallenge). The excellent prophylactic efficacy of fluconazole, a fungistatic agent, underscores the importance of microbistatic mechanisms in endocarditis prophylaxis.

Endovascular infections caused by *Candida* species are increasing in frequency (7, 14, 17, 24). Such infections typically involve prosthetic heart valves (14) and indwelling, central catheters (17, 24), emphasizing the propensity of *Candida* species to adhere to prosthetic materials (13, 18, 19). Anti-*Candida* prophylactic strategies are particularly relevant for prosthetic valve recipients, since the patient has an increased risk of prothesis colonization by direct implantation at the time of surgery (14). Fluconazole is a new triazole antifungal agent with little toxicity even at high-dosage regimens, a favorable human pharmacokinetic profile, and good in vitro activity against most *Candida* strains (4, 6, 20), making this agent an ideal prophylactic candidate. We have previously confirmed the prophylactic and therapeutic efficacy of amphotericin B and fluconazole in the rabbit model of *C. albicans* endocarditis (27). Moreover, we recently showed that both agents were effective in the therapy of endocarditis due to non-*C. albicans* strains *C. tropicalis* and *C. parapsilosis* in the same rabbit model (28). These two *Candida* strains have emerged recently as important human pathogens, especially in endocarditis (1) as well as in nosocomial fungemia in the postoperative or immunocompromised patient (12, 13, 25). Additionally, *C. parapsilosis* is the dominant yeast isolated from the hands of health care workers (16, 21). The current study was designed to compare the prophylactic efficacies of amphotericin B and fluconazole in the rabbit model of *Candida* endocarditis caused by these two non-*C. albicans* strains.

The two *Candida* strains used in this study (*C. tropicalis* and *C. parapsilosis*) were clinical isolates from the Clinical Microbiology Laboratory of Harbor-UCLA Medical Center and were used in a prior study of treatment efficacy in the rabbit endocarditis model (28). For preparation of the infecting fungal inoculum, organisms were grown overnight to stationary phase in yeast extract peptone broth (Difco, Detroit, Mich.) supplemented with 1% glucose at 30°C on a rotating platform. Following sonication (to ensure singlet blastospore suspensions [12]), pelleting of the cells, washing, and resuspension in 0.85% NaCl, the cell preparation was counted and adjusted with a hemocytometer to achieve the final appropriate infecting fungal inoculum ($\sim 2 \times 10^7$ CFU/ml). Confirmation of the infecting fungal inoculum was made by serial dilution plate counts in yeast nitrogen base agar (Difco) supplemented with 5% glucose.

Fluconazole, provided by Pfizer Central Research (Groton, Conn.), was reconstituted in Cremophor EL (Sigma Chemicals, St. Louis, Mo.) according to the manufacturer's directions to a final concentration of 12.5 mg/ml for intraperitoneal administrations (27). Amphotericin B was purchased from commercial sources and prepared following the manufacturer's directions for intravenous (i.v.) administrations.

The antimicrobial susceptibilities to amphotericin B and fluconazole of *C. parapsilosis* and *C. tropicalis* were determined by the broth macrodilution method with synthetic amino acid medium for fungi (courtesy of M. Rinaldi, San Antonio, Tex.). The 24-h in vitro antifungal susceptibilities of the two strains were determined in parallel with final inocula of $\sim 10^4$ and 10^6 CFU/ml to encompass the range of *Candida* densities ordinarily achieved within experimental vegetations in the rabbit endocarditis model (27, 28). These susceptibility data, which have been previously reported (28), are listed in Table 1 and are typical for most non-*C. albicans* strains at our center (9a). As noted for amphotericin B, both strains were susceptible to the fungistatic and fungicidal action of this agent, with no apparent in vitro inoculum effect being observed after a comparison of the 10^4- and 10^6-CFU/ml inocula. In contrast, fluconazole was fungistatic against these two *Candida* strains, with a significant disparity between the minimal fungistatic and fungicidal concentrations for both in vitro inocula.

We used the same dose regimens for amphotericin B and fluconazole in this study as in two previously published experimental endocarditis studies (27, 28). Therefore, no drug levels in serum were measured in the current investigation.

Experimental aortic valve endocarditis was induced in rabbits as previously described (27, 28). 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 the 95% infective dose inoculum ($\sim 2 \times 10^7$ CFU) of either strain (28).

* Corresponding author. Mailing address: Division of Adult Infectious Diseases, Harbor-UCLA Medical Center, 1000 W. Carson St. (Bldg. RB2/Room 225), Torrance, CA 90509. Phone: (310) 222-3815. Fax: (310) 782-2016. Electronic mail address: BAYER@HUMC.EDU.

TABLE 1. In vitro susceptibilities of *C. parapsilosis* and *C. tropicalis* to the fungistatic and fungicidal activities of amphotericin B and fluconazole[a]

Organism	MIC/MFC (μg/ml)[b]			
	Amphotericin B		Fluconazole	
	10^4-CFU/ml inoculum	10^6-CFU/ml inoculum	10^4-CFU/ml inoculum	10^6-CFU/ml inoculum
C. parapsilosis	<0.14/0.58	<0.14/1.16	2.5/10	2.5/>80
C. tropicalis	<0.14/0.58	<0.14/1.16	<1.25/>80	<1.25/>80

[a] These data have been previously published (28).
[b] MIC and MFC, minimal fungistatic and fungicidal concentrations, respectively.

Two distinct prophylactic regimens were utilized: amphotericin B (1 mg/kg of body weight, i.v., given 1 h before i.v. challenge with *Candida* species, with or without a second dose administered at 24 h postchallenge) and fluconazole (100 mg/kg, intraperitoneal, given 1 h before challenge with *Candida* species, with or without a second dose administered at 24 h postchallenge). The two-dose regimens were designed to mirror the two-dose regimens currently recommended by the American Heart Association for the prevention of bacterial endocarditis during bacteremia-inducing procedures (e.g., dental surgery [5]). A separate group of rabbits with *C. parapsilosis* or *C. tropicalis* endocarditis did not receive antifungal prophylaxis and served as controls.

