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Efficacy of SCH-56592 in a Temporarily Neutropenic Murine Model of Invasive Aspergillosis with an Itraconazole-Susceptible and an Itraconazole-Resistant Isolate of *Aspergillus fumigatus*

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SCH-56592 (SCH) is a novel triazole antifungal agent with excellent in vitro activity against *Aspergillus*. We compared three doses (5, 10, and 25 mg/kg of body weight) of SCH with itraconazole (ITZ; 25 mg/kg) and amphotericin B (AB; 5 mg/kg) in a temporarily neutropenic murine model of disseminated aspergillosis (lungs and kidneys) against one ITZ-susceptible (AF71) and one ITZ-resistant (AF90) isolate of *Aspergillus fumigatus*. Treatment started 24 h after infection and lasted for 10 days. Dosing regimens for SCH were once daily for 10 days, those for ITZ were three times daily for 2 days and then twice daily for 3 to 10 days, and those for AB were once daily on days 1, 2, 4, and 7. Both isolates killed 90% of control mice. Kidneys and lungs from survivors were cultured on day 11. Against AF71, all three doses of SCH and ITZ yielded a 90 to 100% survival rate and AB yielded 40% survival ($P \leq 0.01$ to 0.0001 for all treatment groups compared with the controls). All three doses of SCH were superior to AB in cultures of lung and kidney tissue samples ($P \leq 0.01$ to 0.0002) and SCH at 25 mg/kg was superior to ITZ in cultures of kidneys ($P = 0.01$). Against AF90, the highest dose of SCH (25 mg/kg) resulted in a 100% survival rate, compared with 60 and 20% survival rates for the groups treated with SCH at 10 and 5 mg/kg, respectively. Treatment with ITZ yielded no survivors. AB therapy achieved a 50% survival rate. SCH at 25 mg/kg ($P < 0.001$), SCH at 10 mg/kg ($P \leq 0.005$), and AB ($P < 0.05$) were superior to ITZ in cultures of lungs and kidneys. There was a correlation between the MICs of SCH and quantitative organ culture results and between the minimum fungicidal concentration of AB with quantitative organ culture results. SCH appears to be a highly effective anti-*Aspergillus* compound in this model. There appears to be a degree of cross-resistance between itraconazole and SCH.

Infections caused by opportunistic fungal pathogens have increased substantially over the past two decades, and invasive fungal infections are now an important cause of morbidity and mortality (6). This is due to the increasing number of immunosuppressed patients; for example, individuals with hematological malignancies, organ transplants, and bone marrow transplants. The rise in the frequency of fungal infection has also been due to the fact that more AIDS patients are advancing to late-stage AIDS and have a more prolonged risk of airborne fungal infection (8).

Amphotericin B (AB) is the standard therapy for invasive aspergillosis, although it is still far from ideal (2). Itraconazole (ITZ) is the only other antifungal agent which is partially effective for various manifestations of aspergillosis and has advantages over AB in that it can be administered orally and is less toxic (1). However, the bioavailability of ITZ is variable and is especially poor in certain patient groups. We have also recently described resistance to ITZ in *Aspergillus fumigatus* (4). Hence, the search for newer and better antifungal agents continues.

SCH-56592 (SCH) is a new triazole antifungal agent with a broad spectrum of tissue samplesactivity against a wide range of fungi including *Aspergillus* spp. (9–12). Previous experiments in our laboratory demonstrated that SCH is more active than ITZ and AB in vitro against *Aspergillus* spp. (10). In this study we tested SCH in an immunocompromised animal model of pulmonary and disseminated aspergillosis and compared its efficacy to the efficacies of ITZ and AB. We have used one ITZ-susceptible and one ITZ-resistant isolate of *A. fumigatus.*

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MATERIALS AND METHODS

Organisms. Two *A. fumigatus* isolates, isolates AF71 (NCPF 7098) and AF90, were used in this study. AF71 was a pulmonary isolate from an allogenic bone marrow transplant patient who responded to ITZ therapy (2), and AF90 was from a percutaneous lung aspirate from a patient with AIDS who did not respond to ITZ therapy (4). The in vitro activities of AF71 and AF90 were determined by a broth microdilution format (10) prior to the in vivo studies. The MICs and minimum fungicidal concentrations of SCH, ITZ, and AB for AF71 were 0.01 and >8 μg/ml, 0.25 and >16 μg/ml, and 1 and 2 μg/ml, respectively; and those for AF90 were 0.5 and >8 μg/ml, >16 and >16 μg/ml, and 2 and >16 μg/ml, respectively.

