ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1991, p. 1985–1988
0066-4804/91/101985-04$02.00/0

Vol. 35, No. 10

Efficacy of SCH 39304 in Treatment of Experimental Invasive Aspergillosis

THOMAS F. PATTERSON,* DAVID GEORGE, ROXANN INGERSOLL, PEGGY MINITER, AND VINCENT T. ANDRIOLE

Department of Medicine, Section of Infectious Diseases, Yale University School of Medicine, New Haven, Connecticut 06510

Received 5 April 1991/Accepted 19 July 1991

The efficacy of SCH 39304 (SCH) against *Aspergillus fumigatus* was assessed with an immunosuppressed, temporarily leukopenic rabbit model of invasive aspergillosis. Therapy with SCH at 10 or 15 mg/kg of body weight per day was begun 24 h after lethal challenge and compared with therapy with amphotericin B at 1.5 mg/kg/day. Compared with untreated controls, SCH reduced mortality and also reduced the tissue burden of *A. fumigatus* 100- to 1,000-fold in liver, kidney, and lung tissues. SCH at 15 mg/kg/day and amphotericin B eliminated *A. fumigatus* in liver, kidney, and lung tissues. In addition, both dosages of SCH significantly eliminated the organism from brain tissues, compared with controls. Both SCH and amphotericin B decreased or eliminated circulating aspergillus antigen. These results show that new azoles can be as effective as amphotericin B in eradicating the organism from tissues and offer promise in improving the treatment of invasive aspergillosis.

Amphotericin B remains the drug of choice for the treatment of invasive aspergillosis, but therapy with amphotericin B is often toxic and may not be effective, particularly in immunosuppressed hosts (1, 14). Newer antifungal therapies with improved efficacy and reduced toxicity are needed to improve the treatment of invasive aspergillosis.

One approach to improving antifungal therapies has been the development of newer azoles. These agents offer several potential advantages over amphotericin B, including oral as well as intravenous therapy, reduced toxicity, and a broad therapeutic index (5). One of the newer azoles is SCH 39304 (SCH), a compound with a broad spectrum of antifungal activity, a long terminal half-life, and excellent absorption following oral administration (3).

We have developed a rabbit model of invasive aspergillosis to evaluate the efficacy of antifungal therapy against this disease (11). In our lethal, immunosuppressed model, rabbits are made leukopenic and are further immunocompromised with steroid therapy. Extensive infection in liver, kidney, lung, and brain tissues similar to the clinical dissemination of invasive aspergillosis develops (9, 10). The efficacy of therapy is assessed by mortality, semiquantitative organ cultures, and *Aspergillus* antigen measurement. In the present study we used SCH in our rabbit model of invasive aspergillosis to assess the activity of this class of compounds in an experimental model of lethal infection.

MATERIALS AND METHODS

Rabbit model. New Zealand White rabbits were immunosuppressed as previously described (10, 11) with a single dose of cyclophosphamide (200 mg) given intravenously on day 1 of the model. In addition, triamcinolone acetonide (10 mg) (Westwood Pharmaceuticals, Buffalo, N.Y.) was given subcutaneously each day. With this immunosuppressive regimen, the rabbits have reduced total leukocyte counts through day 7, as previously reported (11). At 24 h after immunosuppression (day 2 of the model), seven groups of five to eight rabbits were challenged intravenously with a lethal inoculum of 10^6 *Aspergillus fumigatus* conidia. Each group contained at least one untreated control rabbit. Antifungal therapy was given as described below. Blood was obtained daily for determining total leukocyte counts and aspergillus antigen levels in serum. Ceftazidime (200 mg) (SmithKline Beecham, Philadelphia, Pa.) was administered intramuscularly each day beginning on the day of challenge to prevent intercurrent bacterial infection. Renal toxicity does not develop in the model (10). When rabbits were untreated, this lethal challenge was fatal within 7 days, with a mean (± standard error) survival of 4.3 ± 0.4 days (range, 3 to 7 days) after challenge.

Therapy with amphotericin B (Fungizone; E. R. Squibb & Sons, Princeton, N.J.) or SCH (Schering-Plough Research, Bloomfield, N.J.) was begun 24 h after challenge. Amphotericin B was diluted with 5% glucose in sterile water at a ratio of 1 mg of drug to 10 ml of diluent and was given intravenously over 30 to 60 min through a lateral ear vein at a dosage of 1.5 mg/kg of body weight per day for 4 to 7 days (mean, 5.2 ± 0.3 days). SCH was dissolved in sterile water and administered as an oral suspension via gastric gavage tube (American Pharmaseal Company, Valencia, Calif.) at dosages of 10 or 15 mg/kg/day (SCH 10 and 15, respectively) for 5 days.

