ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1999, p. 589–591 Vol. 43, No. 3
0066-4804/99/$04.00+0

Treatment of Murine Fusariosis with SCH 56592

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Received 1 October 1998/Returned for modification 9 November 1998/Accepted 4 December 1998

Doses of 10 to 100 mg of the azole antifungal agent SCH 56592/kg of body weight/day were studied in immunocompetent mice as therapy for systemic infection by *Fusarium solani*. Treatment was begun 1 h after intravenous infection and continued daily for 4 or 13 doses. Prolongation of survival and organ clearance were dependent on both the dose and the duration of SCH 56592 therapy, with the best results seen at 50 and 100 mg/kg/day. The results at the highest doses of SCH 56592 used (50 or 100 mg/kg/day) were comparable to those obtained with amphotericin B at 1 mg/kg/day. SCH 56592 has potential for therapy of systemic infections caused by *F. solani*.

Fusarium species, traditionally considered plant pathogens, are now known to produce life-threatening infections in patients with compromised immune responses (13, 24). Of the various species of *Fusarium*, the major species isolated from humans has been *Fusarium solani*, followed by *F. oxysporum* and *F. moniliforme* (3–5, 8, 10, 14, 18, 20, 22). In all, as many as 15 different *Fusarium* species have been reported as causing infections in humans and animals (8).

Although the status of the host and the degree of tissue invasion remain the most important factors in predicting the outcome of *Fusarium* infections, the availability of effective antifungal agents is desirable (1). Unfortunately, the in vitro activity of the available agents is not very good. Polyenes such as amphotericin B and natamycin have in vitro activity, but their use is limited by toxicity (1, 17, 19, 21). Flucytosine and the currently available azoles (fluconazole and itraconazole) have no meaningful activity in vitro, and resistance is practically universal (17, 19, 21), although the combination therapy of flucytosine and ketoconazole has been reported to have activity against extensive subcutaneous hyphomycosis caused by *F. solani* (4). SCH 56592 is a novel triazole antifungal with in vitro and in vivo activity against a variety of fungi, including *Candida* spp., *Cryptococcus neoformans*, *Coccidioides immitis*, *Aspergillus fumigatus*, and many other opportunistic yeasts and molds (9, 11, 15, 16, 23). In this work we have studied the activity of SCH 56592 in the treatment of disseminated fusarial infection in nonneutropenic mice.

(This work was presented in part at the 98th General Meeting of the American Society for Microbiology, 1998 [10a].)

MATERIALS AND METHODS

Organism. A single isolate of *F. solani* (FS 1184; *Fusarium* Research Center Culture Collection, Pennsylvania State University) was used for all experiments. This organism was isolated from the blood of a cancer patient with invasive fusariosis. Stock lyophilized vials from the fungal strain were obtained from the *Fusarium* Research Center and kept at 4°C until use (7). For both susceptibility testing and animal studies, FS 1184 was grown in *Fusarium* culture medium (1 liter of 5% glucose in water, 1 liter of sterile saline solution [0.85% NaCl], 2 g of potassium chloride [KCl], and 2 ml of Tween 80 [polyoxyethylene-20-sorbitan monooleate]) for 72 h. The conidia were then harvested and prepared as described by Anaissie et al. (2).

Susceptibility testing. By using an adaptation of the microdilution variant of the National Committee for Clinical Laboratory Standards procedure M27-A (12), FS 1184 was tested two different times against SCH 56592, fluconazole, itraconazole, and amphotericin B. Fluconazole was dissolved in water, and itraconazole, SCH 56592, and amphotericin B were dissolved in dimethyl sulfoxide, according to the M27-A protocol. The conidial inoculum was adjusted to 80 to 82% of transmittance at 530 nm, by following the methodology of Espinel-Ingroff et al. (6), to produce a stock inoculum of 0.5×10^6 to 3.0×10^6 CFU/ml. The MIC for the azoles was defined as the concentration of the well which visually showed growth that was less than 20% of growth in the control well after 48 h of incubation. The MIC for amphotericin B was defined as the lowest concentration at which no growth was visible.

