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Evaluation of Possible Correlations between Antifungal Susceptibilities of Filamentous Fungi In Vitro and Antifungal Treatment Outcomes in Animal Infection Models

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Nine isolates of filamentous fungi previously tested in 11 different laboratories for their susceptibilities to amphotericin B and itraconazole in vitro were injected intravenously into mice and guinea pigs, and responses to treatment with both agents were studied. The experiments were done in a single laboratory. Mean survival times, the percentages of animals surviving 12 days after infection, and culture results for samples of deep organs obtained postmortem were used as markers of antifungal efficacy. Because of variations in organism pathogenicity, interpretable test systems in vivo could not be established for *Fusarium* spp. in mice or guinea pigs or for *Pseudallescheria boydii* in mice, even with the use of immunosuppressive pretreatments. Among the infections that could be evaluated, some degree of response to the corresponding treatment in vivo was seen in animals infected with each of two *Rhizopus arrhizus* isolates susceptible to amphotericin B at <0.5 μg/ml and *Aspergillus* spp. isolates susceptible to itraconazole at <1.0 μg/ml. Conversely, no responses were apparent with infecting strains for which MICs were ≥2 μg/ml (amphotericin B) or ≥1 μg/ml (itraconazole). However, the limitations of the intravenous challenge systems studied mean that no firm conclusion relating MICs in vitro to the lowest effective doses in vivo could be drawn.

The National Committee for Clinical Laboratory Standards Subcommittee on Antifungal Susceptibility Tests has published a standard method, method M27-A, for determining the MICs of five antifungal agents for yeasts of clinical importance (15). Interpretive breakpoints for *Candida* spp. have been proposed for fluconazole and itraconazole on the basis of a comparison of the clinical outcome of treatment of infected patients with these agents at the MICs for the yeast(s) isolated (16). The importance of susceptibility testing with pathogenic filamentous fungi (molds) has been less thoroughly studied than the importance of susceptibility testing with yeasts, but the topic has not been overlooked. Studies of standardization of conidial inoculum suspensions (9) have progressed to collaborative interlaboratory evaluations of the M27 reference method adapted to the testing of molds (11). A large-scale study involving 11 laboratories and 30 isolates representing six species of opportunistic mold pathogens has shown a high level of interlaboratory agreement among the MICs determined by a broth microdilution adaptation of the M27 method (10).

The clinical importance of mold infections in immunocompromised hosts cannot be overstated (8, 14, 18). However, the incidence of infections with most opportunistic mold pathogens is too low to permit a large-scale prospective comparison of antifungal MICs for molds with the clinical results of antifungal treatment. For this reason we sought confirmation of the relevance of mold susceptibilities in vitro with the results of treatment in animal test systems for mold infections. The nine isolates studied were selected from those already tested in vitro in 11 different centers (10) to represent ranges of mold species and susceptibilities in vitro that were as broad as possible. Two antifungal agents, amphotericin B and itraconazole, were tested in the experiments since these are the two drugs in current clinical use for the treatment of opportunistic mold infections. The experiments were carried out in the laboratory of F. C. Odds; the experimental design and evaluation of the results were done in collaboration with the National Committee for Clinical Laboratory Standards Subcommittee on Antifungal Susceptibility Testing.

MATERIALS AND METHODS

Fungi. *Aspergillus flavus* 01, *Aspergillus fumigatus* 10, *Fusarium oxysporum* 15, *F. oxysporum* 15a, *Fusarium solani* 16, *Pseudallescheria boydii* 24, *P. boydii* 25, *Rhizopus arrhizus* 26, and *R. arrhizus* 27 were received in the course of a collaborative study on mold susceptibility testing (10). They were chosen to represent strains for which the MICs of amphotericin B and itraconazole in vitro covered a broad range, as indicated in Table 1. The fungi were grown at 30°C on potato-dextrose agar (Difco Laboratories, Detroit, Mich.) until they were judged to have formed maximal numbers of conidia (4 to 14 days). The conidia were suspended in sterile physiologic saline containing one drop of Tween 80 per 100 ml. The numbers of CFU per milliliter in the suspensions were determined by plating dilutions of the suspensions on Sabouraud agar (Oxoid, Basingstoke, United Kingdom). Suspensions were stored at −70°C in small lots, and individual lots were thawed for use.

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TABLE 1. MICs of amphotericin B and itraconazole for nine mold isolates used to treat infections in animals[a]

Fungal isolate	Amphotericin B MIC (μg/ml)		Itraconazole MIC (μg/ml)	
	Mode	Range	Mode	Range
A. flavus 01	1	0.5–4	0.5	0.12–0.5
A. fumigatus 10	1	0.5–2	0.25	0.12–1
F. oxysporum 15	2	1–4	>16	4–>16
F. oxysporum 15a	2	1–4	>16	8–>16
F. solani 16	1	0.5–2	>16	>16
P. boydii 24	4	2–8	1	0.25–1
P. boydii 25	4	4–16	1	0.5–4
R. arrhizus 26	0.25	0.12–0.5	2	0.5–4
R. arrhizus 27	0.25	0.25–0.5	>16	2–>16

[a] The modal MICs and ranges were recorded in 11 laboratories after 24 h of incubation (*R. arrhizus*) or 48 h of incubation (other species) and colorimetric endpoint determination (10). At least 76% and usually >90% of each set of MIC determinations were within ±1 dilution of the mode MIC.

