ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1999, p. 1256–1257 Vol. 43, No. 5
0066-4804/99/$04.00+0

In Vitro and In Vivo Experimental Activities of Antifungal Agents against *Fusarium solani*

J. GUARRO,[1]* I. PUJOL,[2] AND E. MAYAYO[3]

Unitat de Microbiologia, Facultat de Medicina, Universitat Rovira i Virgili,[1] and Laboratori de Microbiologia, Hospital Universitari de Sant Joan de Reus,[2] 43201 Reus, and Servei de Patologia, Hospital Universitari de Tarragona Joan XXIII, Tarragona,[3] Tarragona, Spain

Received 28 September 1998/Returned for modification 21 December 1998/Accepted 11 February 1999

In the treatment of disseminated *Fusarium* infections, amphotericin B either alone or in combination with flucytosine and rifampin is the drug therapy most frequently used. The efficacy of these antifungal drugs was evaluated in a murine disseminated-infection model, with five strains of *Fusarium solani*. All the treatments were clearly ineffective.

In recent years *Fusarium solani* has become one of the most important fungi causing hyalohyphomycosis in humans (1). The optimal treatment regimen for patients with disseminated infections has not yet been established, but rapid diagnosis and recovery of the neutrophil count seem to be essential for survival. Despite its low activity in vitro (9–11), amphotericin B remains the drug of choice for the treatment of *Fusarium* infections, sometimes together with flucytosine and rifampin and less frequently with azoles or exogenous growth factors (5). However, the efficacy of such combinations has not been proved in animal models, so the advisability of their use in the clinical setting is debatable. The toxicity of these drugs must be taken into account when they are used in a combined therapy (4, 6).

In this study we evaluated the use of combinations of amphotericin B with rifampin and with flucytosine in comparison with the use of amphotericin B alone in the treatment of experimental hyalohyphomycosis by *F. solani*. We also observed whether the treatment outcome correlated with the in vitro results.

MICs of amphotericin B in combination with flucytosine and with rifampin and MICs of each of these drugs alone were determined by a checkerboard microdilution method, with serial twofold dilutions of each drug or drug combination. Five clinical isolates of *F. solani* were used. Four strains were from skin infections, and one was isolated from blood. Stock solutions of amphotericin B (intravenous Fungizone; E. R. Squibb & Sons, Barcelona, Spain) and rifampin (intravenous Rifadin; Marion Merrell Dow, S.A., Madrid, Spain), each at 1,000 μg/ml, were prepared with sterile distilled water. Flucytosine was provided by Hoffmann-La Roche (Basel, Switzerland) as standard powder, and a stock solution of 5,000 μg/ml was also prepared with sterile distilled water. The drug dilutions were prepared with sterile distilled water to provide 10 (to test a single drug) and 20 (to test combinations of drugs) times the final drug concentration, and they were further diluted 1:5 with RPMI 1640 medium. To test a single drug, 100-μl volumes of the 2× rifampin, flucytosine, and amphotericin B dilutions were dispensed into the wells of the first column and row of microplates. To test combinations of drugs, 50 μl each of the 4× rifampin or flucytosine and amphotericin B dilutions was dispensed into the appropriate wells, to yield 100 μl per well. This procedure effectively diluted each drug 1:2. Each well of the microdilution plate was inoculated with 100 μl of the inoculum suspension containing 1×10^4 to 5×10^4 conidia/ml prepared as described previously (9). This step brought the drug dilutions to the final test concentrations (0.07 to 36.94 μg/ml for amphotericin B, 1.25 to 40 μg/ml for rifampin, 10.08 to 322.75 μg/ml for flucytosine, 0.07/1.25 to 36.94/40 μg/ml for the combination of amphotericin B and rifampin, and 0.07/10.08 to 36.94/322.75 μg/ml for the combination of amphotericin B and flucytosine) and yielded an inoculum of 1×10^3 to 5×10^3 conidia/ml. The inoculated plates were incubated at 30°C without agitation. After 48 h of incubation, MIC readings of each drug and drug combination were taken. Drug interaction was classified as synergistic, additive, indifferent, or antagonistic on the basis of the fractional inhibitory concentration index (3, 7).

OF_1 male mice (Charles River, Griffa S.A., Barcelona, Spain) weighing 30 g were used. A 200-μl volume of inoculum suspension at 2.5×10^7 conidia/ml (5×10^6 conidia/mouse) of each strain of *F. solani* was injected into the lateral tail vein. The inoculated mice were randomly housed, 10 per cage, and assigned to one of five treatment groups: amphotericin B (1.5 mg/kg of body weight/day intraperitoneally); amphotericin B (1.5 mg/kg/day intraperitoneally) plus flucytosine (150 mg/kg/day given once a day by gavage with a blunt metal cannula); amphotericin B (1.5 mg/kg/day intraperitoneally) plus rifampin (20 mg/kg/day given once a day by gavage); control group 1, saline solution (0.1 ml/day intraperitoneally); and control group 2, saline solution (0.1 ml/day intraperitoneally) plus sterile distilled water (0.2 ml/day by gavage). Therapy began 2 h after challenge and continued for 10 days, and mortality was recorded daily for 30 days. The significance of the differences in survival among groups was analyzed by the Kaplan-Meier product limit.

