ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1999, p. 1716–1718 Vol. 43, No. 7
0066-4804/99/$04.00+0

Activities of Sordarins in Murine Histoplasmosis

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Received 30 December 1998/Returned for modification 26 February 1999/Accepted 23 April 1999

Sordarins are new antifungals which inhibit fungal protein synthesis by blocking elongation factor 2. Three compounds were evaluated in a murine model of histoplasmosis. Immune-competent mice were infected intravenously with 10^6 to 10^8 CFU of *Histoplasma capsulatum* yeast cells. Mice were treated either orally with sordarins or fluconazole from day 2 through 8 after infection or intraperitoneally with amphotericin B during the same period. Protection was measured by increased rates of survival for 30 days after infection or reduction of lung or kidney tissue counts 9 days after infection. All three of the antifungal drugs tested were protective compared with controls. Sordarins were effective at doses as low as 2 mg/kg of body weight/day. This novel class of drugs compared favorably with amphotericin B and fluconazole for the treatment of histoplasmosis.

Histoplasmosis has been recognized as a life-threatening opportunistic infection associated with AIDS (9, 10, 12, 14, 16). Treatment with amphotericin B is effective, but toxicity is a significant side effect (10). Treatment with triazoles is also effective, but because antifungal drug action is slower, these drugs are reserved for less critically ill patients (13, 15). There remains an interest in identifying new classes of antifungal drugs that are rapidly acting, potent, and well tolerated. Sordarins have the potential of being such a class of agents. They interfere with protein synthesis in fungi through a novel mechanism of inhibiting protein elongation factor 2 (4–7, 17). Sordarins have been shown to inhibit a variety of fungal pathogens, including *Candida* species and *Pneumocystis carinii* (1, 2, 8). In the present studies, we evaluated the activities of three compounds against infection with *Histoplasma capsulatum*.

MATERIALS AND METHODS

Pathogen. *H. capsulatum* clinical isolate 93-255 was obtained from the Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio. The organism was maintained in the yeast phase on Sabouraud's agar at 37°C for infection of mice. In vitro testing of conidia was performed by the National Committee for Clinical Laboratory Standards macrobroth method but with incubation continued until the control tube showed visible growth and an end point of 80% inhibition could be determined (11).

Antifungals. Three sordarins, GM211676A (GM1), GM237354A (GM2), and GM193663A (GM3), were obtained from Glaxo Wellcome, Tres Cantos, Madrid, Spain, and were prepared by dissolving in water. Fluconazole (Pfizer, New York, N.Y.) was dissolved in 0.3% Noble agar. Amphotericin B (Bristol-Myers Squibb, Wallingford, Conn.) was suspended in sterile water.

Animals. Outbred immune-competent ICR male mice, 25 to 30 g, were obtained from Charles River Laboratories (Boston, Mass.). Prior to infection, *H. capsulatum* colonies were scraped from the agar, agitated, washed three times in sterile saline, and suspended in sterile saline for infection. Cells were counted in a hemacytometer for an estimate of the inoculum, and the counts were confirmed by serial colony count dilutions in the yeast phase. Inocula are reported as viable cells. Unanesthetized mice were infected intravenously with 10^6 to 10^8 CFU.

Treatment. The infection was allowed to become established for 2 days before the mice were randomized for treatment. Groups of mice were treated either by gavage with water (controls), fluconazole at 5 or 10 mg/kg of body weight twice daily, or one of the GM compounds, in doses ranging from 0.06 to 50 mg/kg twice daily, or intraperitoneally with amphotericin B at 0.06, 0.3, 1.25, 2.5, or 5 mg/kg once daily. Drugs were administered in 0.2-ml volumes per dose.

Protection measures and statistics. For survival studies, groups of 10 mice were treated from day 2 through 8 after infection and observed through day 30. The log rank and Wilcoxon tests of life tables were used for comparisons. Because of multiple comparisons, a P value of <0.001 was considered significant for survival studies. For tissue burden studies, groups of 7 mice were treated from day 2 through 8 and sacrificed on day 9 after infection. The lungs and kidneys were harvested by an aseptic technique. The organs were homogenized, and serial 10-fold colony count dilutions were plated on brain heart infusion agar plates supplemented with 10% sheep blood. These were incubated at 37°C for 2 weeks, and yeast cell colonies were counted. Dunnett's one-tailed t test was used for comparisons of tissue counts, with a P value of <0.05 determining significance.

RESULTS

In vitro, at 7 days of incubation, the drug concentrations to which *H. capsulatum* was found to be susceptible were <0.1 μg/ml for GM1, GM2, and GM3; 0.125 μg/ml for amphotericin B; and 4 μg/ml for fluconazole.

For each drug, a toxicity study was performed, in which 10 uninfected mice were treated with 50 mg/kg twice daily for 7 days. In the group treated with GM1, 1 mouse died, but the other 9 mice showed no evidence of illness. There was no mortality or apparent illness in the mice treated with the other two drugs. Three studies of survival were done. In the first screening study (Table 1), where high doses of a drug were

TABLE 1. Mean days of survival of mice infected with *H. capsulatum*[a]

Drug or treatment	Dose (mg/kg)	No. of days of survival		No. alive after 30 days
		Mean	SEM	
Control	None	9.5	2.4	1
GM1	50	27.7[b]	2.5	8
	10	25.2[b]	3.1	7
GM2	50	25.7[b]	3.5	8
	10	28.4[b]	2.6	9
GM3	50	30[b]	0.3	9
	10	30[b]	0	10

[a] Mice were infected intravenously with 1.2×10^7 CFU of *H. capsulatum*, treated from day 2 through 8, and observed for their rate of survival through day 30. Ten mice were in each treatment group.
[b] $P < 0.001$ compared with the control group.