Growth and storage. The isolates were stored at −70°C in 15% glycerol. For each experiment fresh subcultures from the frozen stock were used. The inoculum was prepared in a tissue culture flask by growing a loopful of stock on potato dextrose agar for a period of 10 days or until there was a heavy growth of *Aspergillus*. A stock solution of conidia was then collected into sterile phosphate-buffered saline (PBS) containing 0.05% Tween 80 (PBS-Tween). The viability of the stock was determined by serial dilutions, and the stock was diluted to 2×10^6 conidia/ml for AF71 infection and 1×10^7 conidia/ml for AF90 for infection.

Preparation of drugs. Both SCH (Schering-Plough Research Institute, Kenilworth, N.J.) and ITZ (Janssen Research Foundation, Beerse, Belgium) drug powders were solubilized in hydroxypropyl-β-cyclodextrin to produce a stock solution of 25 mg/ml (7). Both drugs were diluted in sterile water and were stored at 4°C until use. AB was supplied as the sodium desoxycholate form of the drug (Fungizone; Bristol-Myers Squibb, Hounslow, United Kingdom) and was dissolved in 5% dextrose to produce a stock solution of 5 μg/ml. The drug stock was diluted in sterile water to produce the required concentrations, and these stock solutions were stored at 4°C in the dark for the duration of the experiment.

Mice. Four- to 5-week-old male CD-1 mice were obtained from Charles River UK Ltd. The mice used were virus-free and weighed between 22 and 26 g. They were housed at 10 mice per cage and were allowed food and water ad libitum.

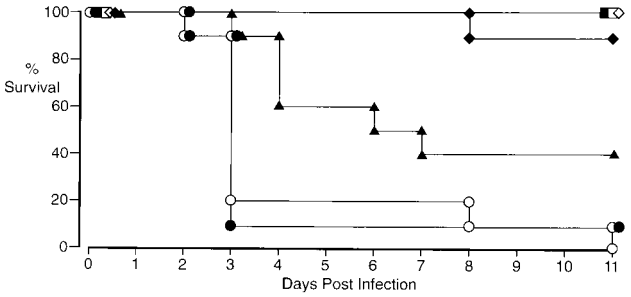

FIG. 1. Survival curve for CD-1 mice infected with isolate AF71 and treated with various dosing regimens. ○, dextrose given i.p.; ●, dextrose given by gavage; □, SCH at 25 mg/kg; ◇, SCH at 10 mg/kg; ◆, SCH at 5 mg/kg; ■, ITZ at 25 mg/kg; ▲, AB at 5.0 mg/kg.

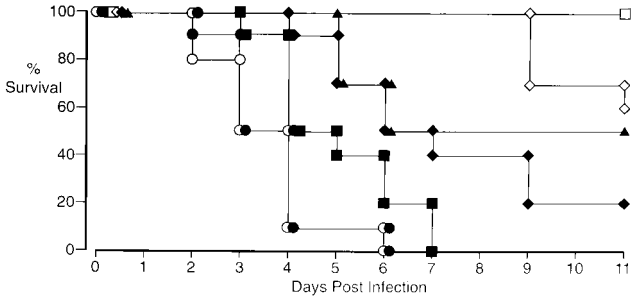

FIG. 2. Survival curve for CD-1 mice infected with isolate AF90 and treated with various dosing regimens. ○, dextrose given i.p.; ●, dextrose given by gavage; □, SCH at 25 mg/kg; ◇, SCH at 10 mg/kg; ◆, SCH at 5 mg/kg; ■, ITZ at 25 mg/kg; ▲, AB at 5.0 mg/kg.

Immunosuppression. All mice were immunosuppressed with a single dose of cyclophosphamide (200 mg/kg of body weight) which was administered intravenously via the lateral tail vein on day −3. This yielded neutropenia for 4 days beginning on day 1 (3).

Inoculum-finding studies. Prior to each experiment inoculum-finding studies for each isolate were performed with three different quantities of the inoculum. The inoculum producing a 90% lethal dose was used in the study. For AF71 this was 2×10^6 conidia/ml, and for AF90 this was 1×10^7 conidia/ml.

Infection. Mice were infected on day 0 via the lateral tail vein. Postinfection viability counts were performed on blood agar to ensure that the correct inoculum had been given.