Antifungal agents. Amphotericin B was purchased as Fungizone intravenous suspension (Bristol Myers-Squibb, Brussels, Belgium) and was resuspended for use in sterile water according to the manufacturer's instructions. Itraconazole (Janssen Pharmaceutica, Beerse, Belgium) was formulated in 40% hydroxypropyl-β-cyclodextrin. A 40% solution of hydroxypropyl-β-cyclodextrin was used as a placebo. All agents were administered intraperitoneally (i.p.) in these experiments. This route of administration was chosen to allow both agents to be given by the same route and to overcome the known problems of toxicity when amphotericin B is given to mice intravenously (i.v.) (5, 12). In guinea pigs, repeated i.v. dosing is technically impossible, so that i.p. injections were also deemed the most appropriate way to allow both drugs to be given to this animal by a common route.

Animal test systems. Albino Pirbright guinea pigs and outbred Swiss White mice were used as animal hosts for the fungal challenges. Guinea pigs were housed individually and were supplied with guinea pig food pellets (Hope Farms, Woerden, The Netherlands) and water ad libitum. Mice were housed in groups of 10 and were supplied with rodent food pellets (Pavan Service, Oud Turnhout, Belgium) and water ad libitum.

In a series of pilot experiments, different concentrations of conidial suspensions were injected i.v. via a lateral vein of the penis in guinea pigs and a lateral tail vein in mice to establish the mean survival time for each inoculum concentration. The goal was to determine the inoculum levels that gave a mean survival time of approximately 7 days. With the *Fusarium* spp. in both animal hosts and with *P. boydii* in mice, even concentrations of conidia as high as 5×10^7 to 1×10^8 per kg of body weight given i.v. failed to establish a lethal infection. Animals were therefore subjected to immunosuppressive pretreatments. In mice, cyclophosphamide was injected at a dose of 150 mg/kg i.p. 24 h before infection (13). In guinea pigs, pretreatments with cyclophosphamide (20 mg/kg given i.p.) and mechlorethamine hydrochloride (0.25 mg/kg given i.p.) were used. Cyclophosphamide was injected on days 5, 4, and 1 before infection and on the day of infection itself. Mechlorethamine hydrochloride was injected on days 5 and 4 before infection.

Antifungal treatments were begun 1 h after infection in all experiments, and the treatments were repeated once daily for 10 days. Animals still alive on day 12 were killed. All animals were autopsied. Entire lungs, liver, spleen, and kidneys were homogenized in sterile saline, and samples were plated on Sabouraud agar. The cultures were incubated at 30°C for 5 days and were recorded as positive if one or more colonies of the fungus used for infection appeared in the culture. Guinea pigs were tested in groups of 5, and mice were tested in groups of 10. For some combinations of fungus and antifungal agent, experiments were repeated up to four times. Run-to-run reproducibility was generally good; data from all experiments were therefore pooled for analysis.

Three measurements were used to assess the outcome of infection and treatment: the mean survival time, the percentage of infected animals that survived to day 12, and the percentage of tissues negative for the infecting fungus at autopsy.

Measurement of itraconazole concentrations in plasma. Samples of blood were taken from the retroorbital plexus of groups of two animals before and at intervals after i.p. injection of itraconazole. Levels of itraconazole and its active metabolite, hydroxy-itraconazole, in plasma prepared from these samples were determined by high-performance liquid chromatography as described previously (25).

RESULTS

In guinea pigs, inocula of *Fusarium* spp. as high as 1×10^8 CFU/kg (an average of 5×10^7 CFU per animal) failed to induce either a lethal infection or any fungus-positive cultures from liver, spleen, lung, or kidney samples obtained postmortem, even in animals pretreated with cyclophosphamide or mechlorethamine hydrochloride to induce neutropenia. Antifungal treatment effects could therefore not be assessed in guinea pigs with the *F. solani* and the two *F. oxysporum* isolates.

Amphotericin B given by repeated i.p. administration has been found to be safe and efficacious for a large range of experimental fungal infections in mice (see, e.g., references 1, 5, and 12). A dose of 10 mg/kg given i.p. to mice produces peak levels in serum of 1.94 μg/ml (1). Both amphotericin B and itraconazole have previously been demonstrated to have efficacy in many guinea pig models of fungal infection when given by the i.p. route (17). In the present studies, measurement of combined levels of itraconazole plus hydroxy-itraconazole after i.p. treatment showed a mean peak concentration in mouse serum of 3.53 μg/ml 2.5 h after injection of 20 mg/kg and 0.30 μg/ml after injection of 5 mg/kg, falling to 1.72 and 0.05 μg/ml, respectively, at 8 h. In guinea pigs, the corresponding figures were 2.25 and 0.37 μg/ml (peak) and 1.21 and 0.14 μg/ml at 8 h.

Table 2 lists the results of challenge and treatment of guinea pigs with the test isolates of *A. flavus*, *A. fumigatus*, *P. boydii*, and *R. arrhizus*. The *Aspergillus* and *Rhizopus* isolates caused fatal infections by i.v. administration at the doses indicated in Table 2, whereas the two isolates of *P. boydii* seldom killed infected animals but led to uniformly culture-positive internal organs. For *A. flavus* 01, none of the test doses of amphotericin B led to the prolongation of survival, an increase in the percentage of animals surviving, or an increase in the frequency of negative cultures of samples from organs obtained at autopsy relative to the results for placebo-treated controls. Itraconazole, by contrast, showed positive effects for all three parameters. With *A. fumigatus* 10, both antifungal agents at all test doses prolonged survival and led to increases in the percentage of culture-negative organs.