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TABLE 2. Survival of mice infected intravenously with *H. capsulatum*[a]

Study and inoculum (CFU)	Drug or treatment	Dose (mg/kg)	No. of days of survival		No. alive at day 30
			Mean	SEM	
Study A (2.0×10^6)	Control	None	15.2	2.9	2
	GM2	20	30[b]	0	10
		10	28.4[b]	2.6	9
		5	29.1[b]	1.9	9
		2	30[b]	0	10
	Amphotericin B	1	30[b]	0	10
		0.5	30[b]	0	10
	Fluconazole	10	23.2	4.0	7
		5	20.1	3.7	5
Study B (10^8)	Control	None	4.0	0.14	0
	GM3	5	6.7[b]	0.9	0
		1.25	4.9	0.4	0
		0.3	4.0	0.3	0
		0.06	4.1	0.2	0
	Amphotericin B	1.25	28.4[b]	2.6	9
		0.3	4.8	0.2	0
	Fluconazole	10	13.1[b]	3.2	2

[a] Mice were treated from day 2 through 8 after infection, and their rates of survival were observed through day 30. Ten mice were in each treatment group.
[b] $P < 0.001$ compared with the control group.

administered orally twice daily, all three drugs markedly prolonged survival beyond that of controls. In two subsequent studies, two sordarins were compared with amphotericin B and fluconazole (Table 2). In this study, GM2 was highly effective in prolonging survival at doses down to the lowest tested, 2 mg/kg, while GM3 was ineffective at 5 mg/kg and below. However, the inoculum in the GM3 study was much larger than that for GM2.

An additional study was done to measure the tissue burden of *H. capsulatum* after treatment with GM3. In this study, mice

TABLE 3. Lung and kidney tissue burdens[a]

Tissue	Drug or treatment	Dose (mg/kg)	Mean burden	95% CI		No. of mice with counts of <20 CFU/g
				Lower	Upper	
Kidney	Control	None	2.3×10^7	1.2×10^7	5.7×10^7	0
	GM3	10	4.8×10^{4b}	2.5×10^4	1.2×10^5	0
		5	8.0×10^{5b}	4.3×10^5	2.0×10^6	0
		1.25	1.3×10^8	6.9×10^7	3.2×10^8	0
	Amphotericin B	2.5	2.3×10^{2c}	1.2×10^2	5.7×10^2	3
	Fluconazole	5	3.4×10^{6b}	1.8×10^6	8.3×10^6	0
Lung	Control	None	5.9×10^7	3.1×10^7	1.6×10^8	1
	GM3	10	1.1×10^{5b}	5.7×10^4	2.6×10^5	0
		5	2.9×10^{6b}	1.5×10^6	7.8×10^6	1
		1.25	1.9×10^8	1.0×10^8	4.8×10^8	0
	Amphotericin B	2.5	1.2×10^{2c}	6.7×10^1	3.1×10^2	6
	Fluconazole	5	1.3×10^{7b}	6.8×10^6	3.1×10^7	0

[a] Values are expressed as CFU per gram of tissue. Each treatment group consisted of seven mice. CI, confidence interval.
[b] $P < 0.05$ compared with control group.
[c] $P < 0.01$ compared with control group or groups receiving fluconazole or GM3.

were infected with a relatively small inoculum, 3.4×10^6 CFU. The lungs and kidneys were cultured. As shown in Table 3, GM3 was effective in reducing cell counts at 5 or 10 mg/kg but not 1.25 mg/kg. The positive control drugs, fluconazole and amphotericin B, were also effective.

DISCUSSION

The sordarins are representative of a class of antifungal agents with a target independent of the cell membrane (targeted by polyenes and triazoles) or the cell wall (targeted by pneumocandins). At a lethal infective dose of approximately 10^6 CFU of inoculum, GM1 was effective in prolonging survival at 50 and 10 mg/kg. At the same infecting dose, GM2 was effective to the lowest dose tested, 2 mg/kg. At the higher infecting dose of 10^8 CFU, GM3 was effective at 5 mg/kg but not 1.25 mg/kg. These results were confirmed in studies of the reduction of tissue burden, in which GM3 was effective in reducing tissue counts at 5 mg/kg but not 1.25 mg/kg.

In terms of relative potency, amphotericin B administered at doses of >1 mg/kg prolonged survival and reduced tissue counts much more than the other agents. The sordarins were generally active at 5 mg/kg and higher. In contrast, fluconazole was variably effective at 5 and 10 mg/kg. Fluconazole was also fungistatic, with much less effect on kidney and lung tissue burdens than either the sordarins or amphotericin B. These results suggest that the sordarins are quite potent antifungals with excellent activity against *H. capsulatum*. On a milligram-for-milligram basis, they may be less potent than amphotericin B, but they are more potent than fluconazole. Further, they can be given orally, unlike amphotericin B.

Due to the limited availability of drugs, the present studies were not sufficient to distinguish among the three sordarins or to permit comparison of all three sordarins with amphotericin B and fluconazole. In the absence of a bioassay or high-pressure liquid chromatography assay, we were not able to study the pharmacokinetics of the drugs. However, others have done so and have reported a correlation between the area under the concentration-time curve and a therapeutic benefit in mice (3). The present studies indicate that sordarins are highly effective against *H. capsulatum* in this murine model and suggest that they may be further evaluated in vivo for antifungal potency and spectrum. The particular compounds studied herein were not highly active against *Aspergillus*, but other analogues are under development.

ACKNOWLEDGMENT

Glaxo Wellcome supported these studies.

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