Dosing regimens. Treatment with all drugs began 18 h after infection on day 1 and continued for 10 days. Three doses of SCH were given in 0.25-ml volumes: 25, 10, and 5 mg/kg. The drug was administered orally by gavage once daily. ITZ was given at 25 mg/kg per dose by gavage. For the first 2 days ITZ was given three times a day at approximately 8-h intervals in 0.25-ml volumes, and for days 3 to 10 the drug was given twice daily separated by approximately 12 h. AB was given as one concentration (5 mg/kg) once daily on days 1, 2, 4, and 7 by intraperitoneal (i.p.) injection (0.1 ml), which is the optimal regimen in this model on the basis of careful dose-ranging work.

Control mice (also 10 mice per group) were infected but received no active treatment. One control group received 5% dextrose by gavage, and the second control group received 5% dextrose by i.p. injection.

Cultures. Mice surviving to day 11 were sacrificed, and their lungs and kidneys were cultured. The organs were removed, transferred into 5 ml of sterile PBS-Tween containing penicillin at 100 IU/ml and streptomycin at 100 μg/ml, homogenized in a tissue grinder for 15 to 30 s, and diluted 10^{-1} and 10^{-2}. A total of 0.5 ml of each dilution (including the neat dilution) was spread onto Sabouraud dextrose agar (Lab M, Bury, United Kingdom), and the plates were incubated at 37°C. The plates were examined daily for up to 5 days, and *A. fumigatus* colonies were counted. One colony or less was defined as no growth.

Pharmacokinetics. Drug concentrations were monitored for all doses of SCH (25, 10, and 5 mg/kg) and ITZ (25 mg/kg). Four groups of three mice were immunosuppressed but not infected in each experiment and were treated according to the dosing regimen for 7 days. At 6 h after dosing on day 7, mice were bled by cardiac puncture. The drug concentrations in mouse serum were measured by bioassay by using *Candida pseudotropicalis* (San Antonio strain) as the test organism (3).

Statistics. Each experiment was conducted once with 10 mice in each group. Mortality and culture data were analyzed by the Mann-Whitney U test or the Kruskal Wallis test if the Mann-Whitney test was not possible (i.e., all values were identical for one group). Mice which died before 10 days were assumed to have quantitative counts in their organs at least as high as the highest counts in the organs of surviving mice when calculating the culture result statistics. All analyses were done with the computer package Minitab (Minitab Data Analysis Software, Philadelphia, Pa.).

RESULTS

Mortality results. The survival curves in Fig. 1 and 2 indicate that lethal *Aspergillus* infections were caused by *A. fumigatus* AF71 and AF90. The *Aspergillus* infection caused the untreated mice to display weight loss, "staring" of the fur, and neurological symptoms such as leaning to one side and circling the cage. Figures 1 and 2 show the good activity of SCH in prolonging the survival of the mice infected with ITZ-susceptible isolate AF71 and ITZ-resistant isolate AF90.

The survival curve for mice inoculated with isolate AF71 showed 90 and 100% mortalities for control mice receiving dextrose by gavage and i.p., respectively. In comparison, there was 100% survival for mice receiving the two highest doses of SCH and for those receiving ITZ and a 90% survival for mice receiving the lowest dose of SCH (5 mg/kg). Treatment with any dose of SCH and ITZ was significantly better at reducing mortality than no antifungal therapy ($P < 0.005$ for SCH at 25, 10, and 5 mg/kg and ITZ at 25 mg/kg compared to the controls (Table 1). Treatment with AB at 5 mg/kg gave a 40% survival rate. Mortality was significantly reduced in mice treated with AB compared to that in mice treated with dextrose i.p. and by gavage ($P < 0.01$ and 0.005, respectively). However, the two highest concentrations of SCH (25 and 10 mg/kg) and ITZ were significantly better than AB ($P \leq 0.005$) or SCH at 5 mg/kg ($P \leq 0.01$) at reducing mortality.

The survival curve for mice infected with isolate AF90 showed that there was 100% mortality in mice that received no antifungal therapy. In comparison, none of the mice receiving SCH at 25 mg/kg died, but there were 60 and 20% mortality rates in the groups receiving SCH at 10 and 5 mg/kg, respectively. Treatment with all doses of SCH significantly prolonged the survival rates compared with those for untreated mice ($P < 0.005$) (Table 1). Fifty percent of the mice receiving AB therapy at 5 mg/kg survived to day 11. Survival was significantly better for these mice compared to that for the control mice ($P < 0.005$). Although all mice treated with ITZ at 25 mg/kg died, statistically, treatment did prolong survival ($P < 0.05$) compared to that for the dextrose-treated controls (median survival, 4.5 days for ITZ-treated mice compared to 3.5 days for both dextrose-treated groups of mice). The two highest doses of SCH (25 and 10 mg/kg) were significantly better than ITZ at increasing survival ($P < 0.005$), and to a lesser degree so were SCH at 5 mg/kg and AB ($P < 0.05$). SCH at 25 mg/kg was also superior to the lowest dose of SCH (5 mg/kg) ($P < 0.005$) and AB ($P < 0.01$).

