Efficacy of SCH39304 and Fluconazole in a Murine Model of Disseminated Coccidioidomycosis

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The efficacies of SCH39304 (SCH) and fluconazole (FLU) were tested in a murine model of coccidioidomycosis. CD-1 mice were infected with Coccidioides immitis and dosed with SCH at 2, 10, 25, or 50 mg/kg per day or FLU at 10 or 100 mg/kg per day. Survival was enhanced (P < 0.001) by both drugs at all doses. Residual burdens of C. immitis in the organs of mice treated with SCH at 25 or 50 mg/kg per day were lower than in mice treated with FLU at 100 mg/kg per day (P < 0.001). These results indicate that SCH is an effective therapy for coccidioidomycosis and is superior to FLU in this comparison.

Disseminated coccidioidomycosis is a mycotic disease which requires therapy. Amphotericin B has been the standard therapeutic agent despite its well-described toxicities and dosage limitations (4). More recently, several azoles have been demonstrated to be effective in the treatment of coccidioidomycosis. These include ketoconazole (3, 4, 10, 11) and two triazoles presently being tested in clinical trials, itraconazole (13) and fluconazole (FLU) (12). However, while encouraging response rates have been reported, some toxicities as well as disease relapses have occurred (10, 11, 13). Because of these data, the search continues for safer and more efficacious antifungal therapies.

SCH39304 (SCH) is a new triazole which can be administered orally. This compound appears, in preliminary studies, to have a broad spectrum of antifungal activity and good pharmacokinetic characteristics. Similarly, FLU has been shown to have good pharmacokinetics, especially in cerebrospinal fluid, and minimal toxicity (12). Initial studies with FLU have indicated that it is efficacious in experimental murine as well as human coccidioidal meningitis (1, 5, 12). In the present study, we compared the efficacy of SCH with that of FLU in the treatment of acute systemic coccidioidomycosis in a murine model. In addition, the pharmacokinetics of SCH and the in vitro activity of SCH and FLU against Coccidioides immitis were examined.

C. immitis (strain Silveira) was obtained from Barbara Zimmer (University of California at Davis, Davis). Stock cultures were maintained in the mycelial phase on slants of 2% glucose–1% yeast extract–2% agar (GYE) incubated at an ambient temperature. Arthroconidia for mouse inoculation and in vitro studies were harvested in sterile saline from four-week-old GYE cultures. Suspensions were shaken by hand with glass beads (6 mm) to disperse cell clumps, filtered through several layers of sterile gauze, and enumerated by hemacytometer counting. The arthroconidia were further diluted in saline to the desired number, and viability was assessed by plate counts on GYE.

Six-week-old specific-pathogen-free female CD-1 mice (Charles River Breeding Laboratories, Inc., Portage, Mich.) were used for the model of systemic coccidioidomycosis as described previously (2). Randomized groups of 10 mice (average weight, 21.2 g) were inoculated intravenously with 252 viable arthroconidia in 0.25 ml of saline. This inoculum was a 90% lethal dose, with no deaths before 12 days and most deaths occurring between 12 and 22 days postinfection. All mice were provided sterilized food and acidified water ad libitum.

SCH (Schering Corp., Kenilworth, N.J.) was supplied as a powder, suspended in 0.3% agar as a stock of 21.2 mg/ml, and stored at 4°C. FLU (Pfizer Inc., Groton, Conn.) was supplied as a powder, suspended in 0.3% agar as a 42.4-mg/ml stock, and stored at 4°C. Dilutions of each drug for administration were made in 0.3% agar. Groups of 10 mice were given SCH or FLU by gavage (0.05 ml per dose) once daily on days 2 to 19 postinfection. SCH was given at 50, 25, 10, or 2 mg/kg per day, and FLU was given at 100 or 10 mg/kg per day. One group of infected mice received no therapy and served as untreated controls. Deaths were recorded through 52 days postinfection. At the end of this period, all survivors were killed by cervical dislocation and necropsied immediately. The lungs, liver, and spleen were removed and weighed. Each organ was homogenized in 5 ml of sterile saline with a Tissuemizer (Tekmar Co., Cincinnati, Ohio) and further diluted in saline. The number of viable CFU was determined by plate counts. Organ burdens were expressed as the number (log10) of CFU per organ. Differences in cumulative mortality were analyzed by the Wilcoxon rank sum test, and organ burdens were analyzed by the Mann-Whitney U statistic (9).

