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Comparative Efficacies of Amphotericin B, Triazoles, and Combination of Both as Experimental Therapy for Murine Trichosporonosis

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We assessed the activities of amphotericin B deoxycholate, liposomal amphotericin B, fluconazole, and SCH 39304 against 10 strains of *Trichosporon beigelii* in mice with hematogenous infections. Cyclophosphamide-immunosuppressed CF1 male mice were challenged intravenously with a lethal inoculum of *T. beigelii* (5×10^6 conidia per mouse) and were assigned to different treatment groups or were left untreated. Amphotericin B deoxycholate (1 mg/kg of body weight and liposomal amphotericin B (1, 5, and 10 mg/kg) were given parenterally once daily. Escalating doses (5, 10, and 20 mg/kg/day) of fluconazole and SCH 39304 were tested. We also compared the activity of amphotericin B deoxycholate plus fluconazole (1 and 10 mg/kg/day, respectively) with that of each agent alone. Fluconazole significantly prolonged the survival of mice infected with each of the 10 strains tested. Amphotericin B deoxycholate achieved various responses, improving the outcomes in mice infected with seven of the strains. Liposomal amphotericin B was not more effective than amphotericin B deoxycholate against the two strains tested. Both fluconazole and SCH 39304 reduced the kidney fungal counts in a dose-dependent pattern, with SCH 39304 being more active than fluconazole against one of the two strains tested. The activity of the combination of amphotericin B deoxycholate plus fluconazole appeared to be superior to that of either agent alone, especially in reducing the kidney fungal burden. Fluconazole is more active than amphotericin B deoxycholate against experimental murine trichosporonosis.

Trichosporon beigelii is increasingly recognized as a fungal opportunistic pathogen. Infection with this pathogen is frequently disseminated and carries a high mortality rate, particularly in the setting of immunosuppression (3, 6–11, 13, 16–26, 28, 32). The treatment of choice for disseminated trichosporonosis remains to be established. While amphotericin B is active against a variety of fungal infections, the agent appears to have limited activity against *T. beigelii* (32). In recent years, several antifungal agents have become available. These include liposomal amphotericin B and the triazoles fluconazole and SCH 39304 (2, 4, 5). In the present work, we evaluated these antifungal agents and the role of combination antifungal chemotherapy in the treatment of disseminated murine trichosporonosis. We sought to determine whether triazoles were as efficacious as amphotericin B deoxycholate and whether liposomal amphotericin B was superior to amphotericin B deoxycholate. Additionally, we tried to determine whether a dose response to triazole therapy was present and whether the combination of a triazole with amphotericin B was associated with antagonism.

MATERIALS AND METHODS

Animals. Outbred CF1 male mice (average weight, 25 g; Harlan Breeding Laboratories, Indianapolis, Ind.) were used. All mice were housed in standard boxes with corncob bedding and were given food and water ad libitum. Five mice were housed in each box.

Organisms and culture conditions. Ten *T. beigelii* isolates were obtained from the following sources: The University of Texas M. D. Anderson Cancer Center, Houston (isolates 3001, 008, 009, 147, 341, M83, M220, and M236); M. Rinaldi at the Fungus Testing Laboratory, Department of Pathology, The University of Texas Health Science Center at San Antonio (isolate TCM); and T. Walsh at the National Institutes of Health, Bethesda, Md. (isolate TSA). The organisms were subcultured from water stocks onto Sabouraud dextrose agar plates. Twenty-four hours after incubation at 35°C, 5 ml of saline was added to the plates. A bent glass rod was gently used to dislodge the colonies from the medium in the plate. The suspension was then filtered through 12 layers of sterile gauze to remove the hyphae and the remaining clumps of organisms. Sterile normal saline was added to each suspension as needed to obtain a 40% transmittance by spectrophotometry (Spectronic 20; Bausch & Lomb, Rochester, N.Y.). Hemacytometric and spectrophotometric readings were verified by serial dilution on Sabouraud dextrose agar plates that were incubated at 35°C for 24 h. All final suspensions contained $1.1 \times 10^7 \pm 0.4 \times 10^7$ CFU/ml, with more than 90% of the organisms being in conidial forms.