Rabbits were sacrificed at 24 h after the last antifungal dose (to mitigate in vivo antimicrobial carryover effects). In animals with properly placed transaortic valve catheters, cardiac vegetations from each animal were removed, weighed, serially diluted (to further limit antimicrobial carryover effects), and quantitatively cultured in yeast nitrogen glucose agar at 30°C for 48 h to calculate the $\log_{10}$ CFU per gram of vegetation. Only animals with culture-negative vegetations were considered to have had *Candida* endocarditis prevented.

Differences in endocarditis induction rates for the antifungal regimens were compared by Fisher's exact test or the chi-square test, with the Yates correction factor being used as appropriate. A probability value of 0.05 was considered statistically significant.

In the absence of antifungal prophylaxis, all catheterized rabbits challenged with either *Candida* strain developed endocarditis. For both *Candida* strains, neither amphotericin B nor fluconazole was effective in preventing experimental endocarditis when administered in single-dose regimens, yielding prophylactic efficacies of from 0 to 29% (Table 2). In contrast, both amphotericin B and fluconazole were effective prophylactically when administered in two-dose regimens. For amphotericin B, prophylactic efficacies of 65 and 79% were observed for *C. parapsilosis* and *C. tropicalis*, respectively. Similarly, for fluconazole, prophylactic efficacies of 85 and 81% were observed for *C. parapsilosis* and *C. tropicalis*, respectively. For controls without prophylaxis and animals failing prophylaxis, vegetation fungal densities were not significantly different (mean of ~6 $\log_{10}$ CFU/g of vegetation).

As opposed to amphotericin B, fluconazole is an attractive agent for anti-*Candida* prophylaxis because of its oral bioavailability, wide tissue distribution, excellent in vitro antifungal activity against most candidal species, and lack of significant toxicities (4, 6, 20). Recent studies have demonstrated the excellent prophylactic efficacy of fluconazole in preventing *Candida* mucositis in patients seropositive for human immunodeficiency virus (15, 22) and in preventing mucosal and visceral candidiasis in cancer chemotherapy and transplant patients (11, 26). Emergence of non-*C. albicans Candida* strains as important causes of fungemia and disseminated infection, especially in immunocompromised patients (13, 25), prompted the current study to examine the prophylactic efficacy of fluconazole in a rabbit model of *Candida* endocarditis. This model represents a rigorous test for antimicrobial prophylactic efficacy because of the high challenge inoculum used to induce infection as well as the high densities of fungal organisms achieved within experimental endocarditis vegetations. Moreover, the paucity of inflammatory cell influx within infected endocarditis vegetations (1) and the nonparticipation of polymorphonuclear leukocytes in mediating antibiotic-induced endocarditis prophylaxis (3) place the burden for prophylactic efficacy upon the antimicrobial agent.

The present studies showed that both fluconazole and amphotericin B were active in preventing the induction of *Candida* endocarditis caused by *C. tropicalis* and *C. parapsilosis* when the drugs were administered in two-dose (but not single-dose) regimens. The fact that fluconazole, a predominantly fungistatic agent, exhibited prophylactic efficacy equivalent to that of amphotericin B, a fungicidal agent, emphasizes the role of growth inhibition in achieving successful prophylaxis against endocarditis, as noted above. Previous studies with animal models of bacterial endocarditis have clearly documented that the mechanism of antibiotic prophylaxis is largely independent of bactericidal effects (3, 10). Instead, antibiotic prophylaxis is dependent on two interrelated pharmacodynamic parameters: time above MIC and the duration of inhibitory activity in serum induced by the antibiotic of interest (2, 8). In this regard, our previous pharmacokinetic data generated in the same rabbit endocarditis model used in this current study predicted that fluconazole would be an ideal prophylactic agent against the induction of *Candida* endocarditis (27). Thus, the two-dose fluconazole regimen of 100-mg/kg doses would be expected to achieve supra-MICs in serum for both *Candida* strains utilized in the present investigation for ~48 h postinfection.

In contrast to fluconazole, amphotericin B exhibited potent fungicidal effects in vitro against the two non-*C. albicans Candida* strains used in the present study. As in our prior study showing successful prophylaxis against induction of *C. albicans* endocarditis by amphotericin B (27), the current study documented the prophylactic efficacy of this agent against the two non-*C. albicans Candida* strains. However, as opposed to those of fluconazole, the pharmacokinetics and pharmacodynamics of amphotericin B remain incompletely characterized (9, 23). Therefore, it is not currently possible to correlate pharmacodynamic parameters with the excellent prophylactic efficacy

TABLE 2. Fluconazole and amphotericin B in the prophylaxis of experimental *Candida* endocarditis due to non-*C. albicans* strains *C. parapsilosis* and *C. tropicalis*

Agent	No. of doses[a]	No. with endocarditis prevented/no. challenged (%)	
		C. parapsilosis	*C. tropicalis*
Fluconazole	1	0/14 (0)	0/14 (0)
	2	12/14 (85)[b]	16/19 (81)[b]
Amphotericin B	1	0/14 (0)	4/14 (29)
	2	13/20 (65)[b]	10/13 (79)[b]
None		0/20 (0)	0/16 (0)

[a] Fluconazole was administered intraperitoneally at 100 mg/kg, and amphotericin B was administered i.v. at 1 mg/kg.
[b] $P < 10^{-4}$ versus controls.

exhibited by amphotericin B against induction of *Candida* endocarditis.

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