The pharmacokinetics of SCH were studied in both acute and chronic models of treatment. Age-matched uninfected mice were given a single dose of SCH at 50 mg/kg for the acute model studies. For the chronic model studies, uninfected mice received the same 19-day regimen of SCH at 50 mg/kg per day as their infected counterparts. At various times after the final dose, mice were killed and the blood from two animals was pooled for the collection of serum. Levels of SCH in serum were determined by a bioassay with Candida pseudotropicalis as a test organism as described previously (13).

In vitro susceptibility studies were performed for both SCH and FLU against C. immitis Silveira. Two milligrams of SCH was solubilized in 18 ml of a synthetic medium (6) at 35°C with agitation for 3 to 4 h. FLU was solubilized in sterile distilled water and stored frozen as a 2-mg/ml stock. Both drugs were further diluted in the culture medium used in the assays. The MIC and the minimum fungicidal concentration (MFC) were determined for each drug against C.
immitis. A modification of the previously described broth dilution method was used (13). In brief, MICs were determined in a 3-ml total volume with 1,000 arthroconidia of C. immitis per ml. All tubes were incubated statically at an ambient temperature, and the MICs were determined after 72 h. MFCs were determined by plating 50-μl samples from MIC tubes with no growth onto GYE. MFC plates were incubated at an ambient temperature and examined for growth after 7 and 14 days of incubation.

The cumulative mortalities of the mice in various therapy groups are shown in Fig. 1. Most deaths occurred between days 12 and 22 postinfection. By day 52 postinfection, 90% (9 of 10) untreated control mice had died, whereas 70% (7 of 10) treated with SCH at 2 mg/kg per day and 50% (5 of 10) treated with FLU at 10 mg/kg per day had died (Fig. 1). All mice treated with SCH at 10, 25, and 50 mg/kg per day and FLU at 100 mg/kg per day survived through the 52-day experimental period (Fig. 1). In all cases, survival was prolonged over that of untreated controls (P < 0.001) by both drugs at all doses.

On day 52 postinfection, the residual organ burden of C. immitis in the spleen, liver, and lungs was quantified in all survivors. When compared with the residual CFU of C. immitis in the organs of mice treated with FLU at 100 mg/kg per day, mice which had received SCH at 25 or 50 mg/kg per day had significantly lower burdens in all organs (P < 0.0001) (Table 1). The burdens of C. immitis recovered from the organs of survivors which had been treated with SCH at 2 or 10 mg/kg per day and FLU at 10 mg/kg per day were not different from the burdens recovered from mice treated with FLU at 100 mg/kg per day (P > 0.05) (Table 1). C. immitis was not recovered from the organs of 80% (6 of 10) and 10% (1 of 10) mice treated with SCH at 50 or 25 mg/kg per day, respectively; all other treated and untreated surviving mice had residual infections with C. immitis.

The pharmacokinetics of SCH after a single 50-mg/kg dose and after chronic dosing (50 mg/kg per day for 19 days) are shown in Fig. 2. After a single 50-mg/kg dose, two peaks were observed. The first, of 45 μg/ml, occurred 2 h postdose and was followed by a decline to 21 μg/ml at 4 h. The second, smaller peak, of 35 μg/ml, occurred 8 h postdose and possibly was a result of enterophepatic circulation. These data indicate that the half-life of SCH may be prolonged, with a minimum of 5 h (Fig. 2). After chronic dosing with 50 mg/kg per day, a trough of 6.2 μg/ml occurred at 24 h and a peak of 64 μg/ml occurred at 2 h postdose. As in the single-dose profile, a decline in the level at 4 h (22.5 μg/ml) was followed by a slight increase to 29 μg/ml at 8 h postdose (Fig. 2).