Drugs. Fluconazole was received as powder from Pfizer Inc. (Groton, Conn.). SCH 39304 was provided by Schering-Plough Corp. (Kenilworth, N.J.) in powder form. Amphotericin B deoxycholate was purchased as Fungizone from E. R. Squibb & Sons (Princeton, N.J.). Liposomal amphotericin B (Ambisome) was provided by Vestar, Inc. (San Dimas, Calif.). Fluconazole and SCH 39304 were prepared as solutions in normal saline. Amphotericin B deoxycholate and liposomal amphotericin B were prepared according to the manufacturers' recommendations. The concentrations of the antifungal agents were adjusted for intraperitoneal injection (amphotericin B deoxycholate, SCH 39304, and fluconazole) and intravenous injection (liposomal amphotericin B). When the activ-

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ity of amphotericin B deoxycholate was directly compared with that of liposomal amphotericin B, the solutions were administered intravenously.

Immunosuppression of animals. Twenty-four hours before fungal inoculation all animals received one intraperitoneal injection of cyclophosphamide (Bristol Laboratories, Syracuse, N.Y.) at a dose of 150 mg/kg of body weight. Leukocyte and differential counts from the peripheral blood of randomly selected animals (15%) were monitored at the baseline and then every other day for 7 days after cyclophosphamide injection as described previously (12).

Experimental design. The conidia of *T. beigelii* (5×10^6 CFU per mouse) were inoculated into the lateral tail veins of transiently immunosuppressed CF1 mice. Control animals were immunosuppressed with cyclophosphamide but were not infected. One hour after receiving a fungal inoculation, groups of 20 animals each received either one of the antifungal agents or no drug. All drugs were given for 10 days; amphotericin B and liposomal amphotericin B were given once daily and the triazoles were given twice daily. Five mice from each experimental group, inoculated as described previously, were killed 4 days after being infected. The kidneys of each of these animals were removed aseptically, transferred into sterile polyethylene bags, and homogenized in 5 ml of sterile saline. Samples were removed, serially diluted in 0.9% NaCl, plated onto Sabouraud dextrose agar plates, incubated at 35°C, and examined at 48 h. The colonies were counted, and the numbers of CFU per gram of tissue were calculated. The remaining 15 animals from each experimental group were observed for mortality for up to 30 days after the challenge. The activities of the various agents were compared by examining the survival rates of infected mice and/or the clearance of fungi from the kidneys. Experiments were repeated to confirm the reproducibility of the results.

In the first series of experiments, we compared the survival of mice infected with any of the 10 strains of *T. beigelii* and left untreated with that of mice treated with either fluconazole (10 mg/kg twice daily) or amphotericin B (1 mg/kg/day). Using survival rate and the clearance of fungi from the kidneys, we then compared the activity of amphotericin B (1 mg/kg/day against two strains [*T. beigelii* 008 and 009]) with that of liposomal amphotericin B (1, 5, and 10 mg/kg/day). Finally, we tested escalating doses (5, 10, and 20 mg/kg/day) of fluconazole and SCH 39304 against two strains (*T. beigelii* 009 and TCM) and compared the survival rate and the clearance of fungi from the kidneys.

In a second series of experiments we compared the activity of 4 days of therapy with amphotericin B (1 mg/kg/day) plus fluconazole (10 mg/kg/day) with that of each agent alone (same dosage schedule) against *T. beigelii* 009.

Statistical analysis. The results of the organ clearance and survival experiments were analyzed by the Mann-Whitney U test, and significance was defined as $P \leq 0.05$.

RESULTS

Both amphotericin B and the triazoles had activity against *T. beigelii*, although mice infected with 3 of the 10 strains failed to respond to amphotericin B (Table 1). Overall, fluconazole appeared to be superior to amphotericin B (Table 1). Liposomal amphotericin B produced no significant prolongation of survival compared with amphotericin B (data not shown), although a dose-related decrease in the kidney fungal burden in mice infected with isolate 008 was observed (Table 2). When escalating doses of either fluconazole or SCH 39304 were studied, a dose-dependent improvement in clearing kidney infections was seen with one of the two strains tested (strain 009) (Table 3). SCH 39304 was more active than fluconazole against one isolate (isolate 009) but not against the other (isolate TCM). For isolate 009 in particular, triazole therapy resulted in up to a 2-log-unit reduction in the fungal titers, while the effect of amphotericin B was marginal (Tables 2 and 3). The combination of amphotericin B and fluconazole prolonged survival ($P = 0.08$) and significantly reduced the burden of infection in the kidneys of infected animals ($P < 0.005$) compared with the results obtained with either drug administered as a single agent (Fig. 1 and Table 4).

