Comparison of SCH 39304, Fluconazole, and Ketoconazole for Treatment of Systemic Infections in Mice

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SCH 39304 was compared with fluconazole and ketoconazole in a systemic Candida albicans infection in mice (10^6 CFU per mouse). Results were based on survival rates and CFU in kidneys following once-daily oral treatment of 2, 5, or 10 days duration. In normal mice, SCH 39304 (dose to reduce kidney counts by 4 log units, 0.5 mg/kg of body weight) was 3 and 200 times more active than fluconazole and ketoconazole, respectively. In immunocompromised mice (gamma irradiation, 600 rads), SCH 39304 (dose to reduce kidney counts by 4 log units, 1.3 mg/kg) was 35 and greater than 100 times more active than fluconazole and ketoconazole, respectively. In normal mice, when the infecting inoculum varied from 10^5 to 10^7 CFU, only a fivefold increase in the dose to reduce kidney counts by 4 log units was observed with SCH 39304. Excellent protection was also seen when mice were treated with a single oral dose of SCH 39304 up to 24 h prior to infection with C. albicans. Studies in a systemic C. albicans infection model indicated that SCH 39304 is equally efficacious following either oral or intravenous administration. In a systemic Aspergillus flavus infection, mice treated with SCH 39304 (5 mg/kg) survived twice as long (16 days) as those treated with fluconazole (50 mg/kg) or controls.

In the search for less toxic, more efficacious antifungal agents that are useful parenterally as well as orally, the azoles have proved to be a significant alternative to amphotericin B. Ketoconazole, itraconazole, and fluconazole have all shown efficacy against various fungal diseases in humans. SCH 39304 is a new triazole antifungal agent with broad-spectrum activity in vitro (2, 7, 8) and in vivo activities, and it is active orally as well as parenterally. It has excellent pharmacokinetics, is well absorbed, and has a long serum half-life in mice, rabbits, and monkeys (5b), and it has wide tissue distribution in rabbits (5a). In this study, SCH 39304 was shown to be effective treatment in normal and immunocompromised mice infected with Candida albicans. Other studies have shown the efficacy of SCH 39304 in other animal models infected with C. albicans (9, 16), Aspergillus fumigatus (3b, 11a), Cryptococcus neoformans (9, 10), Histoplasma capsulatum (5), Coccioides immitis (1a, 3), Blastomyces dermatidis (12), and Fusarium solani (1). On the basis of these results, SCH 39304 may become an important drug for the treatment and prophylaxis of systemic fungal infections in patients with normal or compromised immune function.

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MATERIALS AND METHODS

Growth of strains. C. albicans Wisconsin (laboratory strain C 43) was grown on Sabouraud dextrose agar slant for 48 h at 28°C and was then suspended in saline and adjusted to 46% transmission at 550 nm on a spectrophotometer. The inoculum was adjusted with a hemacytometer and was confirmed by plate counts to be approximately 10^7 CFU/ml. Aspergillus flavus (laboratory strain ND 83) was grown on malt extract agar for 1 week at 28°C. Spores were washed from the agar surface, and the inoculum was adjusted as described above.

Mice. Harlan Sprague-Dawley, Inc. (Indianapolis, Ind.), CF-1 mice (white; male; weight, ca. 20 g) were used in these studies.

Antifungal agents. SCH 39304 was obtained from Sumitomo Pharmaceuticals Co., Ltd., Hyogo, Japan, and is identical to SM-8668 (13). Fluconazole was obtained from Pfizer Inc., Kent, England, and ketoconazole was obtained from Janssen Pharmaceutica, Beerse, Belgium. All drugs were prepared in a vehicle consisting of ethanol-Emulphor EL-719P (115 ml/liter of water; GAF, Wayne, N.J.) and lactic acid (5 ml of a 20% [wt/vol] solution per liter of water) (10:90 [vol/vol]). In this vehicle, SCH 39304 was a suspension at concentrations above 0.1 mg/ml. Ketoconazole was also a suspension at the levels tested. Fluconazole was in solution.

C. albicans infection studies. Normal and/or immunocompromised (gamma irradiation, 600 rads) mice were infected with C. albicans by injecting 10^6 CFU into the tail vein. In most studies, antifungal agents were administered orally by gavage 4 h postinfection and once daily thereafter for 2, 5, or 10 days. For therapeutic equivalence studies, SCH 39304 was administered either intravenously or orally. For prophylaxis studies, antifungal agents were administered at various times prior to infection. In all studies, survival was monitored daily. Survivors were sacrificed either 24 h after or at various times after the last treatment. Kidneys of individual mice were homogenized in sterile saline, diluted, and spread onto Mycosel agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.). Colony counts were determined after 48 h at 37°C. For calculation of geometric means, mice that died during the experiment were considered to have 10^0 CFU per pair of kidneys (on the basis of previous experimental data). The 50% protective dose (PD50) was defined as that dose which allowed for 50% survival of mice.

