ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1995, p. 1345–1348 Vol. 39, No. 6
0066-4804/95/$04.00+0
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Evaluation of SCH51048 in an Experimental Model of Pulmonary Aspergillosis

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Received 4 October 1994/Returned for modification 6 January 1995/Accepted 27 March 1995

The efficacy of a novel triazole, SCH51048, was assessed with a murine model of pulmonary aspergillosis and was compared with those of SCH39304 and itraconazole. A wide range of doses of SCH51048 (5 to 50 mg/kg of body weight) was evaluated. Mortality was significantly delayed in mice treated with doses of 5 mg of SCH51048 per kg or greater in comparison with mortality in controls ($P < 0.05$). Both SCH51048 and SCH39304 at higher doses (30 and 50 mg/kg) reduced the number of viable *Aspergillus fumigatus* organisms in lung tissue ($P < 0.05$). In the present model, itraconazole neither delayed mortality nor significantly reduced the counts in tissue at the doses used. We conclude that SCH51048 is an effective therapy for murine pulmonary aspergillosis.

Invasive aspergillosis is a prevalent problem in leukopenic patients and is increasing in frequency (7). Therapeutic success is dependent on early diagnosis and the early initiation of treatment (1), but despite standard therapy with amphotericin B, mortality remains high (4) and the clinical outcome finally depends on the resolution of granulocytopenia (6). Additionally, therapy is complicated by the toxicities associated with amphotericin B (8).

As alternatives, triazoles have been developed; they offer the advantage of either oral or parenteral administration and seem to be less toxic. One of those newer triazoles, SCH39304, was demonstrated to have excellent efficacy in animals (3, 12) and in humans in phase I clinical trials, but further development has been stopped because of its oncogenicity. Nevertheless, as a positive control we included this antifungal agent in our study. Another triazole antifungal agent, itraconazole, has entered clinical trials and seems to be efficacious against invasive aspergillosis (5). There is still a need for the development of additional effective antifungal drugs for the treatment of aspergillosis. SCH51048 is a new triazole with broad-spectrum antifungal activity and excellent in vitro activity against *Aspergillus* species (13). However, little is known about the in vivo efficacy of the compound.

In the present study we evaluated the efficacy of SCH51048 in our previously reported murine model of invasive pulmonary aspergillosis (9).

MATERIALS AND METHODS

Animals. Female BALB/c *nu*/+ mice (age 6 weeks; weight, approximately 20 g) from our own breeding colony were used throughout the experiments. They were mouse hepatitis virus and mycoplasma free, were housed in cages of five per group, and had access to food and water ad libitum.

Organism. *Aspergillus fumigatus* H11-20 was kindly provided by D. Armstrong, Sloan Kettering Institute, New York, N.Y. It was maintained on Sabouraud dextrose agar slants at 4°C. For each study, the fungus was grown on potato dextrose agar plates at 25°C, and 7- to 10-day-old conidia were harvested for each study. Conidia were overlaid with Tween 80 at 0.02% in water and were gently removed with a cell scraper, washed three times in saline, counted on a hemacytometer, and adjusted to the desired concentration (10^8/ml) in sterile saline. Viability was confirmed by quantitative culture for each experiment. The viability, on average, was 75%, ranging from 63 to 91%.

Method of immunosuppression and infection. For all experiments, mice were immunosuppressed by subcutaneous injection of cortisone acetate (150 mg/kg) for 4 consecutive days beginning 1 day before inoculation. On the day of inoculation the animals were anesthetized intramuscularly with sodium pentobarbital at 50 mg/kg of body weight. The inoculum, a 50-μl droplet, was placed into the nares of the mice at a concentration of 5×10^6 conidia per mouse. The procedure caused less than 5% mortality, and data for mice that died within 24 h of challenge were not included in the results.