Animal model. Four- to six-week-old healthy male CF1 mice with a mean weight of 24 g (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) were used. No immunosuppressant agents were used. The inoculum for infection was prepared by adjusting the organism to a transmittance at 530 nm of 10%, which produced 1×10^7 to 3×10^7 CFU/ml, and was quantitated by plating onto Sabouraud dextrose agar. Infection was established by injecting 0.2 ml of the adjusted inoculum via the tail vein. After infection, mice were observed twice daily, and animals exhibiting profound inanition or an inability to reach food and water were sacrificed. SCH 56592 was prepared for administration by heating 4 g of methylcellulose (Sigma, St. Louis, Mo.) at 80°C for 90 min in 1 liter of distilled water. After approximately 45 min, 5.6 ml of Tween 80 (Fisher Scientific, Fair Lawn, N.J.) and 9 g of NaCl (EM Industries, Gibbstown, N.J.) were added. This solution was autoclaved. SCH 56592 was added after cooling, and the mixture was stored at 4°C until use. For amphotericin B, a vial containing 50 mg of amphotericin B USP (Pharma-Tek, Huntington, N.Y.) for injection was reconstituted per the manufacturer's instructions. SCH 56592 was given orally by gavage once daily in a final volume of 0.1 ml at daily doses of 10, 25, 50, or 100 mg/kg of body weight. Amphotericin B was administered intraperitoneally once daily at a final dose of 1 mg/kg in a final volume of 0.1 ml. Treatment with both drugs was begun 1 h after intravenous infection and continued daily for a total of 4 or 13 doses. Groups of 16 to 20 animals each were treated with either amphotericin B, SCH 56592, or methylcellulose diluent or were left untreated. On days 5 and 14 (24 h after the last dose of drug), five mice from each group were sacrificed for determination of the numbers of CFU of *F. solani* per gram in the liver and kidney. This determination was made by removing and weighing the organs, homogenizing the organs in 10 ml of sterile saline with a Stomacher 80 (A. J. Seward, UAC House, London, England), and plating appropriate dilutions on Sabouraud dextrose agar. The plates were incubated at 35°C for 48 h, and the number of colonies was then counted. All surviving animals were sacrificed at 21 days after infection. All animal care procedures were supervised and approved by the University of Texas—Houston Animal Welfare Committee.

Statistical methods. Survival times were estimated by the Kaplan-Meier method and were compared by the log rank test. Organ clearance results were subjected to analysis of variance. Calculations were performed by using SPSS for Windows, version 7.5.1 (SPSS, Inc., Chicago, Ill.).

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TABLE 1. Effects of treatment on survival

Treatment		Expt 1		Expt 2		Expt 3	
Drug, daily dose (mg/kg)	Total no. of doses[a]	Survival[b]	P[c]	Survival	P	Survival	P
Control (no treatment)		5.9 ± 0.8		9.5 ± 0.7		8.8 ± 0.6	
Methylcellulose diluent	4	6.2 ± 0.7	0.896	8.4 ± 0.7	0.232		
	13			9.1 ± 0.6	0.629	7.9 ± 0.8	0.624
SCH 56592							
10	4	7.1 ± 1.3	0.309	11.6 ± 0.5	0.057		
	13					12.5 ± 1.8	<0.014
25	13					14.3 ± 1.6	0.002
50	4	11.8 ± 0.9	0.0001	14.6 ± 1.0	0.0005		
	13			14.7 ± 1.0	0.0002	18.7 ± 1.2	<0.0001
100	4	9.4 ± 0.9	0.008	10.7 ± 1.3	0.143		
	13			14.9 ± 1.3	0.0009		
Amphotericin B, 1	4			13.5 ± 1.0	0.0025		
	13					16 ± 1.4	0.0005

[a] At one dose per day.
[b] Kaplan-Meier estimate of mean survival in days ± standard error (SE).
[c] For comparison with the control group by the log rank test. Not all conditions were tested in all experiments. Experiments 1 and 2 used 16 animals in each study group; experiment 3 used 20 animals in each study group.

RESULTS

In vitro susceptibility testing results. The MIC of fluconazole and itraconazole against FS 1184 was >64 μg/ml, that of amphotericin B was 1.0 μg/ml, and that of SCH 56592 was 4.0 μg/ml. These results suggested that FS 1184 was resistant to fluconazole and itraconazole but potentially susceptible to amphotericin B and SCH 56592.

In vivo study. (i) Survival and mortality. The effects of treatment on the survival of mice infected with FS 1184 are shown in Table 1. In all, three different experiments, using various combinations of doses and treatment durations, were performed. Mice treated with the methylcellulose diluent had the same duration of survival (6.2 to 8.4 days) as untreated mice (5.9 to 9.5 days). A significant and dose-dependent effect of therapy on prolongation of survival was apparent for SCH 56592. The most consistently increased survival results were obtained with SCH 56592 at 50 mg/kg/day, suggesting that the peak of the dose-response curve had been attained. The etiology of the slight decrease in survival noted when 4 daily doses of SCH 56592 were given at 100 mg/kg/day is not clear, and this effect was not observed when 13 doses were given. The results of treatment with SCH 56592 at 25, 50, and 100 mg/kg/day were not statistically different from those produced with amphotericin B at 1 mg/kg/day.

(ii) Response to therapy. Data on the reduction in the numbers of CFU per gram of kidney and liver are shown in Tables 2 and 3, respectively. Reduction in the numbers of CFU per gram was a function of the dose and duration of therapy in both the kidney and the liver, with maximal reductions produced after 13 daily doses of therapy with SCH 56592 at 50 to 100 mg/kg. In the liver in particular, this duration of therapy resulted in complete clearance of the infecting organism. The organ clearance results after four doses of therapy with SCH 56592 were similar to the clearance seen with four doses of amphotericin B.