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and the 50% effective dose was defined as that dose which lowered kidney counts to $10^6$ CFU, which was 4 log units below those of control mice.

**A. flavus infection studies.** Normal mice were infected with *A. flavus* by injecting $10^6$ CFU into the tail vein. SCH 39304 or fluconazole was administered orally 4 h postinfection and once daily for 10 days. Survivors were determined each day.

**RESULTS**

**Oral efficacy in systemic Candida infections.** The efficacy of SCH 39304 was compared with those of fluconazole and ketoconazole in normal and immunocompromised mice infected with *C. albicans*. The percent survival at 24 h after 10 days of treatment at various doses is shown in Fig. 1. Treatment with SCH 39304 or fluconazole resulted in 100% survival of normal mice at doses of 0.3 mg/kg of body weight and above. The PD$_{50}$ of both SCH 39304 and fluconazole in normal mice was 0.15 mg/kg. Ketoconazole was less effective in normal mice, with a PD$_{50}$ of 20 mg/kg. In immunocompromised mice treated with SCH 39304 at doses from 0.6 to 10 mg/kg, 80 to 100% of the mice survived, with a PD$_{50}$ of 0.45 mg/kg. Immunocompromised mice treated with fluconazole at doses of 2.5 to 10 mg/kg had only 50 to 70% survival, with a PD$_{50}$ of 2.5 mg/kg. All mice treated with ketoconazole (100 mg/kg) died.

A more quantitative measure of the efficacy of SCH 39304 was shown (Fig. 2) by using the dose to reduce kidney counts by 4 log units (CFU per kidney). In normal mice, SCH 39304 (dose to reduce kidney counts by 4 log units, 0.45 mg/kg) was 3-fold more effective than fluconazole (dose to reduce kidney counts by 4 log units, 1.25 mg/kg) and was 200-fold more effective than ketoconazole (dose to reduce kidney counts by 4 log units, 100 mg/kg). In immunocompromised mice, SCH 39304 (dose to reduce kidney counts by 4 log units, 1 mg/kg) was 35-fold more effective than fluconazole (dose to reduce kidney counts by 4 log units, 35 mg/kg) and was at least 100-fold more effective than ketoconazole.

In normal mice treated for only 5 or 2 days (data not shown), the results were similar to those obtained in the 10-day treatment studies.

**Effect of inoculum.** The efficacy of SCH 39304 was determined in normal and immunocompromised mice that were infected with *C. albicans* at various inoculum levels ($10^5$, $10^6$, and $10^7$ CFU per mouse) and that were then treated for 10 days. In normal mice (Fig. 3), the efficacy of SCH 39304, based on CFU per pair of mouse kidneys, was greater than that of fluconazole at each of the inoculum levels tested. In immunocompromised mice (Fig. 4), the efficacy of SCH 39304 was again greater than that of fluconazole at inoculum levels of $10^5$ and $10^6$ CFU per mouse. Neither drug showed good efficacy in immunocompromised mice infected with an inoculum of $10^7$ CFU per mouse, although some mice treated
with SCH 39304 at doses of as low as 1.25 mg/kg survived, while no mice treated with fluconazole at doses below 80 mg/kg survived.

**Prophylactic treatment.** The efficacies of SCH 39304 and fluconazole in normal mice administered a single oral dose (1.0, 2.5, 5.0, or 10 mg/kg) to 72 h prior to infection with *C. albicans* were also compared. At 5 days postinfection, SCH 39304 at doses of 2.5 mg/kg and above given 24 h prior to infection (Fig. 5) protected 70 to 100% of the mice. Fluconazole administered 24 h prior to infection failed to protect mice, even at 10 mg/kg. However, fluconazole protected 70 to 100% of the mice when it was given as a pretreatment (2.5 mg/kg and above) up to 6 h prior to infection.

**Therapeutic equivalence.** Figure 6 shows the results of a study in which normal mice infected with *C. albicans* were treated for 4 days with SCH 39304 (0.31 to 1.0 mg/kg), which was administered either orally or intravenously (using an intravenous formulation). SCH 39304 was equally efficacious by either route, as shown by the number of CFU per kidney.

**A. flavus infection.** The efficacies (Fig. 7) of SCH 39304 (5 mg/kg) and fluconazole (5 or 50 mg/kg) were also determined in mice infected with *A. flavus* and treated orally for 10 days. The results showed that all mice treated with SCH 39304 (5 mg/kg) survived for 12 days postinfection. However, all mice treated with fluconazole (5 mg/kg) were dead by day 9 (similar to vehicle-treated controls), and only 50% of those treated with 50 mg of fluconazole per kg survived to day 12.