Chemotherapy. SCH51048 and SCH39304 were provided by Schering Plough Research Institute, Kenilworth, N.J. The compounds were suspended in hydroxypropyl β-cyclodextrin (40% in 5% glucose in water) with sonication and heating at 50°C for 30 min. Itraconazole was obtained from Janssen Pharmaceuticals, Beerse, Belgium, and was dissolved in hydroxypropyl β-cyclodextrin solution according to the manufacturer's specification. All compounds were given orally, by gavage, in a volume of 0.2 ml per dose. Control groups received hydroxypropyl β-cyclodextrin. For mortality studies, SCH51048 was given at 5, 10, 15, 20, and 50 mg/kg. Doses of SCH39304 were administered at 15 and 75 mg/kg. Itraconazole was dosed at 30, 60, and 100 mg/kg. All triazoles were administered once daily. For tissue burden studies, SCH51048 was given at 15, 30, and 50 mg/kg and SCH39304 was given at 50 and 75 mg/kg. Itraconazole was administered at 40 and 50 mg/kg.

Assessment of antifungal efficacy. (i) Mortality studies. Treatment was started 24 h postinoculation after mice had been randomized into treatment groups of 8 to 12 animals each. Therapy was given for 10 consecutive days, and mortality was recorded daily for 20 days of infection. Efficacy of antifungal treatment was assessed by measuring the delay of mortality.

(ii) Quantitative culture studies. Mice were randomly assigned to groups of five to eight animals each 24 h postinoculation and prior to the initiation of antifungal therapy. Treatment was given for 5 consecutive days. Animals were sacrificed by cervical dislocation 48 h after administration of the last dose, and the lungs were removed, weighed, and homogenized manually with tissue grinders in 2 ml of 0.9% saline supplemented with 60 μg of piperacillin and amikacin per ml. The homogenates were serially diluted 10-fold, the dilutions were plated onto Sabouraud dextrose agar plates, and the plates were incubated at 37°C for 48 h. The numbers of CFU were counted, and the number of CFU per total weight of tissue was calculated.

Histopathology. The remaining animals at the end of one of the mortality studies were killed by cervical dislocation. Their lungs were removed, fixed in buffered formalin, and embedded in paraffin, and sections were stained with hematoxylin and eosin and by the periodic acid Schiff technique.

Pharmacokinetics. Healthy BALB/c *nu*/+ mice ($n = 5$) were given 100 mg of itraconazole per kg by gavage once daily for 3 continuous days. Six hours after administration of the last dose, the lungs were removed and serum was obtained by centrifuging the blood for 10 min at 1,500 × *g*. The material was stored at −70°C until it was assayed. The levels of itraconazole in plasma and lungs were measured by a modified bioassay described previously (2). This assay measures both itraconazole and hydroxyitraconazole.

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TABLE 1. In vitro susceptibilities of SCH51048, SCH39304, amphotericin B, and itraconazole against *A. fumigatus* H11-20

Antifungal agent	MIC (μg/ml) at 48 h/72 h	MLC (μg/ml) at 48 h/72 h
Amphotericin B	0.029/0.29	0.29/0.58
Itraconazole	0.035/0.07	0.07/2.5
SCH51048	≤0.018/0.035	0.035/2.5
SCH39304	10/40	>80

In vitro susceptibility tests. MICs and minimal lethal concentrations (MLCs) of SCH51048, SCH39304, itraconazole, and amphotericin B were determined by a macrobroth dilution method as described previously (14). Itraconazole, SCH51048, and SCH39304 were dissolved in polyethylene glycol. Amphotericin B was obtained as a powder (E. R. Squibb & Sons, Princeton, N.J.) and was dissolved in sterile water. Twofold serial dilutions of SCH51048, SCH39304, and itraconazole were incorporated into synthetic amino acid medium-fungal (American BioOrganics, Inc.), and antibiotic medium (Difco) was used for amphotericin B. *Paecilomyces variotii* was used as the control organism. Positive control cultures contained drug-free medium plus polyethylene glycol. Cultures were incubated at 25°C, and the MIC was read by visual observation of turbidity at 48 h and 72 h. MLCs were determined by subculturing broth tubes showing no visible growth at 48 h and again at 72 h.