The combinations of amphotericin B and each of the other two drugs showed a synergistic effect for only one strain (FMR 5207). Interactions of the combined drugs were indifferent for the rest of the strains tested (Table 1). In all cases, the in vitro inhibitory action of both flucytosine and rifampin was greatly enhanced by the addition of amphotericin B.

Survival data for the five isolates tested are presented in Table 2. No statistically significant differences were observed between the median survival times for mice treated with am-

* Corresponding author. Mailing address: Unitat de Microbiologia, Facultat de Medicina, Universitat Rovira i Virgili, Carrer Sant Llorenç 21, 43201 Reus, Tarragona, Spain. Phone: 977-759359. Fax: 977-759322. E-mail: umb@astor.urv.es.

TABLE 1. MICs of amphotericin B, rifampin, and flucytosine alone and in combination[a]

Strain	MIC (μg/ml)					FIC index	
	AMB	RIF	5FC	AMB + RIF	AMB + 5FC	AMB + RIF	AMB + 5FC
FMR 5207	2.31	>40	>322.75	1.16/0.62	1.16/5.04	0.51	0.51
FMR 4391	1.16	>40	>322.75	1.16/0.62	1.16/5.04	1.01	1.01
FMR 4928	1.16	>40	>322.75	1.16/0.62	1.16/5.04	1.01	1.01
FMR 4927	1.16	>40	>322.75	1.16/0.62	1.16/5.04	1.01	1.01
FMR 4931	1.16	>40	>322.75	1.16/0.62	1.16/5.04	1.01	1.01

[a] Tests were performed with fungal inocula of 10^3 conidia/ml at 30°C and 48 h of incubation. FIC, fractional inhibitory concentration; AMB, amphotericin B; RIF, rifampin; 5FC, flucytosine.

photericin B and those for the controls with any of the five strains tested ($P > 0.5$), with the exception of strain FMR 4928, in which case the survival time was lower in treated animals (6 days) than in controls (13 days) ($P = 0.0391$). No significant differences were noticed when the combination therapies of amphotericin B with rifampin and amphotericin B with flucytosine were tested.

Despite the increasing number of *Fusarium* infections, only two studies on the correlation between in vitro and in vivo infections in an experimental model have been performed (2, 8). In the more recent study, Odds et al. (8) failed to establish a model for *Fusarium* infection in mice and guinea pigs, while being successful in establishing models for other molds. In the study of Anaissie et al. (2), two isolates of *F. solani* were used and the infected mice received amphotericin B intraperitoneally in daily doses of 0.5, 1, and 2 mg/kg for 10 days. These therapeutic regimens did not prolong survival of treated animals or have a significant effect on fungal burden. Amphotericin B did not exhibit in vivo activity against any of the five strains tested in our study either. Neither was in vivo activity observed in any combination tested against the five strains used. In general, this is in agreement with in vitro results. However, further experimental studies with a number of *F. solani* strains for which the MICs are considerably lower would be needed to corroborate this correlation. Finding such strains is very difficult; the in vitro studies performed on the majority of *Fusarium* isolates have shown high MICs and high minimal fungicidal concentrations (9–13).

TABLE 2. Survival of mice infected with *F. solani* and treated with amphotericin B alone or combined with rifampin or flucytosine

Strain	Treatment group[a]	No. of animals that died/ no. tested	Survival time (days)	
			Median (SE)	Range
FMR 5207	Control 1	10/10	2 (0.5)	1–14
	Control 2	10/10	2 (0.7)	1–22
	AMB 1.5 ip	10/10	3 (1.0)	1–8
	AMB 1.5 ip + RIF 20 or	10/10	2 (3.6)	1–27
	AMB 1.5 ip + 5FC 150 or	10/10	4 (1.1)	0–13
FMR 4391	Control 1	10/10	9 (2.1)	1–16
	Control 2	10/10	9 (2.1)	1–13
	AMB 1.5 ip	9/10	13 (2.6)	7–>30
	AMB 1.5 ip + RIF 20 or	9/10	13 (2.0)	4–>30
	AMB 1.5 ip + 5FC 150 or	10/10	10 (1.5)	1–22
FMR 4928	Control 1	9/10	13 (3.1)	2–>30
	Control 2	10/10	2	2–16
	AMB 1.5 ip	10/10	6[b] (2.6)	2–13
	AMB 1.5 ip + RIF 20 or	10/10	3 (0.5)	2–14
	AMB 1.5 ip + 5FC 150 or	10/10	3 (0.7)	2–16
FMR 4927	Control 1	9/9	7 (4.4)	2–15
	Control 2	8/8	7 (4.9)	2–19
	AMB 1.5 ip	10/10	3 (1.1)	2–19
	AMB 1.5 ip + RIF 20 or	9/10	3 (0.3)	2–>30
	AMB 1.5 ip + 5FC 150 or	10/10	6 (1.5)	3–14
FMR 4931	Control 1	8/8	14 (0.4)	11–19
	Control 2	8/8	14 (0.9)	11–18
	AMB 1.5 ip	9/10	14 (3.3)	3–>30
	AMB 1.5 ip + RIF 20 or	10/10	14 (2.1)	2–26
	AMB 1.5 ip + 5FC 150 or	9/10	14 (4.6)	2–>30