**DISCUSSION**

Fungal infections pose a very serious threat to patients with impaired immune systems (4). Therefore, new antifungal drugs targeted against systemic mycoses should be evaluated for their potential efficacy in immunocompromised animal models. In the present study, the efficacy of the new triazole SCH 39304 was compared with those of fluconazole and ketoconazole in fungal infection models in both normal and immunocompromised (gamma-irradiated) mice. Treatment with all three compounds was once daily, despite differences in the pharmacokinetics of the drugs.

Richardson et al. (11) and Troke et al. (14) previously showed that fluconazole is more efficacious than ketoconazole in both normal and immunocompromised mice. In our studies, we also found fluconazole to be much more effective than ketoconazole. However, SCH 39304 was more efficacious than either fluconazole or ketoconazole in *C. albicans*-infected normal mice and was especially more efficacious in immunocompromised mice. The efficacy of oral SCH 39304 in immunocompromised *C. albicans*-infected mice was shown by increased survival rates and decreased *C. albicans* counts in kidneys in comparison with the results obtained with fluconazole. Ketoconazole failed to protect immunocompromised mice even at 100 mg/kg. Welsh et al. (16) showed that SCH 39304 is as effective as fluconazole alone or amphotericin B plus flucytosine in granulocytopenic rabbits with disseminated candidiasis. Many organs were completely cleared, and in other organs the numbers of organisms were reduced significantly. Perfect et al. (9) also found that SCH 39304 has excellent activity against hematogenously disseminated candidiasis in rabbits. SCH 39304 cleared the vitreous and choroid-retina and significantly reduced the numbers of organisms in the renal cortex. SCH 39304 showed equal efficacy against *C. albicans* in mice whether it was administered orally or intravenously, which is consistent with pharmacokinetic data showing that orally administered SCH 39304 is 100% bioavailable in mice (5b).

SCH 39304 was also more effective than fluconazole prophylactically. When it was administered to normal mice up to 24 h prior to infection with *C. albicans*, SCH 39304 protected ≈70% of mice 5 days postinfection. Fluconazole failed to protect mice when it was administered 24 h prior to infection, but it did protect mice (40 to 100%) when treatment was given up to 6 h prior to infection. This occurred despite the fact that SCH 39304 (half-life, 5.5 h) and fluconazole (half-life, 5.1 h) have similar half-lives in mice (5b).

SCH 39304 was also effective against systemic *A. flavus* infections in normal mice. In that model, SCH 39304 at 5 mg/kg was more efficacious than fluconazole at 50 mg/kg. Schmitt et al. (11a) also reported that SCH 39304 is more effective than fluconazole against disseminated *C. albicans* infections in immunocompromised mice.
active than fluconazole, itraconazole, or ketoconazole in rats infected intratracheally with *A. fumigatus*. In immunocompromised mouse models of pulmonary aspergillosis initiated with either *A. fumigatus* (3b) or *A. flavus* (5c), SCH 39304 was more efficacious than other antifungal drugs, as demonstrated by increased survival and decreased lung counts.

Restrepo et al. (10) reported that SCH 39304 prolongs survival significantly longer than fluconazole does in mice infected either intranasally or intracerebrally with *C. neoformans*. SCH 39304 significantly lowered brain, lung, and spleen colony counts during treatment. Perfect et al. (9) also found that SCH 39304 is very efficacious against menigitis produced by *C. neoformans* in rabbits. Yeast counts in cerebrospinal fluid were markedly reduced. Kobayashi et al. (5) reported that SCH 39304 is more effective than fluconazole in both normal and immunosuppressed mice infected with *H. capsulatum*.

In a murine model of systemic coccidioidomycosis, SCH 39304 was superior to fluconazole in promoting mouse survival and clearing of *C. immitis* from organs (1a). SCH 39304 was also more effective therapy than itraconazole and fluconazole in mice infected intracerebrally with *C. immitis* (3). In a mouse model of pulmonary blastomycosis, SCH 39304 was more effective treatment than fluconazole (12). In addition, the efficacy of treatment with SCH 39304 was similar to that with itraconazole in an athymic mouse model of chromoblastomycosis (3a).

The mechanism of action of SCH 39304 has not yet been determined. Azole drugs are known to inhibit fungi by blocking ergosterol biosynthesis (6, 15, 17), and SCH 39304 probably acts in this pathway. However, whether this mechanism alone or an additional mechanism could account for the increased efficacy of SCH 39304 relative to those of fluconazole and ketoconazole in immunocompromised mice is under investigation.

SCH 39304 has shown encouraging results in a number of in vivo infection studies with normal and immunocompromised animal models. These results, coupled with its good pharmacokinetic qualities, suggested that SCH 39304 is a potentially effective antifungal drug in humans. However, recent information from prolonged (18 to 24 months) toxicological studies indicates that hepatocellular adenomas and carcinomas have been observed in rats and mice. On the basis of these findings, the clinical program for SCH 39304 has been terminated.

REFERENCES


