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In Vitro and In Vivo Activities of SCH 56592 against *Blastomyces dermatitidis*†

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The new triazole derivative SCH 56592 has been tested in a National Committee for Clinical Laboratory Standards-adapted in vitro susceptibility test, and its activity against 12 isolates of *Blastomyces dermatitidis* yeast-like forms has been compared with those of amphotericin B, itraconazole, and fluconazole. SCH 56592 was the most active of the four compounds, with an MIC at which 90% of the isolates are inhibited of 0.06 μg/ml and a minimal fungicidal concentration at which 90% of the isolates are inhibited of 4 μg/ml. The results of the treatment of mice infected with *B. dermatitidis* with three different doses of SCH 56592 (25, 5, or 1 mg/kg of body weight), amphotericin B (1 mg/kg), or itraconazole (150 mg/kg) confirmed the potent activity of SCH 56592. Survival was prolonged at each dose of SCH 56592, and sterilization of the lungs occurred in the high-dose group but not in the groups treated with itraconazole or fluconazole. SCH 56592 is a promising new azole antifungal drug that should be studied in humans with blastomycosis.

The triazole derivative SCH 56592 has been shown to be active against a variety of different fungal pathogens (1, 6, 7). Other triazoles have been found to be effective in the treatment of murine and human pulmonary blastomycosis (3, 5, 8, 10), but agents with improved activity, pharmacokinetic parameters, and safety would provide additional means to treat blastomycosis as well as other mycoses. In this study, we evaluated the in vitro activity of SCH 56592 using an adaptation of the National Committee for Clinical Laboratory Standards proposed standard method for antifungal susceptibility testing (9) and the in vivo efficacy in a well-described murine model of pulmonary blastomycosis (2).

The standard for antifungal susceptibility testing of yeasts proposed by the National Committee for Clinical Laboratory Standards (4) was adapted for use with the yeast-like form of *Blastomyces dermatitidis* (9). Twelve different laboratory (n = 4) and clinical (n = 8; kindly provided by R. Bradsher) isolates were studied. The assay was performed according to the previously described protocol (4), using RPMI 1640, with the exception that endpoints were determined after 5 days of incubation. The MIC of the azoles was defined as the concentration of drug in the first tube in which the growth of 80% of the isolates was inhibited, i.e., scored +1 growth. The concentration of drug contained in the first clear tube was defined as the MIC. Minimal fungicidal concentrations (MFCs) were determined by subculturing 1-ml aliquots from the tubes that were clear onto blood agar plates and then incubating the plates for 5 days at 37°C. The MFC was the concentration of drug at which no colonies grew on the subculture.

SCH 56592 (Schering-Plough Research Institute, Kenilworth, N.J.), fluconazole (Pfizer Central Research, Groton, Conn.), and itraconazole (Janssen Pharmaceutica, Titusville, N.J.) were obtained as powders from their manufacturers, and amphotericin B was obtained as Fungizone from Bristol-Myers Squibb (Wallingford, Conn.). SCH 56592 was initially dissolved in dimethyl sulfoxide (4%), amphotericin B-deoxycholate and fluconazole were reconstituted in sterile distilled water, and itraconazole was dissolved in polyethylene glycol 400. Subsequent dilutions were made in RMPI 1640.

Male outbred ICR mice were obtained from Harlan Sprague-Dawley and weighed approximately 20 g at the start of the experiment. Mice were infected with ca. 5×10^6 CFU of *B. dermatitidis* (ATCC 26199) yeast cells by intranasal inoculation while the mice were anesthetized. Azoles were suspended in methyl cellulose (0.4%), and amphotericin B was obtained as Fungizone and diluted in 5% dextrose in water. Therapy consisted of once-daily administration of the following: amphotericin B, 1 mg/kg of body weight by intraperitoneal injection; SCH 56592, 25, 5, or 1 mg/kg by oral gavage; and itraconazole, 150 mg/kg by oral gavage. Therapy was begun 5 days following infection and continued for 21 days. Mortality was assessed over a 45- or 60-day period.

The lungs were removed from sacrificed mice at specified time points and were homogenized, and the entire right lung of each mouse was cultured on Sabouraud agar plates. Colonies were counted after 5 to 6 days of incubation at 37°C.

Survival and lung culture results were analyzed by analysis of variance, according to the Student-Newman-Keuls method. Significance was defined as $P \leq 0.05$.

The results of in vitro susceptibility testing of the 12 yeast-form isolates are shown in Table 1. The MIC and MFC of SCH 56592 were consistently lower than those of the other agents.