TABLE 1. Survival of mice infected with *T. beigelii* and treated with amphotericin B or fluconazole

Infecting isolate	Survival (median [range] days)		
	No therapy	Amphotericin B (1 mg/kg/day)	Fluconazole (20 mg/kg/day)
008	5 (3–8)	5 (3–>30)	30[a] (>30)
009	6 (5–9)	7 (5–>30)	30[a] (>30)
TCM	5 (3–8)	5[b] (5–>30)	30[a] (>30)
M236	4 (2–6)	7[b] (5–>30)	15[a] (7–>30)
M220	5 (2–7)	8[b] (5–>30)	15[c] (11–>30)
M147	4 (2–6)	30[b] (5–>30)	16[c] (7–>30)
M83	5 (1–5)	30[b] (5–>30)	30[b] (7–>30)
TSA	5 (2–7)	6 (2–>30)	13[b] (7–>30)
3001	5 (4–6)	30[b] (4–>30)	30[c] (>30)
M341	5 (3–7)	30[b] (>30)	30[c] (9–>30)

[a] $P \leq 0.01$ versus no therapy and amphotericin B.
[b] $P \leq 0.05$ versus no therapy.
[c] $P \leq 0.05$ versus no therapy and amphotericin B.

DISCUSSION

Our results indicate that amphotericin B, fluconazole, and SCH 39304 have in vivo activity against experimental murine disseminated trichosporonosis. Fluconazole appeared to be more active than amphotericin B, whereas SCH 39304 was more active than fluconazole. This apparent greater efficacy of SCH 39304 may be related to the longer half-life of this drug in mice (8 h) compared with that of fluconazole (4.5 h). Unfortunately, SCH 39304 is no longer available because of its association with liver carcinogenicity in rodents. The activity of amphotericin B varied, with some strains exhibiting a good in

TABLE 2. Effects of amphotericin B and liposomal amphotericin B on clearance of fungi from kidneys of mice with disseminated trichosporonosis

Treatment group (dose [mg/kg/day])	Isolate 009		Isolate 008	
	Mean ± SE $\log_{10}$ CFU/g of kidney tissue[a]	*P* value vs no therapy	Mean ± SE $\log_{10}$ CFU/g of kidney tissue[a]	*P* value vs no therapy
No therapy	5.93 ± 0.33		6.87 ± 0.18	
Amphotericin B (1)	5.75 ± 0.04	NS[b]	5.88 ± 0.13	<0.05
Liposomal amphotericin B				
1	6.05 ± 0.08	NS	6.27 ± 0.11	<0.05
5	5.66 ± 0.39	NS	6.13 ± 0.07	<0.05
10	5.38 ± 0.35	<0.05	5.76 ± 0.23	<0.05

[a] Results represent data from three experiments with five mice in each experimental group.
[b] NS, not statistically significant.

TABLE 3. Effects of escalating doses of triazoles on kidney fungal burden of mice with disseminated trichosporonosis[a]

Treatment group (dose [mg/kg/day])	Isolate 009		Isolate TCM	
	Mean ± SE $\log_{10}$ CFU/g of kidney tissue[a]	*P* value vs no therapy	Mean ± SE $\log_{10}$ CFU/g of kidney tissue[a]	*P* value vs no therapy
No therapy	6.12 ± 0.18		6.39 ± 0.27	
Fluconazole				
5	5.84 ± 0.42	NS[b]	4.97 ± 0.37	NS
10	5.39 ± 0.42	<0.05		
20	5.01 ± 0.47[c]	<0.05	4.79 ± 0.29	<0.05
SCH 39304				
5	5.32 ± 0.38	<0.05	4.92 ± 0.42	<0.05
10	4.72 ± 0.48	<0.05		
20	4.13 ± 0.13[c]	<0.05	4.74 ± 0.27	<0.05

[a] Results represent data from three experiments with five mice per experimental group.
[b] NS, not statistically significant.
[c] $P \leq 0.05$ versus 5 mg/kg/day.

TABLE 4. Effect of treatment with amphotericin B, fluconazole, and a combination of both agents on clearance of fungi from kidneys of mice infected with *T. beigelii* 009

Treatment group (dose [mg/kg/day])	Mean ± SE $\log_{10}$ CFU/g of kidney tissue[a]
No therapy	6.53 ± 0.30
Amphotericin B (1)	5.34 ± 0.35[b]
Fluconazole (10)	5.29 ± 0.24[b]
Amphotericin B (1) + fluconazole (10)	4.88 ± 0.20[b,c]

[a] Results represent data from three experiments with five mice per experimental group.
[b] $P \leq 0.001$ versus no therapy.
[c] $P < 0.01$ versus either agent alone.

vivo response and others showing relative resistance to this agent. Of interest is the limited benefit obtained by the use of escalating doses of liposomal amphotericin B, perhaps indicating an intrinsic resistance of some strains to polyenes, regardless of the dosage used. These data suggest that the *T. beigelii* species may be a heterogeneous group of organisms, with important differences among the strains yet to be determined (15). This heterogeneity seems to be quite different from the pattern observed with *Candida* species.