For both test isolates of *P. boydii*, neither amphotericin B nor itraconazole treatments led to any significant increase in negative cultures of samples from the organs of infected animals. Almost all animals, regardless of their treatment, survived for the 12 days of the experiment. With the two *R. arrhizus* isolates, a rapidly fatal infection was induced, with placebo-treated guinea pigs surviving only for an average of 3 to 4 days after infection. Treatment with amphotericin B at 0.63 mg/kg and higher doses prolonged survival in this model, although few animals survived for the full 12 days of observation. Amphotericin B treatment did not lead to negative cultures of samples from any organs processed postmortem. Itraconazole treatments caused no changes in any of the parameters studied at any of the doses tested.

The results of experimental mold infections and antifungal treatments in mice are presented in Table 3. In the murine host, *A. flavus* 01 induced a level of infection comparable to that in guinea pigs, and also in this model, itraconazole but not amphotericin B showed a positive effect on the outcomes of survival and resulted in culture-negative organs.

Without immunosuppressive pretreatment of mice, the two isolates of *P. boydii* produced only a mildly lethal infection even at an inoculum of 5×10^7 CFU/kg (approximately 1 million to 2 million conidia per animal). Higher inocula could not be used because the amount of conidia produced by these isolates was too small. Treatment with itraconazole and amphotericin B was attempted for *P. boydii* 24 and *P. boydii* 25, respectively, but with equivocal results since the mean survival times for placebo-treated animals were already high (Table 3). When mice were pretreated with cyclophosphamide, they became highly susceptible to infection with both *P. boydii* isolates.

TABLE 2. Effects of infection and antifungal treatment for guinea pigs inoculated with molds i.v.

Organism (inoculum [CFU/kg]) and treatment and dose (mg/kg)	No. of animals infected	MST ± SEM (days)	% Survivors on day 12	% Negative organs
A. flavus 01 (2.5×10)				
Placebo (0)	15	5.9 ± 0.5	0	0
Amphotericin B				
0.31	5	3.8 ± 0.2	0	0
0.63	5	4.2 ± 0.2	0	0
1.25	5	4.4 ± 0.3	0	0
2.5	5	4.6 ± 0.7	0	0
Itraconazole				
2.5	15	9.3 ± 1.0	67	18
5.0	15	9.6 ± 1.0	67	27
10	15	11.3 ± 0.5	87	33
20	15	11.7 ± 0.2	87	47
A. fumigatus 10 (1×10^7)				
Placebo (0)	15	6.7 ± 0.8	13	3
Amphotericin B				
0.31	10	11.4 ± 0.3	70	25
0.63	10	11.4 ± 0.4	80	35
1.25	10	10.9 ± 0.8	80	38
2.5	10	11.1 ± 0.9	90	45
Itraconazole				
2.5	5	10.6 ± 1.0	60	25
5.0	5	10.6 ± 1.6	80	50
10	5	12.0 ± 0.0	100	70
20	5	11.4 ± 0.4	60	65
P. boydii 24 (5×10^7)				
Placebo (0)	10	12.0 ± 0.0	100	0
Amphotericin B				
0.31	ND	ND	ND	ND
0.63	5	12.0 ± 0.9	100	15
1.25	5	11.2 ± 0.0	80	5
2.5	5	12.0 ± 0.0	100	15
Itraconazole				
2.5	5	12.0 ± 0.0	100	0
5.0	10	12.0 ± 0.0	100	5
10	10	12.0 ± 0.0	100	5
20	10	11.7 ± 0.3	90	0
P. boydii 25 (5×10^7)				
Placebo (0)	10	12.0 ± 0.0	100	0
Amphotericin B				
0.31	5	12.0 ± 0.0	100	0
0.63	10	12.0 ± 0.0	100	0
1.25	10	12.0 ± 0.0	100	0
2.5	10	12.0 ± 0.0	100	0
Itraconazole				
2.5	ND	ND	ND	ND
5.0	5	12.0 ± 0.0	100	0
10	5	12.0 ± 0.0	100	0
20	5	11.4 ± 0.7	80	0
R. arrhizus 26 (2.5×10^7)				
Placebo (0)	5	3.0 ± 0.6	0	0
Amphotericin B				
0.31	5	5.4 ± 0.3	0	0
0.63	5	6.6 ± 1.6	20	0
1.25	5	6.2 ± 1.7	20	0
2.5	5	5.0 ± 0.7	0	0
Itraconazole				
2.5	5	3.2 ± 0.4	0	0
5.0	5	3.0 ± 0.4	0	0
10	5	3.0 ± 0.7	0	0
20	5	3.8 ± 0.7	0	0
R. arrhizus 27 (2.5×10^7)				
Placebo (0)	10	3.9 ± 0.3	0	0
Amphotericin B	5	5.0 ± 1.0	0	0
0.31	5	6.8 ± 1.7	20	0
0.63	5	5.6 ± 2.9	20	0
2.5	5	7.2 ± 2.8	20	0
Itraconazole				
2.5	5	3.6 ± 0.3	0	0
5.0	5	3.8 ± 0.2	0	0
10	5	3.4 ± 0.3	0	0
20	5	4.6 ± 0.4	0	0

[a] Results are for daily i.p. treatments with 40% cyclodextrin placebo and with amphotericin B and itraconazole at the indicated doses. MST, mean survival time; ND, not determined.