In one experiment, mice were infected with 3.1×10^5 CFU of *B. dermatitidis* yeast (Fig. 1). The time to reach the 50% lethal dose (LD_{50}) was 20 days for the control mice; only 8% survived to 60 days. In contrast, 92, 58, and 42% of the mice treated with SCH 56592 (25, 5, or 1 mg/kg/day, respectively) survived to day 60 ($P < 0.05$ [all groups compared with control and itraconazole]). The survival of amphotericin B-treated mice (67%) was similar to that of mice treated with the middle dose of SCH 56592. Itraconazole (150 mg/kg/day)-treated mice showed delayed mortality compared with control mice, but the total mortality of itraconazole-treated mice was similar to that of the control group.

Thirty days following the discontinuation of therapy, the

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TABLE 1. MICs and MFCs of 12 isolates of *B. dermatitidis* yeast

Isolate	MIC of drug/MFC of drug (μg/ml)			
	SCH 56592[a]	Amphotericin B[b]	Itraconazole[c]	Fluconazole[d]
ATCC 26199	<0.016/4	0.03/8	8/32	8/16
ATCC 26197	0.03/1	0.06/4	2/8	2/8
ATCC 26198	0.03/1	0.125/4	0.125/4	0.25/>32
ATCC 60637	0.03/1	0.06/1	4/8	2/16
86-01	0.03/8	0.03/16	0.03/32	0.06/16
84-03	0.03/2	0.06/4	0.06/8	2/16
91-01	<0.016/1	1/4	0.5/4	2/>32
86-02	0.06/2	1/4	2/32	2/16
86-03	0.06/2	2/4	4/16	2/>32
86-04	0.03/1	0.06/8	1/>32	0.125/>32
86-06	<0.016/1	0.03/16	2/>32	2/>32
91-02	<0.016/1	0.25/1	4/>32	4/>32

[a] The MIC at which 50% of the isolates were inhibited (MIC_{50}), MIC_{90}, MFC at which 50% of the isolates were inhibited (MFC_{50}), and MFC_{90} were 0.03, 0.06, 1, and 4 μg/ml, respectively.
[b] The MIC_{50}, MIC_{90}, MFC_{50}, and MFC_{90} were 0.06, 1, 4, and 16 μg/ml, respectively.
[c] The MIC_{50}, MIC_{90}, MFC_{50}, and MFC_{90} were 2, 4, 16, and >32 μg/ml, respectively.
[d] The MIC_{50}, MIC_{90}, MFC_{50}, and MFC_{90} were 2, 4, 16, and >32 μg/ml, respectively.

lungs of the mice were cultured. Only the highest SCH 56592 dose sterilized the lungs (Table 2). The mice in all treatment groups contained fungal burdens statistically significantly lower than those in the control group. All SCH 56592 dosage groups were statistically superior to itraconazole in reducing fungal colony counts (Table 2).

In a second experiment, mice were infected with 1.1×10^5 CFU of *B. dermatitidis* yeast and observed for 45 days (Fig. 2). The time to reach the LD_{50} was 28 days for the control mice, and 33% survived to day 45. In contrast, 100, 58, and 20% of the mice treated with SCH 56592 (25, 5, or 1 mg/kg/day, respectively) survived to day 45 ($P < 0.05$ [all groups compared with control and itraconazole]). In this experiment, the survival of amphotericin B-treated mice compared favorably with effects of the highest SCH 56592 dose (92 compared with 100% survival on day 45). Once again, itraconazole delayed mortality but did not improve on the ultimate survival of mice compared with that of controls (25 compared with 33%).

TABLE 2. Recovery of *B. dermatitidis* from lungs of mice

Treatment group (dose [mg/kg/day])	CFU/lung (mean ± SD)	
	Expt 1[a]	Expt 2[b]
Control	$4.1 \times 10^6 \pm 3 \times 10^6$	$2.8 \times 10^5 \pm 0.3 \times 10^5$
Amphotericin B (1)	18 ± 21	0
SCH 56592 (25)	0	0
SCH 56592 (5)	$1.5 \times 10^3 \pm 0.5 \times 10^3$	$6.3 \times 10^3 \pm 0.6 \times 10^4$
SCH 56592 (1)	$8.0 \times 10^3 \pm 1.4 \times 10^3$	$9.4 \times 10^4 \pm 0.5 \times 10^4$
Itraconazole (150)	$2.7 \times 10^4 \pm 0.3 \times 10^4$	$1.7 \times 10^5 \pm 0.7 \times 10^5$

[a] Lungs were cultured 30 days after therapy was stopped. For comparisons between all groups P was <0.05, except that no difference between amphotericin B and SCH 56592 (25 mg/kg/day) was observed.
[b] Lungs were cultured 7 days after therapy was stopped. For comparisons between all groups P was <0.05, except that no difference between amphotericin B and SCH 56592 (25 mg/kg/day) was observed.