TABLE 1. Survival times for each treatment group for two experiments

Isolate	Median survival time (days) for mice receiving the following treatments[a]:					
	Controls	ITZ at 25 mg/kg	SCH at 25 mg/kg	SCH at 10 mg/kg	SCH at 5 mg/kg	AB at 5 mg/kg
AF71	3	11***	11***	11***	11***	6.5**
AF90	3.5	4.5*	11***	11***	6.5***	8.5***

[a] *, $P < 0.05$ (compared with dextrose); **, $P < 0.01$ (compared with dextrose); ***, $P < 0.005$ (compared with dextrose).

TABLE 2. Culture results for lungs and kidneys for both experiments[a]

Treatment group (dose [mg/kg])	AF71			AF90		
	No. of survivors/total no. of mice	Mean CFU/organ (10^2)		No. of survivors/total no. of mice	Mean CFU/organ (10^2)	
		Lungs	Kidneys[b]		Lungs	Kidneys[b]
Dextrose given i.p.	0/10	330	39.8	0/10	1,000	1,000
Dextrose G given by gavage	1/10	116.5	17.4	0/10	1,000	1,000
ITZ (25)	10/10	0.14***	0.69***	0/10	1,000	1,000
SCH (25)	10/10	0.021***	0.09***	10/10	3.05***	9.46***
SCH (10)	10/10	0.025***	0.23***	6/10	26.6***	185.7***
SCH (5)	9/10	0.028***	0.73***	2/10	311.8	623.7
AB (5)	4/10	10.2*	17.7*	5/10	103.7*	293.7**

[a] *, $P \leq 0.05$ (compared with dextrose); **, $P \leq 0.01$ (compared with dextrose); ***, $P \leq 0.005$ (compared with dextrose).
[b] Data are for both kidneys.

Culture results. The culture results for both isolates are presented in Table 2. Mice infected with isolate AF71 and treated with all three doses of SCH and with ITZ at 25 mg/kg had significantly reduced fungal burdens in both lungs and kidneys ($P < 0.005$) compared to those in the lungs and kidneys of mice which received no treatment. Mice treated with AB had significantly reduced fungal loads in both organs in comparison with those in the organs of mice receiving dextrose i.p., although the significance was less than that for SCH- and ITZ-treated mice. The highest dose of SCH (25 mg/kg) was significantly better than AB at reducing the fungal burdens in both the lungs and kidneys ($P < 0.005$) and was also significantly better than SCH at 5 mg/kg ($P < 0.05$) and ITZ at 25 mg/kg ($P \leq 0.01$) at reducing the fungal burden in the kidneys.

Mice infected with AF90 and treated with SCH at 25 or 10 mg/kg or with AB had significantly reduced amounts of fungus in their organs ($P < 0.005$ for SCH at 25 and 10 mg/kg for lungs and kidneys; $P < 0.05$ for lungs and $P < 0.01$ for kidneys for AB). Treatment with SCH at 5 mg/kg or ITZ did not significantly reduce the fungal burden in either organ compared to that in controls, although there were only two survivors in these treatment groups.

SCH at 25 mg/kg ($P < 0.001$) and 10 mg/kg ($P \leq 0.005$) for both organs and AB for lungs ($P < 0.05$) and AB for kidneys ($P < 0.01$) were also significantly better than ITZ at reducing the fungal load. SCH at 25 mg/kg was also significantly better than SCH at 5 mg/kg ($P < 0.005$ for kidneys and $P < 0.01$ for lungs).

Pharmacokinetics. The concentrations of all doses of SCH and ITZ in serum are presented in Table 3. The levels varied with the dose and appeared to reflect the range of doses. In both experiments the SCH concentrations obtained were almost identical, showing the reproducibility of the bioavailability of the drug. This was in contrast to the levels obtained for ITZ, which were somewhat different: 1.8 and 5.1 μg/ml for isolates AF71 and AF90, respectively. However, higher concentrations of ITZ did nothing to improve the efficacy of the drug against AF90, as all the mice infected with AF90 died, despite a serum itraconazole concentration of 5.1 μg/ml.