At an i.v. inoculum of 2,000 CFU/g, the mean survival time of the mice was between 9 and 10 days, a figure similar to that measured with a much higher inoculum in unpretreated animals (Table 3). However, at an inoculum doubled to just 4×10^6 CFU/kg, all of the infected mice died within 3 to 4 days, and neither of the antifungal agents tested showed an effect on survival or organ positivity at any dose tested.

The results for the two *R. arrhizus* isolates in studies with mice were similar to those obtained in studies with guinea pigs, with itraconazole being ineffective and with amphotericin B treatment at all test concentrations leading to the prolongation of survival (Table 3). No culture-negative organ from mice infected with either *R. arrhizus* isolate was found, regardless of treatment with amphotericin B or itraconazole.

In mice, unlike guinea pigs, pretreatment with cyclophosphamide rendered the animals susceptible to infection with the three *Fusarium* isolates studied. However, for both *F. oxysporum* isolates few deaths occurred even with an inoculum of 10^8 CFU/kg (Table 3), and most organs were culture positive for the infecting species, regardless of the antifungal treatment. With *F. solani* 16, an inoculum of 10^8 CFU/kg led to fatal disseminated infections in pretreated mice, with a mean survival time of about 7 days. Neither of the antifungal agents tested produced curative effects in animals infected with *F. solani* in terms of either survival or the percentage of negative organs.

DISCUSSION

These experiments have reemphasized the difficulties of achieving reproducible, fatal experimental challenges with molds that resemble the situations that arise naturally in immunocompromised human hosts. The difficulties mean that a fewer than expected number of valid comparisons could be made between antifungal effects in vitro and in vivo. The goal of the study was to allow comparison of the MICs of two antifungal agents for nine molds in vitro with the minimum effective doses of those agents against experimental challenges with the same nine molds in vivo. For this purpose a range of four doses of each agent was chosen, and two animal hosts were used in case different effects of the compounds were found between guinea pigs and mice. Under ideal circumstances and to achieve scientific standardization, for all nine fungi a mean survival time of 7 days would have been achieved with the same infecting dose in unmodified hosts: such circumstances in vivo would have come closest to those used to determine MICs in vitro. In practice, different infecting doses were required for each fungus, and some isolates caused no

TABLE 3. Effects of infection and antifungal treatment for mice infected with molds i.v.[a]