Statistical analyses. The data from mortality studies were analyzed by Wilcoxon's test for life table analysis. The data from quantitative tissue studies were analyzed by Tukey's studentized range test. A P value of less than 0.05 was considered significant.

RESULTS

Antifungal activity in vitro. The MIC and MLC results for the isolate of *A. fumigatus* used throughout the studies are presented in Table 1. Values for itraconazole, amphotericin B, and SCH51048 were similar, with SCH51048 demonstrating the lowest MICs and MLCs. SCH39304 demonstrated less in vitro activity than the other three agents against the isolate tested.

Antifungal activity in vivo. For mortality studies groups of 10 mice each were used. All control animals generally succumbed to infection by day 9. In the first experiment, the results of which are shown in Fig. 1, we compared the activities of two doses of SCH51048 (5 and 15 mg/kg) with those of itraconazole (30 mg/kg) and SCH39304 (15 mg/kg). Only SCH51048 at both doses delayed mortality significantly when compared with control and itraconazole treatments ($P < 0.05$). The next study used doses of 10 and 20 mg of SCH51048 per kg, which were compared with 60 mg of itraconazole per kg. Again, SCH51048 statistically delayed mortality ($P < 0.05$) compared with control and itraconazole treatments; itraconazole treatment did not delay mortality significantly compared with control treatment (data not shown). In the next study, we increased the dose of SCH51048 to 30 mg/kg and that of itraconazole to 100 mg/kg, and the results are shown in Fig. 2. As before, SCH51048 significantly delayed mortality compared with control and itraconazole treatments. Although high levels of itraconazole were obtained in serum and lung tissue (average for serum, 9 μg/ml; average for lung tissue, 2.5 μg/g), no difference in mortality compared with that in untreated animals was found. In an additional experiment we compared SCH51048 given at 50 mg/kg with SCH39304 given at 75 mg/kg (Fig. 3). Both antifungal agents reduced mortality significantly when compared with control treatments ($P < 0.01$), but no difference was demonstrated when the two antifungal agents were compared with each other. Lung sections of survivors in this experiment ($n = 4$ mice from both treated groups) demonstrated a predominantly neutrophilic infiltrate and few, if any, fungal elements (data not shown).

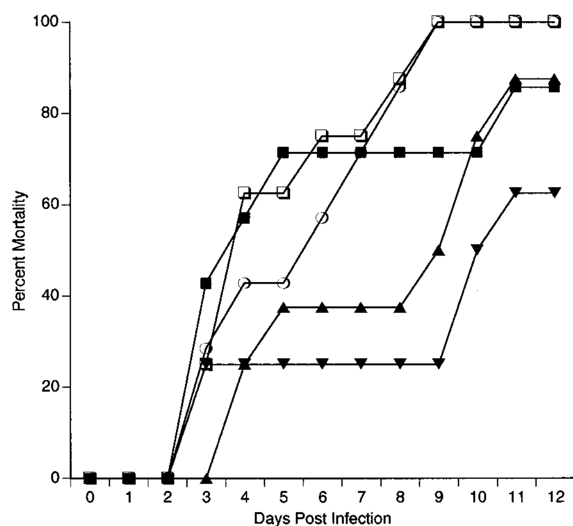

FIG. 1. Percent mortality of BALB/c *nu*/+ mice after intranasal challenge with 5×10^6 conidia of *A. fumigatus*. Mice ($n = 7$ to 8 per group) were treated orally once daily for 10 consecutive days. $P < 0.05$ comparing treatments with SCH51048 at 5 and 15 mg/kg with control treatment. □, control; ○, itraconazole at 30 mg/kg; ■, SCH39304 at 15 mg/kg; ▲, SCH51048 at 5 mg/kg; ▼, SCH51048 at 15 mg/kg.