The in vitro susceptibility of C. immitis to SCH and FLU was tested by broth dilution. MICs of SCH and FLU were determined to be 3.1 and 6.3 μg/ml, respectively. Corre-

![Cumulative mortality of female CD-1 mice infected intravenously with C. immitis. MKD, Milligrams per kilogram per day.](http://aac.asm.org/)

**FIG. 1.** Cumulative mortality of female CD-1 mice infected intravenously with C. immitis. MKD, Milligrams per kilogram per day.

**TABLE 1.** Recovery of C. immitis from the organs of surviving mice

<table>
<thead>
<tr>
<th>Treatment group and dose (mg/kg per day)</th>
<th>Total no. recovering</th>
<th>Culture positive in:</th>
<th>Mean log&lt;sub&gt;10&lt;/sub&gt; CFU per:</th>
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<td></td>
<td>Surviving&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Spleen</td>
<td>Liver</td>
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<td>Untreated control</td>
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<td>1</td>
<td>1</td>
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<td>SCH</td>
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<td>25&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>FLU</td>
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<td>10</td>
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<td>100</td>
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<td>9</td>
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<sup>a</sup> Of a total of 10 mice.

<sup>b</sup> Of the total number surviving.

<sup>c</sup> No C. immitis was recovered from the organs of one mouse.

<sup>d</sup> No C. immitis was recovered from the organs of eight mice.
FIG. 2. Pharmacokinetics of SCH in CD-1 mice given a single 50-mg/kg dose of SCH (○) or after 19 days of therapy with SCH at 50 mg/kg per day (○). Mice were age matched and uninfected.

responding MFCs against C. immitis were determined to be 50 µg/ml for SCH and greater than 50 µg/ml for FLU. Our results indicate that both SCH and FLU prolonged the survival of mice given a lethal challenge of C. immitis arthroconidia. However, SCH proved to be approximately 5- to 10-fold more active than FLU in prolonging survival. In contrast to the 3- to 4-log10 CFU-per-organ residual burden of C. immitis in the organs of FLU-treated survivors (100 mg/kg per day), SCH was approximately 10-fold more active, with 80% of those treated with SCH at 50 mg/kg per day harboring no residual infections. Thus, biological sterilization of C. immitis was effected, indicating in vivo fungicidal activity by SCH but not by FLU.

SCH had desirable pharmacokinetics, with an extended serum half-life. No accumulation was observed over the duration of treatment. After repeated dosing, trough levels of SCH were higher than the MICs for C. immitis and peak levels were higher than the MFCs. These results correlated well with the observed in vivo efficacy of SCH in this model of coccidioidomycosis. Because biological cure was achieved in 80% of the animals treated with SCH at 50 mg/kg per day, it appears that the in vitro susceptibility of C. immitis to SCH might be predictive for in vivo efficacy. Previous studies in our laboratory indicated that FLU is present in lower concentrations in serum than SCH is, after administration of the same doses, although the peak FLU concentration with the doses used in this study would exceed the MIC for C. immitis reported here (D. A. Stevens, E. Brummer, J. G. McEwen, and A. M. Perlman, Rev. Infect. Dis., in press). In addition, we observed no overt toxicities in animals receiving any dose of SCH or FLU during the 19-day regimen.

SCH has a broad spectrum of antifungal activity in vitro and encouraging in vivo efficacy in a variety of experimental fungal infections (7, 8; H. J. Schmitt, E. M. Bernard, M. Hauser, and D. Armstrong, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 171, 1988; G. S. Kobayashi, S. J. Travis, and G. Medoff, 28th ICAAC, abstr. no. 172, 1988). In light of these data as well as our own indicating the capacity of SCH to sterilize C. immitis in tissues, further evaluation of this compound is warranted both in other animal models and for initial clinical trials against human mycoses.

LITERATURE CITED