Organism (inoculum [CFU/kg]) and treatment and dose (mg/kg)	No. of animals infected	MST ± SEM (days)	% Survivors on day 12	% Negative organs
A. flavus 01 (5×10^7)				
Placebo (0)	29	7.6 ± 0.6	17	6
Amphotericin B				
0.31	10	4.5 ± 0.5	0	ND
0.63	10	7.5 ± 1.0	20	ND
1.25	20	7.0 ± 0.9	35	3
2.5	20	5.5 ± 0.7	15	6
Itraconazole				
2.5	10	10.7 ± 0.8	70	18
5.0	10	11.3 ± 0.5	80	10
10	10	10.9 ± 0.6	70	10
20	10	12.0 ± 0.0	100	20
A. fumigatus 10 (5×10^7)				
Placebo (0)	40	10.5 ± 0.3	58	8
Amphotericin B				
0.31	30	11.7 ± 0.2	90	35
0.63	30	10.4 ± 0.5	70	25
1.25	30	10.5 ± 0.5	77	28
2.5	30	11.0 ± 0.5	77	43
Itraconazole				
2.5	20	10.1 ± 0.7	65	16
5.0	20	9.9 ± 0.6	50	18
10	20	11.2 ± 0.5	80	19
20	20	10.0 ± 0.9	75	18
P. boydii 24 (5×10^7)				
Placebo (0)	10	9.4 ± 1.0	50	0
Amphotericin B				
0.31	ND	ND	ND	ND
0.63	ND	ND	ND	ND
1.25	ND	ND	ND	ND
2.5	ND	ND	ND	ND
Itraconazole				
2.5	10	10.6 ± 0.8	70	0
5.0	10	12.0 ± 0.0	100	0
10	10	12.0 ± 0.0	100	0
20	10	10.6 ± 0.7	60	0
P. boydii 24 (2×10^6)[b]				
Placebo (0)	10	9.1 ± 1.0	40	0
Amphotericin B				
0.31	10	10.8 ± 0.7	70	0
0.63	10	8.9 ± 1.2	50	0
1.25	10	10.6 ± 1.0	80	0
2.5	10	7.4 ± 0.9	20	0
Itraconazole				
2.5	10	11.0 ± 0.7	80	0
5.0	10	8.6 ± 1.2	50	0
10	10	10.4 ± 0.9	70	0
20	10	9.6 ± 1.3	70	0
P. boydii 24 (4×10^6)[b]				
Placebo (0)	10	4.2 ± 0.2	0	0
Amphotericin B				
0.31	10	4.3 ± 0.2	0	0
0.63	10	4.2 ± 0.1	0	0
1.25	10	3.7 ± 0.2	0	0
2.5	10	4.0 ± 0.0	0	0
Itraconazole				
2.5	10	4.3 ± 0.2	0	0
5.0	10	4.1 ± 0.1	0	0
10	10	4.4 ± 0.2	0	0
20	10	4.5 ± 0.2	0	0
P. boydii 25 (5×10^7)				
Placebo (0)	10	11.0 ± 1.1	90	0
Amphotericin B				
0.31	10	11.5 ± 0.5	90	0
0.63	10	11.0 ± 0.7	80	0
1.25	10	11.0 ± 0.7	80	0
2.5	10	10.3 ± 0.8	60	0
Itraconazole				
2.5	ND	ND	ND	ND
5.0	ND	ND	ND	ND
10	ND	ND	ND	ND
20	ND	ND	ND	ND
P. boydii 25 (2×10^6)[b]				
Placebo (0)	10	8.5 ± 1.2	40	0
Amphotericin B				
0.31	10	10.6 ± 0.8	70	0
0.63	10	9.4 ± 1.0	50	0
1.25	10	11.9 ± 0.1	90	0
2.5	10	10.8 ± 0.7	70	0
Itraconazole				
2.5	10	10.0 ± 0.8	50	0
5.0	10	7.3 ± 0.9	20	0
10	10	6.9 ± 0.3	0	0
20	10	8.7 ± 0.9	36	0
P. boydii 25 (4×10^6)[b]				
Placebo (0)	10	3.3 ± 0.3	0	0
Amphotericin B				
0.31	10	3.5 ± 0.2	0	0
0.63	10	3.4 ± 0.2	0	0
1.25	10	3.2 ± 0.2	0	0
2.5	10	3.0 ± 0.0	0	0
Itraconazole				
2.5	10	3.2 ± 0.1	0	0
5.0	10	3.1 ± 0.1	0	0
10	10	3.1 ± 0.2	0	0
20	10	2.9 ± 0.1	0	0
R. arrhizus 26 (5×10^6)				
Placebo (0)	20	4.9 ± 0.5	5	0
Amphotericin B				
0.31	10	6.5 ± 0.8	10	0
0.63	10	7.4 ± 0.9	20	0
1.25	10	9.7 ± 0.8	40	0
2.5	10	10.9 ± 0.9	80	0
Itraconazole				
2.5	10	4.3 ± 0.4	0	0
5.0	10	3.6 ± 0.2	0	0
10	10	3.7 ± 0.3	0	0
20	10	4.0 ± 0.4	0	0
R. arrhizus 27 (5×10^6)				
Placebo (0)	10	5.3 ± 0.2	0	0
Amphotericin B				
0.31	10	11.9 ± 0.1	90	0
0.63	10	12.0 ± 0.0	100	0
1.25	10	12.0 ± 0.0	100	0
2.5	10	12.0 ± 0.0	100	0
Itraconazole				
2.5	10	4.1 ± 0.1	0	0
5.0	10	5.1 ± 0.5	0	0
10	10	4.4 ± 0.4	0	0
20	10	4.3 ± 0.3	0	0

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TABLE 3—*Continued*

Organism (inoculum [CFU/kg]) and treatment and dose (mg/kg)	No. of animals infected	MST ± SEM (days)	% Survivors on day 12	% Negative organs
F. oxysporum 15a (1×10^8)[b]				
Placebo (0)	10	10.8 ± 0.9	80	0
Amphotericin B				
0.31	10	8.0 ± 0.9	20	0
0.63	10	9.2 ± 0.9	30	0
1.25	10	10.8 ± 0.7	60	3
2.5	10	10.1 ± 1.0	60	0
Itraconazole				
2.5	10	9.5 ± 0.9	40	0
5.0	10	10.2 ± 1.3	80	8
10	10	10.3 ± 0.9	60	10
20	10	11.2 ± 0.3	60	5
F. oxysporum 15 (1×10^8)[b]				
Placebo (0)	10	12.0 ± 0.0	100	0
Amphotericin B				
0.31	10	11.7 ± 0.3	90	0
0.63	10	11.9 ± 0.1	90	0
1.25	10	11.5 ± 0.4	80	0
2.5	10	10.6 ± 1.0	80	0
Itraconazole				
2.5	10	12.0 ± 0.0	100	0
5.0	10	11.7 ± 0.3	90	0
10	10	11.7 ± 0.3	90	0
20	10	12.0 ± 0.0	100	0
F. solani 16 (1×10^8)[b]				
Placebo (0)	10	6.7 ± 1.4	30	0
Amphotericin B				
0.31	10	4.6 ± 0.4	0	0
0.63	10	6.6 ± 1.1	10	0
1.25	10	6.4 ± 0.9	0	0
2.5	10	8.1 ± 1.3	40	0
Itraconazole				
2.5	10	6.6 ± 1.3	30	0
5.0	10	5.8 ± 1.2	20	0
10	10	5.5 ± 1.1	20	0
20	10	5.7 ± 1.1	20	0

[a] See footnote *a* of Table 2.
[b] Mice were pretreated with cyclosphosphamide at 150 mg/kg i.p. before infection.

disseminated infections or they infected only modified hosts pretreated to achieve immunosuppression.