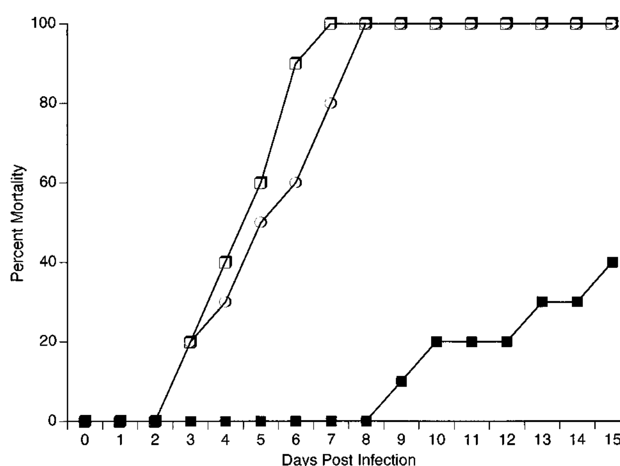

FIG. 2. Percent mortality of BALB/c *nu*/+ mice after intranasal challenge with 5×10^6 conidia of *A. fumigatus*. Mice ($n = 9$ to 10 per group) were treated orally once daily for 10 consecutive days. $P < 0.001$ comparing treatment with SCH51048 at 30 mg/kg with control treatment. □, control; ○, itraconazole at 100 mg/kg; ■, SCH51048 at 30 mg/kg.

For tissue burden experiments, mice were immunosuppressed and infected intranasally with 5×10^6 conidia of *A. fumigatus*. Treatment started 24 h after challenge and was continued for 5 days, and the lungs were removed 48 h after administration of the last dose. Table 2 demonstrates the effect of therapy with SCH51048 given at 15 and 30 mg/kg, SCH39304 given at 50 mg/kg, and itraconazole given at 50 mg/kg on viable *A. fumigatus* in lung tissue. While SCH51048 at the 30-mg/kg dose significantly reduced the fungal burden, at the 15-mg/kg dose it did not achieve a significant reduction in CFU compared with that in controls. SCH39304 at 50 mg/kg was able to lower the fungal burden significantly compared with those in controls, mice given the 15-mg/kg dose of SCH51048, and mice given the 50-mg/kg dose of itraconazole.

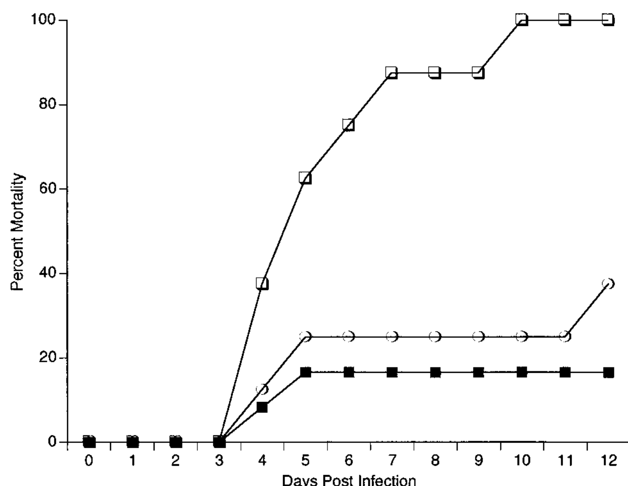

FIG. 3. Percent mortality of BALB/c *nu*/+ mice after intranasal challenge with 5×10^6 conidia of *A. fumigatus*. Mice (n = 8 to 12 per group) were treated orally once daily for 10 consecutive days. P = 0.001 comparing treatment with SCH51048 at 50 mg/kg with control treatment, and P = 0.01 comparing treatment with SCH39304 at 75 mg/kg with control treatment. □, control; ○, SCH39304 at 75 mg/kg; ■, SCH51048 at 50 mg/kg.