DISCUSSION

SCH is a new triazole antifungal agent with excellent in vitro activity against a wide range of pathogenic fungi including *Candida* spp. (9, 12), *Cryptococcus neoformans* (11), *Aspergillus* spp. (10), and dimorphic fungi (5). We have shown in this study the remarkable in vivo activity of SCH in a neutropenic mouse model of pulmonary and disseminated aspergillosis with two isolates of *A. fumigatus*. This activity was seen both in mortality studies and in reduction of fungal burden studies. In both experiments, the efficacy of SCH was compared with the efficacies of the established agents AB and ITZ.

In the first animal experiment with an ITZ-susceptible isolate (isolate AF71), all doses of SCH were clearly as effective as ITZ because among the mice in all three SCH groups, only one mouse died (the mouse was treated with SCH at 5 mg/kg). With this isolate of *A. fumigatus* it was unusual to see that only 40% of mice in the AB group survived, whereas in previous experiments we have nearly always obtained 100% survival rates. This result may have been due to the slightly heavier than usual weight of the mice used in the experiment (22 to 26 g, as opposed to the weight of 18 to 20 g of mice used previously).

The results of the second animal experiment, which used an ITZ-resistant isolate; isolate AF90 [4] showed the good activity of SCH (at the highest concentration of 25 mg/kg), with 100% survival. This is in contrast to the 100% mortality rate for mice treated with ITZ at the same dose of 25 mg/kg, which resulted in serum drug concentrations similar to those for SCH at 25 mg/kg. Treatment with lower doses of SCH was not as effective as that with the highest dose of SCH, although SCH at 10 mg/kg was still significantly better than ITZ. The data are in keeping with some degree of cross-resistance between ITZ and SCH, consistent with the mechanism of resistance that we found in AF90. This mechanism was reduced inhibition of ergosterol synthesis in a cell-free system, suggesting a mutation in the target gene, 14α-demethylase (4). The data are also consistent with an intrinsically higher potency of SCH compared with that of ITZ for *A. fumigatus*. However, the half-life of SCH at 20 mg/kg in serum exceeds 12 h in mice, whereas that of ITZ is less than 6 h in mice, making interpretation of potency difficult.

These in vivo data reflect the in vitro results obtained in our laboratory in which susceptibility results for SCH showed its excellent activity against *Aspergillus* spp. compared to that of ITZ (10). The median geometric mean MIC of ITZ for the

TABLE 3. Concentrations of SCH and ITZ in serum 6 h after dosing on day 7

Isolate	Concn (μg/ml) in serum for the following group[a]:			
	SCH (25)	SCH (10)	SCH (5)	ITZ (25)
AF71	8.0	4.0	1.2	1.8
AF90	7.6	4.2	2.1	5.1

[a] Data are means for three mice. Numbers in parentheses are doses (in milligrams per kilogram).

itraconazole-susceptible isolates was 0.25 μg/ml, and that of SCH was 0.09 μg/ml. The MIC of SCH for AF90 (ITZ-resistant isolate) was found to be higher than that for the majority of the isolates tested (0.5 μg/ml), although it was still substantially less than the ITZ MIC (>16 μg/ml).

Of particular interest in this experiment was the close relationship between the MICs and quantitative counts in the organs. Comparing the results for AF71 and AF90, for the group receiving SCH at 25 mg/kg, there was approximately a 100-fold difference in the counts in both the lungs and the kidneys; the MICs differed by about 50-fold (0.01 and 0.5 μg/ml, respectively). At an SCH dose of 10 mg/kg, the counts in the lungs and kidneys were 1,000-fold higher in the AF90 model than in the AF71 model. With respect to AB there was also a 10-fold difference in the counts in both the lungs and the kidneys between the experiments with AF71 and AF90, consistent with the minimum fungicidal concentration results (2 and >16 μg/ml, respectively). These data are intriguing and warrant further investigation.

The concentrations in serum obtained for SCH given once daily were found to be considerably higher than those obtained for ITZ given twice or three times daily, especially for the experiment involving isolate AF71. The concentrations obtained were very reproducible and were almost identical in the experiments described here as well as in other, duplicate experiments that were also performed. No toxicity was observed in any of the SCH-treated uninfected mice.

In conclusion, this study has shown that oral administration of the new triazole SCH effectively prolonged survival against invasive aspergillosis in mice. However, it is apparent that there is some degree of cross-resistance between SCH and ITZ. The close relationship between MICs and organ counts suggests that in vitro testing of SCH against *Aspergillus* spp. is likely to be clinically meaningful. The data are also consistent with an intrinsically higher potency-treated uninfected mice. compared with that of itraconazole. Further clinical and pharmacokinetic studies are required to assess the full potential of SCH therapy as an alternative to AB therapy for invasive aspergillosis.

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