Others have commented on the difficulties of establishing reproducible disseminated infections in animal hosts by intravenous injection of molds known to be pathogenic only for severely immunocompromised human patients. We copied the cyclophosphamide pretreatment recommended by Legrand et al. (13) for establishing disseminated infections with *F. solani* in mice, but we did not achieve the same high levels of mortality, even though our infecting doses were higher than those used by the previous investigators. One reason for the difference might be our use of outbred rather than defined inbred murine hosts. Since mouse strains vary in their susceptibilities to disseminated infection with moderately pathogenic species such as *Candida albicans* (3, 7), it is likely that they also vary in their susceptibilities to less virulent molds. Moreover, different strains of pathogenic fungi also vary in their inherent virulence for animals (2, 4, 19). Thus, to optimize experimental test systems in vivo for any given fungal isolate, it is necessary to investigate the result of variations in host strain types, pretreatment regimens, infecting doses, and routes of infection. However, the performance of so many titrations on the scale necessary to allow statistical comparisons of the MICs for fungi with treatment outcomes prohibits optimization of the model conditions for logistic, economic, and practical reasons.

Because filamentous fungi tend to be less virulent than species such as *C. albicans* in vivo, endpoints based on survival and the numbers of samples that are culture positive may be too austere to allow detection of differences in response to antifungal therapy, particularly when subtleties in susceptibility are evaluated. More sensitive indicators, such as quantitative organ sample cultures and markers of fungus-mediated tissue injury (e.g., pulmonary infarct scores and lung weights in pulmonary aspergillosis), may reveal more subtle differences in antifungal activity. These markers have been used in a profoundly neutropenic rabbit model of invasive pulmonary aspergillosis (20).

The fact that the infections caused by most filamentous fungi produced minimal lethality may be related to the host response. Patients developing infections due to opportunistic filamentous fungi are usually profoundly immunocompromised, with profound and persistent neutropenia being the most critical factor. Such depths and durations of neutropenia are not usually achievable without supervening bacterial infections analogous to those that develop in patients. Moreover, the higher doses of antineoplastic therapy often require closer hematologic monitoring than is feasible in most models. The next step may be to examine these infections in the profoundly neutropenic rabbit models of *Aspergillus*, *Fusarium*, and *Pseudallescheria* infections. Bacterial infections are prevented and hematologic toxicity is monitored during intensive chemotherapy in these models, resulting in lethality and fungus-mediated tissue injury (20–22).

Our choice of i.p. administration of antifungal treatment was based on a desire to use the same route for both agents and both animal hosts and the logistic considerations of performing repeated parenteral treatment regimens with large numbers of animals. Published data for i.p. amphotericin B treatments in mice and guinea pigs have shown this route to be efficacious (1, 5, 12, 17). The i.p. route has also been used successfully with experimental itraconazole treatments in guinea pigs (17). Our data show that itraconazole administration by this route in mice produces high peak levels of drug in plasma that remain high after dosing at 20 mg/kg. At 5 mg/kg, levels of active antifungal azoles were still detectable, but they were low 8 h after i.p. administration.

The limitations of the infection outcomes markedly reduced the value of comparisons between MICs and minimum effective doses in the present study. From the data presented in Tables 2 and 3, it can be seen that itraconazole given i.p. at 2.5 mg/kg and higher doses showed antifungal activity in vivo, by at least one of the parameters used, against *A. flavus* 01 and *A. fumigatus* 10. It was inactive against two isolates of *R. arrhizus* at all concentrations tested. The results obtained with this agent against infections caused by the other mold isolates were too equivocal to allow an interpretation to be made. The modal itraconazole MICs were less than 1 μg/ml for the two *Aspergillus* isolates and 2 μg/ml or higher for the isolates of *R. arrhizus*. These observations suggest a theoretical breakpoint for the resistance of molds to itraconazole of between 1 and 2 μg/ml if the inherent susceptibility of the fungus to the antifungal agent is assumed to be the only variable affecting the outcome of treatment in the test systems used.

The efficacy of amphotericin B at 0.31 mg/kg was seen in vivo (by at least one parameter) against *A. fumigatus* 10 (MIC, 1 μg/ml) but efficacy was not seen against *A. flavus* 01 (MIC, also 1 μg/ml) at any dose tested. The compound also showed anti-

TABLE 4. Modal MICs of amphotericin B and itraconazole for nine mold isolates used in animal challenges and interpretive summary of the effects of these agents against experimental infections[a]

Results for amphotericin B			Results for itraconazole		
Fungus isolate	MIC (μg/ml)	Response	Fungus isolate	MIC (μg/ml)	Response
R. arrhizus 26	0.25	Active	*A. fumigatus* 10	0.25	Active
R. arrhizus 27	0.25	Active	*A. flavus* 01	0.5	Active
A. flavus 01	1	Not active	*P. boydii* 24	1	Not active
A. fumigatus 10	1	Active	*P. boydii* 25	1	Not active
F. solani 16	1	Not active	*R. arrhizus* 26	2	Not active
F. oxysporum 15	2	Not active	*F. oxysporum* 15	>16	Not active
F. oxysporum 15a	2	?[b]	*F. oxysporum* 15a	>16	?[b]
P. boydii 24	4	Not active	*F. solani* 16	>16	Not active
P. boydii 25	4	Not active	*R. arrhizus* 27	>16	Not active

[a] Results for the two compounds are listed separately, ranked in increasing order of MIC.
[b] Results are too inconclusive for interpretation.

fungal effects in vivo against the two *R. arrhizus* isolates (MICs, 0.2 μg/ml), although with *R. arrhizus* 26 such effects were less evident at the lowest test dose (0.31 mg/kg) than at higher doses in both mice and guinea pigs. Infections with *F. solani* 16 (amphotericin B MIC, 1 μg/ml) in cyclophosphamide-pretreated mice showed no response to amphotericin B treatment apart from a small increase in survival at 2.5 mg/kg (Table 3). These results do not suggest any obvious predictive association between amphotericin B MICs for various molds in vitro and the outcome of treatment of experimental infections with those molds.