Seven days following the discontinuation of therapy in this experiment, the lungs of the mice were cultured. The highest dose of SCH 56592 and amphotericin B sterilized the lungs (Table 2). The mice treated with any of the three doses of SCH 56592 demonstrated statistically significant reductions in lung colony counts compared with those of itraconazole-treated mice. Amphotericin B treatment was superior to itraconazole and the two lower-dose SCH 56592 treatments, but there was no difference between amphotericin B-treated mice and those treated with SCH 56592 (25 mg/kg/day).

These experiments indicate that SCH 56592 is extremely active in vitro against both laboratory and clinical isolates of *B. dermatitidis*. Moreover, this in vitro activity is translated into activity in a well-accepted murine model of pulmonary blastomycosis. While currently clinically available azoles have not effectively sterilized the lungs of mice with blastomycosis, an earlier experimental azole, SCH 51048, could affect the sterilization of infected lungs (10). Similarly, SCH 56592 (25 mg/kg/day) consistently sterilized *B. dermatitidis*-infected lungs in

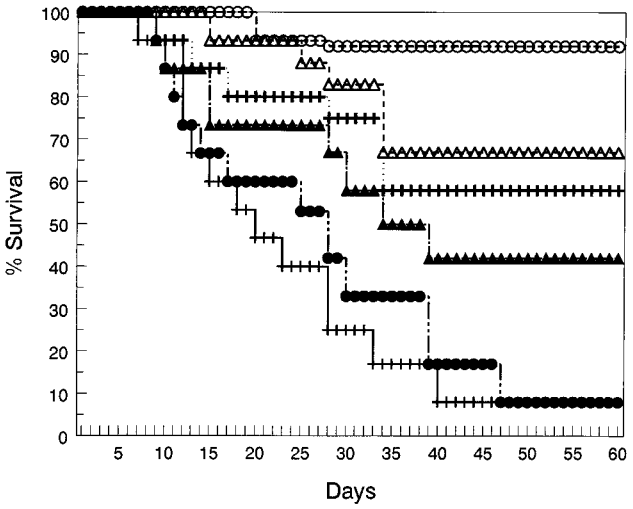

FIG. 1. Survival of mice treated for 21 days, starting on day 5, with 5% dextrose (control) (+); amphotericin B (△); SCH 56592, 25 mg/kg/day (○); SCH 56592, 5 mg/kg/day (+); SCH 56592, 1 mg/kg/day (▲); or itraconazole (●). The mice were observed for 60 days.

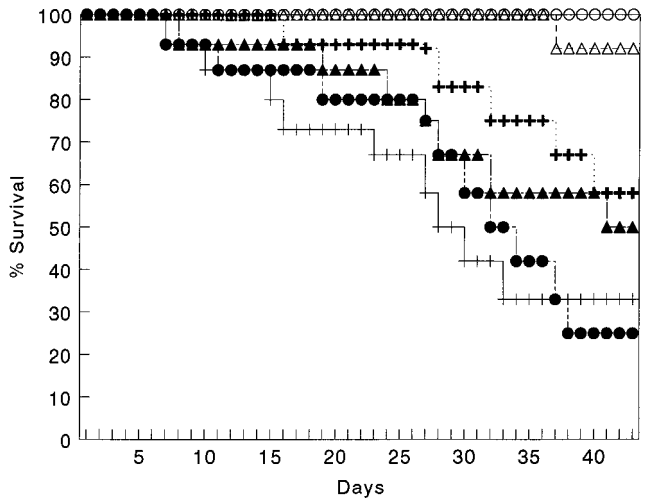

FIG. 2. Survival of mice treated for 21 days, starting on day 5, with 5% dextrose (control) (+); amphotericin B (△); SCH 56592, 25 mg/kg/day (○); SCH 56592, 5 mg/kg/day (+); SCH 56592, 1 mg/kg/day (▲); or itraconazole (●). The mice were observed for 45 days.

these studies, which is similar in effect to amphotericin B. At 1 mg/kg/day, SCH 56592 was more effective than itraconazole at 150 mg/kg/day in prolonging the survival of the *B. dermatitidis*-infected mice.

Itraconazole has been very effective in the treatment of patients with blastomycosis, but this drug is unable to sterilize the lungs, an activity that could be particularly useful in immunocompromised patients. That SCH 56592 is more active than itraconazole in this animal model and that it results in sterilization of the lungs suggest that this new azole might also be effective in treating blastomycosis in patients. The lack of dramatic effect of itraconazole in this study most likely reflects suboptimal dosing, since polyethylene glycol 400 is not as effective as the cyclodextrins in encouraging maximal absorption. These studies indicate that SCH 56592 should be further evaluated in clinical trials for the treatment of patients with blastomycosis.

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