Our results also suggest that amphotericin B and fluconazole may interact favorably against murine trichosporonosis, although only one isolate of *T. beigelii* was examined. This is in contrast to a previously held belief that azoles and polyenes may be antagonistic (29). Our data are in agreement, however, with other studies that have shown a benefit from the addition of these two classes of agents for the treatment of yeast infections (1, 14, 27, 30).

Limited clinical experience in eight patients treated with azoles confirms our experimental data that these antifungal agents may represent effective therapy for trichosporonosis (4). Our experimental results with the triazoles are also in agreement with those obtained from studies on disseminated trichosporonosis in rabbits (31); however, in the latter study, amphotericin B deoxycholate appeared to lack activity against the experimental infections.

While the results of our experiments are encouraging, we would like to point out that only a few strains of *T. beigelii* were tested: 10 strains for the comparative studies between fluconazole and amphotericin B and only 1 or 2 strains for the other studies. In addition, sterilization of the kidneys did not occur

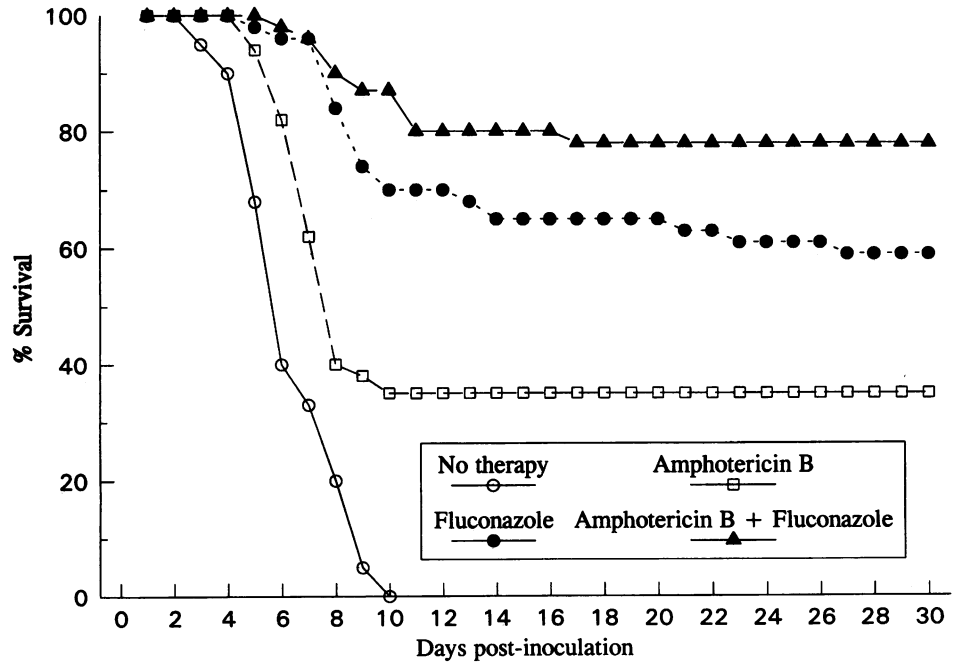

FIG. 1. Effect of 4 days of therapy with amphotericin B deoxycholate (1 mg/kg/day), fluconazole (10 mg/kg/day), or the combination of both agents (at the same dosage schedule) on the survival of mice with disseminated trichosporonosis (isolate 009). Results represent data from three experiments performed with 15 mice each.

with any of the tested agents, implying that none had fungicidal activity against *T. beigelii*, at least at the dosage schedules used. In contrast to the findings in experimental candidiasis with *Candida albicans*, which responds to 0.5 mg of fluconazole per kg/day, much higher fluconazole doses are needed to affect experimental trichosporonosis. This could be explained, at least partly, by the lowest MICs of fluconazole for *C. albicans* (MIC for 90% of isolates tested, 0.5 μg/ml) compared with those for *T. beigelii* (MIC for 90% of isolates tested, 16 μg/ml) (unpublished data).

In conclusion, we demonstrated that amphotericin B and the triazoles fluconazole and SCH 39304 are efficacious against disseminated murine trichosporonosis and that fluconazole may be superior to amphotericin B under the experimental conditions used. At the doses tested, fluconazole showed enhanced therapeutic efficacy when it was combined with amphotericin B. Liposomal amphotericin B did not appear to improve the antifungal activity of the parent compound, although higher doses might have resulted in a better outcome.

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