Perhaps, as has been suggested elsewhere (6, 24), the minimum fungicidal concentrations (MFCs) of amphotericin B may correlate better with the outcome of treatment than the MIC. The in vitro-in vivo correlation has been demonstrated previously for *Trichosporon beigelii*, an organism for which the MIC of amphotericin B is ≤0.5 μg/ml (which is considered susceptible), but for which the MFC is markedly greater (>16 μg/ml; which is considered resistant). Time-kill assays demonstrated that *T. beigelii* was inhibited but not killed by safely achievable levels of amphotericin B (23). Studies in vivo demonstrated that amphotericin B given at 1 mg/kg/day i.v. failed in the treatment of a persistently neutropenic animal model of disseminated *Trichosporon* infection (21). By comparison, similar doses of amphotericin B, also in persistently neutropenic animals, successfully treated disseminated *C. albicans* infections in which both the MIC and the MFC for the infecting isolates were ≤0.5 μg/ml. Thus, perhaps MFCs may have predictive value for profoundly compromised hosts, in which the antifungal agent may be the only means of clearing tissue in the absence of intrinsic host defenses.

Table 4 provides an interpretive summary of the results of the animal experiments in which the activities of the test compounds could be determined in relation to the modal MICs previously determined for the infecting isolates (10). For both amphotericin B and itraconazole the treatment responses judged as showing some activity of the agent were associated with lower MICs than the responses judged as showing no activity in vivo. For the fungi for which the amphotericin B or the itraconazole MIC was less than 1 μg/ml, a response of some kind was seen in the experimental infections. For the fungi for which the amphotericin B MICs were at or above 2 μg/ml or the itraconazole MICs were at or above 1 μg/ml, no response was seen. However, the overlap (amphotericin B) and 1-dilution difference (itraconazole) in MICs associated with a response judged as active and inactive suggest that such MICs could not be interpreted as predicting treatment outcome in these animal models.

The conclusion of this study must be that a limited association between MIC and treatment outcome was seen but that such associations could be determined with confidence for only four of nine test fungi because of the limitations of the models used. More definitive evaluation of the predictive worth of MICs for molds in vitro must await further experimentation in studies with animals and the accumulation of data from case histories for humans with opportunistic mold infections.

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REFERENCES

1. **Albert, M. M., J. R. Graybill, and M. G. Rinaldi.** 1991. Treatment of murine cryptococcal meningitis with an SCH-39304–amphotericin B combination. Antimicrob. Agents Chemother. **35:**1721–1725.
2. **Anaissie, E. J., N. C. Karyotakis, R. Hachem, M. C. Dignani, J. H. Rex, and V. Paetznick.** 1994. Correlation between in vitro and in vivo activity of antifungal agents against *Candida* species. J. Infect. Dis. **170:**384–389.
3. **Ashman, R. B., E. M. Bolitho, and A. Fulurija.** 1995. Cytokine mRNA in brain tissue from mice that show strain-dependent differences in the severity of lesions induced by systemic infection with *Candida albicans* yeast. J. Infect. Dis. **172:**823–830.
4. **Barchiesi, F., L. K. Najvar, M. F. Luther, G. Scalise, M. G. Rinaldi, and J. R. Graybill.** 1996. Variation in fluconazole efficacy for *Candida albicans* strains sequentially isolated from oral cavities of patients with AIDS in an experimental murine candidiasis model. Antimicrob. Agents Chemother. **40:**1317–1320.
5. **Clemons, K. V., and D. A. Stevens.** 1992. Efficacies of amphotericin B lipid complex (ABLC) and conventional amphotericin B against murine coccidioidomycosis. J. Antimicrob. Chemother. **30:**353–363.
6. **Colombo, A. L., D. A. McGough, and M. G. Rinaldi.** 1994. Discrepancies between MIC and MLC values of amphotericin B against isolates of *Aspergillus* species. Mycopathologia **128:**129–133.
7. **Costantino, P. J., N. F. Gare, and J. R. Warmington.** 1995. Humoral immune responses to systemic *Candida albicans* infection in inbred mouse strains. Immunol. Cell. Biol. **73:**125–133.
8. **Denning, D. W., J. Y. Lee, J. S. Hostetler, P. Pappas, C. A. Kauffman, D. H. Dewsnup, J. N. Galgiani, J. R. Graybill, A. M. Suger, A. Catanzaro, H. Galli, J. R. Perfect, B. Dockery, W. E. Dismukes, and D. A. Stevens.** 1994. NIAID mycoses study group multicenter trial of oral itraconazole therapy of invasive aspergillosis. Am. J. Med. **97:**135–144.
9. **Espinel-Ingroff, A., and T. M. Kerkering.** 1991. Spectrophotometric method of inoculum preparation for the in vitro susceptibility testing of filamentous fungi. J. Clin. Microbiol. **29:**393–394.
10. **Espinel-Ingroff, A., J. H. Rex, R. Bowden, P. Menezes, M. Bartlett, M. McGinnis, C. Cooper, F. C. Odds, M. Pfaller, S. A. Messer, P. W. Nelson, M. G. Rinaldi, A. Fothergill, G. Shankland, T. J. Walsh, J. Peter, I. Weitzman, and N. X. Chin.** 1996. Multicenter evaluation of proposed standardized