However, no difference in fungal load reduction was obtained compared with that obtained with SCH51048 given at 30 mg/kg. The second culture study compared the effect of treatment with SCH51048 at 50 mg/kg with that of itraconazole at 50 mg/kg. The fungal load was significantly reduced ($P < 0.05$) by SCH51048 (3.9 ± 0.02 $\log_{10}$ CFU [mean ± standard error]) compared with those in control mice (5.4 ± 0.69 $\log_{10}$ CFU) and itraconazole-treated mice (5 ± 0.24 $\log_{10}$ CFU); the fungal load in itraconazole-treated mice was not different from that in control mice.

DISCUSSION

The present studies indicate that SCH51048 is effective in the treatment of experimental pulmonary aspergillosis. The excellent in vitro activity of SCH51048 correlated well with the in vivo outcome with our model. In mortality studies, the antifungal activity was observed over a broad range of doses. A relatively low dose (5 mg/kg) of the compound delayed the mortalities of the animals. Interestingly, the mortalities of mice treated with 30 mg of SCH51048 per kg were not lower than those of mice treated with 10 or 15 mg/kg. In contrast to mortality studies, a higher dose (30 mg/kg) was needed to reduce the fungal burden in lung tissue. It is not surprising that despite delaying mortality with low doses of the antifungal agent, elimination of the fungus in a short-term organ load experiment is achieved only with higher doses. In addition, the effect of immunosuppression is still more persistent in quantitative studies, in which treatment is given for 5 days.

TABLE 2. Efficacies of SCH51048, SCH39304 and itraconazole against viable *A. fumigatus* in lung tissue[a]

Antifungal agent (dosage [mg/kg/day])	$\log_{10}$ CFU/ total lung wt
Control	5.6 ± 1.02
Itraconazole (50)	5.2 ± 0.49
SCH51048 (15)	5.2 ± 0.44
SCH51048 (30)	4.8 ± 0.14[b]
SCH39304 (50)	4.5 ± 0.16[c]

[a] After intranasal challenge with 5×10^6 conidia of *A. fumigatus*, mice (n = 5 to 8 per group) were treated for 5 consecutive days and were sacrificed on day 7 of infection. Data are means ± standard errors.

[b] $P < 0.05$ comparing treatment with SCH51048 at 30 mg/kg with control treatment.

[c] $P < 0.05$ comparing treatment with SCH39304 with control treatment and treatments with itraconazole and SCH51048 at 15 mg/kg.

One limitation of the present model is that we were unable to compare SCH51048 with amphotericin B as the standard therapeutic drug. Amphotericin B at doses used previously (3 mg/kg given intraperitoneally) was toxic to animals. This is in accord with findings from other investigators (11), who demonstrated a synergistic toxic effect of amphotericin B and cortisone acetate when they are given concomitantly. Therefore, we included SCH39304 as a positive control in our studies. The compound had previously been demonstrated to be effective in animal and clinical trials (3, 11; unpublished data), but further trials were stopped because of the development of hepatic carcinomas in rats and mice. In the present studies, SCH39304 was effective at both prolonging survival and reducing counts in tissue, but at doses higher than those of SCH51048.

The other triazole, itraconazole, included in the present studies has recently been licensed in the United States and has been demonstrated to have clinical efficacy against various fungi, including *Aspergillus* species (5). With the present model, despite good in vitro MICs and high levels in serum and tissue, the compound was not markedly effective in delaying mortality at the doses tested. This finding is consistent with previous studies in mice in which a similar model was used (10), but is in contrast to clinical findings which support therapy with itraconazole (4). After intravenous treatment the slight 1-h half-life of itraconazole contrasted with the 18-h half-life of SCH51048. This may account for the differences in therapeutic results. Different results might have been obtained with more frequent dosing of itraconazole.

With the increases in the numbers of immunocompromised patients, there is a considerable need to develop new antifungal agents. Triazoles offer the advantage of oral administration and they have fewer toxicities than amphotericin B. The present data indicate that the new triazole SCH51048 is efficacious in the treatment of experimental pulmonary aspergillosis, and further studies to evaluate the compound should be considered.

ACKNOWLEDGMENTS

This work was supported by a grant from Schering Plough Research Institute.

We thank Gloria Velez for technical assistance.

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