DISCUSSION

SCH 56592 has previously been shown to have excellent activity both in vitro (9, 16, 23) and in vivo (11, 15, 23) against a variety of fungi, including *Candida* spp., *Blatomyces dermatitidis*, *C. immitis*, and *Aspergillus* spp. In this study, we have shown that SCH 56592 is also active both in vitro and in vivo against *F. solani* in an immunocompetent mouse model of systemic fusariosis.

The effect of SCH 56592 in vivo was dependent on both the dose and the duration of therapy. Optimal effects were produced when 13 doses were given at 50 to 100 mg/kg/day. This dose optimum is similar to, but slightly higher than, the optimum dose in other studies with this compound. Oakley et al. (15) demonstrated that SCH 56592 at 25 mg/kg/day was better than itraconazole or amphotericin B in a neutropenic murine model of aspergillosis. Lutz et al. (11) found that doses of 10 and 25 mg/kg/day cured mice infected with *C. immitis*. Finally, Sugar and Liu (23) have shown that 25 mg/kg/day sterilized the lungs of mice infected with *B. dermatitidis*. Although a slight diminution in survival was noted when four daily doses of SCH

TABLE 2. Effects of treatment on colony counts in the kidney

Treatment		Expt 1		Expt 2	
Drug, daily dose (mg/kg)	Total no. of doses[a]	10^3 CFU/g ± SE[b]	P[c]	10^3 CFU/g ± SE	P
Control (no treatment)	4	27.3 ± 5.0		21.6 ± 2.7	
Methylcellulose diluent	4	23.0 ± 3.2	0.25	19.3 ± 2.5	0.28
SCH 56592					
10	4	13.8 ± 0.8	0.015	22.3 ± 3.3	0.44
50	4	9.7 ± 1.1	0.005	18.0 ± 3.8	0.23
	13			4.9 ± 1.3	<0.001
100	4	5.8 ± 1.0	0.001	13.1 ± 0.9	0.009
	13			5.9 ± 1.7	<0.001
Amphotericin B, 1	4	8.2 ± 4.4	0.014	13.1 ± 1.5	0.013

[a] At one dose per day. Animals were sacrificed 24 h after the last dose of drug.
[b] $n = 5$ for all groups.
[c] To aid in the interpretation of the results, the P value of the t test comparing the CFU per gram for each treatment group (4 and 13 doses) with the CFU per gram for the untreated animals in each experiment is shown. By analysis of variance, the numbers of CFU per gram were significantly different among groups in both experiments ($P < 0.001$). Day 14 organ burden data are not available for the untreated animals because essentially all such animals died prior to 14 days.

TABLE 3. Effects of treatment on colony counts in the liver

Treatment		Expt 1		Expt 2	
Drug, daily dose (mg/kg)	Total no. of doses[a]	10^3 CFU/g ± SE[b]	P[c]	10^3 CFU/g ± SE	P
Control (no treatment)	4	108 ± 20.7		78.5 ± 13.6	
Methylcellulose diluent	4	93.3 ± 10.0	0.27	64.8 ± 8.2	0.207
SCH 56592					
10	4	42.3 ± 3.4	0.007	45.3 ± 2.3	0.021
50	4	23.0 ± 3.6	0.002	29.0 ± 3.3	0.004
	13			0.0 ± 0.0	<0.001
100	4	16.7 ± 3.2	0.001	23.2 ± 1.8	0.002
	13			0.0 ± 0.0	<0.001
Amphotericin B, 1	4	19.5 ± 5.1	0.007	32.9 ± 3.8	0.006

[a] At one dose per day. Animals were sacrificed 24 h after the last dose of drug.
[b] $n = 5$ for all groups.
[c] To aid in the interpretation of the results, the P value of the t test comparing the CFU per gram for each treatment group (4 and 13 doses) with the CFU per gram for the untreated animals in each experiment is shown. By analysis of variance, the numbers of CFU per gram were significantly different among groups in both experiments ($P < 0.001$). Day 14 organ burden data are not available for the untreated animals because essentially all such animals died prior to 14 days.

56592 were given at 100 mg/kg/day, this effect was not observed when therapy was given for a longer period. Thus, the higher doses of SCH 56592 required for treatment of this difficult infection appeared well tolerated.

Of interest, SCH 56592 appeared to completely clear the infection in the liver. The liver appears to be preferentially targeted by the fungus in this model (the numbers of CFU per gram of tissue in the livers of untreated animals are three- to fourfold higher than those in the kidneys), thus making this effect even more apparent. The relatively less effective clearance of the renal infection may be a function of the pharmacokinetics of SCH 56592, as effective concentrations of SCH 56592 in the liver are two- to fourfold higher than those in the kidney (20a).

In conclusion, we found that SCH 56592 increased survival and reduced organ burdens in immunocompetent mice infected with *F. solani*. Prolongation of survival and organ clearance were dependent on both the dose and the duration of SCH 56592 therapy. Similar results were obtained with amphotericin B. SCH 56592 has potential for therapy of systemic infections in humans caused by *F. solani*.

ACKNOWLEDGMENT

This work was supported in part by a grant to M. Lozano-Chiu from the Dirección General de Investigación Cientifica y Enseñanza Superior of Spain.

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