procedure for antifungal susceptibility testing of filamentous fungi. J. Clin. Microbiol. **35:**139–143.
11. **Espinel-Ingroff, A., K. Dawson, M. Pfaller, E. Anaissie, B. Breslin, D. Dixon, A. Fothergill, J. Peter, M. Rinaldi, and T. Walsh.** 1995. Comparative and collaborative evaluation of standardization of antifungal susceptibility testing for filamentous fungi. Antimicrob. Agents Chemother. **39:**314–319.
12. **Graybill, J. R., and R. Bocanegra.** 1995. Liposomal amphotericin B therapy of murine histoplasmosis. Antimicrob. Agents Chemother. **39:**1885–1887.
13. **Legrand, C., E. Anaissie, R. Hashem, P. Nelson, G. P. Bodey, and J. Ro.** 1991. Experimental fusarial hyalohyphomycosis in a murine model. J. Infect. Dis. **164:**944–948.
14. **Matsumoto, T., L. Ajello, T. Matsuda, P. J. Szaniszlo, and T. J. Walsh.** 1994. Developments in hyalohyphomycosis and phaeohyphomycosis. J. Med. Vet. Mycol. **32**(Suppl. 1)**:**329–349.
15. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **Rex, J. H., M. A. Pfaller, J. N. Galgiani, M. S. Bartlett, A. Espinel-Ingroff, M. A. Ghannoum, M. Lancaster, F. C. Odds, M. G. Rinaldi, T. J. Walsh, and A. L. Barry.** 1997. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro-in vivo correlation data for fluconazole, itraconazole, and *Candida* infections. Clin. Infect. Dis. **24:**235–247.
17. **Van Cutsem, J.** 1990. Oral and parenteral treatment with itraconazole in various superficial and systemic experimental fungal infections. Comparisons with other antifungals and combination therapy. Br. J. Clin. Pract. Suppl. **71:**32–40.
18. **Vartivarian, S. E., E. J. Anaissie, and G. P. Bodey.** 1993. Emerging fungal pathogens in immunocompromised patients: classification, diagnosis, and management. Clin. Infect. Dis. **17**(Suppl. 2)**:**S487–S491.
19. **Velez, J. D., R. Allendoerfer, M. Luther, M. G. Rinaldi, and J. R. Graybill.** 1993. Correlation of in vitro azole susceptibility with in vivo response in a murine model of cryptococcal meningitis. J. Infect. Dis. **168:**508–510.
20. **Walsh, T. J., K. Garrett, E. Feuerstein, M. Girton, M. Allende, J. Bacher, A. Francesconi, R. Schaufele, and P. A. Pizzo.** 1995. Therapeutic monitoring of experimental invasive pulmonary aspergillosis by ultrafast computerized tomography, a novel, noninvasive method for measuring responses to antifungal therapy. Antimicrob. Agents Chemother. **39:**1065–1069.
21. **Walsh, T. J., J. W. Lee, G. P. Melcher, E. Navarro, J. Bacher, D. Callender, K. D. Reed, T. Wu, G. Lopez-Berestein, and P. A. Pizzo.** 1992. Experimental *Trichosporon* infection in persistently granulocytopenic rabbits—implications for pathogenesis, diagnosis and treatment of an emerging opportunistic mycosis. J. Infect. Dis. **166:**121–133.
22. **Walsh, T. J., J. W. Lee, E. Roilides, P. Francis, J. Bacher, C. A. Lyman, and P. A. Pizzo.** 1992. Experimental antifungal chemotherapy in granulocytopenic animal models of disseminated candidiasis—approaches to understanding investigational antifungal compounds for patients with neoplastic diseases. Clin. Infect. Dis. **14:**S139–S147.
23. **Walsh, T. J., G. Melcher, M. Rinaldi, J. Lecciones, D. McGough, J. Lee, D. Callender, M. Rubin, and P. A. Pizzo.** 1990. *Trichosporon beigelii*: an emerging pathogen resistant to amphotericin B. J. Clin. Microbiol. **28:**1616–1622.
24. **Walsh, T. J., J. Peter, D. A. McGough, A. W. Fothergill, M. G. Rinaldi, and P. A. Pizzo.** 1995. Activity of amphotericin B and antifungal azoles alone and in combination against *Pseudallescheria boydii*. Antimicrob. Agents Chemother. **39:**1361–1364.
25. **Woestenborghs, R., W. Lorreyne, and J. Heykants.** 1987. Determination of itraconazole in plasma and animal tissues by high-performance liquid chromatography. J. Chromatogr. Biomed. Appl. **413:**332–337.