[a] Control 1, saline solution; control 2, saline solution plus sterile distilled water; AMB 1.5 ip, amphotericin B at 1.5 mg/kg/day intraperitoneally; RIF 20 or, rifampin at 20 mg/kg/day orally; 5FC 150 or, flucytosine at 150 mg/kg/day orally.
[b] $P < 0.5$ versus control value (Wilcoxon rank sum).

The lack of efficacy of amphotericin B was already known (2), but the efficacy of therapy with this drug combined with either rifampin or flucytosine, commonly used to treat severe invasive infections caused by *Fusarium*, had not been tested in vitro. The treatment of disseminated fusarial infections, which is critical in neutropenic patients, is still an unsolved problem. In theory, the use of lipid-associated formulations of amphotericin B or the use of new triazole antifungal agents or of cytokines still gives hope for possible new approaches to the treatment of such infections. However, before treatments are used in the clinical setting, proof of their efficacy in adequate animal models is required. The possible presence of antagonistic effects between polyene antifungal agents and other antifungal drugs means that they should not be used together indiscriminately until the effects of their use in combination have been demonstrated experimentally to be favorable.

This work was supported by a grant from the Fundació Ciència i Salut of Spain.

We thank F. C. Odds (Janssen Research Foundation, Beerse, Belgium) for his critical comments.

REFERENCES

1. **Anaissie, E., H. Kantarajan, J. Ro, R. Hopfer, K. Rolston, V. Fainstein, and G. Bodey.** 1988. The emerging role of *Fusarium* infections in patients with cancer. Medicine **67:**77–83.
2. **Anaissie, E. J., R. Hachem, C. Legrand, P. Legenne, P. Nelson, and G. P. Bodey.** 1992. Lack of activity of amphotericin B in systemic murine fusarial infection. J. Infect. Dis. **165:**1155–1157.
3. **Berenbaum, M. D.** 1978. A method for testing for synergy with any number of agents. J. Infect. Dis. **137:**122–130.
4. **Gallis, H. A., R. H. Drew, and W. W. Pickard.** 1990. Amphotericin B: 30 years of clinical experience. Rev. Infect. Dis. **12:**308–329.
5. **Guarro, J., and J. Gené.** 1995. Opportunistic fusarial infections in humans. Eur. J. Clin. Microbiol. Infect. Dis. **14:**741–754.
6. **Hoeprich, P. D.** 1992. Clinical use of amphotericin B and derivatives: lore, mystique, and fact. Clin. Infect. Dis. **14**(Suppl. 1)**:**S114–S119.
7. **Nguyen, M. H., F. Barchiesi, D. A. McGough, V. L. Yu, and M. G. Rinaldi.** 1995. In vitro evaluation of combination of fluconazole and flucytosine against *Cryptococcus neoformans* var. *neoformans*. Antimicrob. Agents Chemother. **39:**1691–1695.
8. **Odds, F. C., F. Van Gerven, A. Espinel-Ingroff, M. S. Bartlett, M. A. Ghannoum, M. V. Lancaster, M. A. Pfaller, J. H. Rex, M. G. Rinaldi, and T. J. Walsh.** 1998. Evaluation of possible correlations between antifungal susceptibilities of filamentous fungi in vitro and antifungal treatment outcomes in animal infection models. Antimicrob. Agents Chemother. **42:**282–288.
9. **Pujol, I., J. Guarro, J. Gené, and J. Sala.** 1997. In-vitro antifungal susceptibility of clinical and environmental *Fusarium* spp. strains. J. Antimicrob. Chemother. **39:**163–167.
10. **Reuben, A., E. Anaissie, P. E. Nelson, R. Hashem, C. Legrand, D. H. Ho, and G. P. Bodey.** 1989. Antifungal susceptibility of 44 clinical isolates of *Fusarium* species determined by using a broth microdilution method. Antimicrob. Agents Chemother. **33:**1647–1649.
11. **Rotowa, N. A., H. J. Shadomy, and S. Shadomy.** 1990. In vitro activities of polyene and imidazole antifungal agents against unusual opportunistic fungal pathogens. Mycoses **33:**203–211.
12. **Sekhon, A. S., A. A. Padhye, A. K. Garg, H. Ahmad, and N. Moledina.** 1994. In vitro sensitivity of medically significant *Fusarium* species to various antimycotics. Chemotherapy (Basel) **40:**239–244.
13. **Stern, G. A.** 1978. In vitro antibiotic synergism against ocular fungal isolates. Am. J. Ophthalmol. **86:**359–367.