Tissue samples were cultured and histopathological examinations were performed at the time of autopsy or sacrifice (48 to 72 h after completion of therapy for the treated rabbits). After being anesthetized with 35 mg of ketamine per kg (Bristol Laboratories, Syracuse, N.Y.) and 10 mg of xylazine per kg (Mobay Corp., Shawnee, Kans.), rabbits were sacrificed by lethal exsanguination. Cultures were obtained by placing minced organ samples directly on blood agar and on Sabouraud dextrose agar plates. Samples were considered positive when more than one colony of *A. fumigatus* was present on ≥1 g of minced organ tissues plated directly on Sabouraud dextrose and blood agar plates or when semiquantitative cultures of tissue homogenates contained over 10 CFU/g of tissue (11). The tissue burden of *A. fumigatus* was evaluated with a modification (10, 11) of

* Corresponding author.

the semiquantitative culture technique of Graybill and Kaster (6). Samples of liver, kidney, lung, and brain tissues were manually chopped, weighed, diluted 1:10 (wt/vol) with sterile saline, and homogenized for 25 s with an electric tissue homogenizer (TRI-R Instruments, Rockville Center, N.Y.). Then 1.0- and 0.1-ml samples of each organ homogenate were plated in duplicate on Sabouraud dextrose and blood agar plates. The plates were incubated for 48 h at 37°C, and colonies were counted. The combination of these methods detected from 2 to 20,000 CFU/g of tissue.

SCH levels. Levels of SCH in serum were measured by using modifications of previously reported bioassay techniques (13). Briefly, *Candida kefyr* (kindly provided by Patrick Robinson) was grown overnight in Sabouraud dextrose broth at 37°C and diluted with saline to a concentration of 5×10^5 cells with a hemacytometer. A 1:50 dilution of cells was made with High Resolution medium (Pfizer Central Research, Groton, Conn.) prepared according to the manufacturer's specifications. A 45-ml sample of the inoculated medium was poured into plastic plates (150 by 25 mm) and wells were cut with a 4-mm punch. A standard curve was made by using known concentrations of drug ranging from 3.125 to 50 μg/ml. Samples and standards were placed in duplicate into wells, and the plates were incubated at 30°C for 24 h. Inhibition zones were measured, and sample zones were compared with known standards. Blood for peak drug levels in serum was drawn from immunocompromised and challenged rabbits 1.5 to 2 h after the third dose of drug (7). Trough levels were obtained from the same rabbits 48 h after five doses of drug (which coincided with the time of sacrifice).

Inhibition ELISA for serum aspergillus antigen in serum. The procedures for the inhibition enzyme-linked immunosorbent assay (ELISA) and for its required antigen and antibody preparation were performed as previously reported (10–12).

Statistical analysis. The Fisher exact test and the Wilcoxon rank sum test were used when appropriate. Statistical significance was defined as $P < 0.05$.

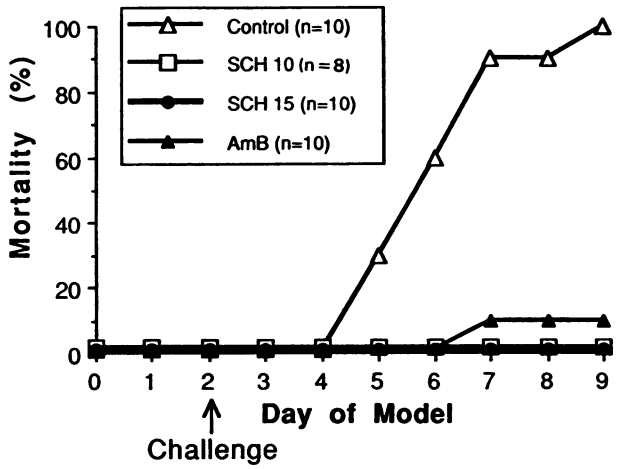

FIG. 1. Treatment studies. Cumulative mortality of lethally challenged rabbits treated with SCH and amphotericin B (AmB). Rabbits were challenged on day 2. Controls received no antifungal therapy. Treated rabbits had AmB (1.5 mg/kg/day) or SCH 10 or SCH 15 therapy initiated 24 h after challenge.

TABLE 1. Semiquantitative organ cultures from lethally challenged rabbits given antifungal therapy[a]

Treatment group (*n*)[b]	Colony counts (mean $\log_{10}$ CFU/g of tissue ± SE) in[c]:			
	Liver	Lung	Kidney	Brain
Control (10)	3.6 ± 0.2	2.8 ± 0.3	3.3 ± 0.2	1.1 ± 0.3
SCH 10 (8)	0.8 ± 0.4*	1.6 ± 0.3†	1.3 ± 0.4*	<0.3
SCH 15 (10)	<0.3*	0.6 ± 0.4*	<0.3*	<0.3
AmB 1.5 (10)	<0.3*	0.7 ± 0.3*	<0.3*	0.9 ± 0.6

[a] Therapy was begun 24 h after challenge.
[b] *n*, number of rabbits per group; AmB 1.5, amphotericin B at 1.5 mg/kg/day.
[c] *, $P < 0.001$; †, $P < 0.01$ (compared with controls).

RESULTS

Following three doses of therapy, peak levels of SCH 10 in serum were 8.4 ± 0.8 μg/ml, and levels of SCH 15 in serum were 12.0 ± 0.7 μg/ml (mean ± standard error). Mean trough levels, measured 48 h after five doses of therapy, for both SCH 10 and SCH 15 were below the limits of detection by bioassay (the minimum level of detection of this assay is 3.125 μg/ml).

The survival of rabbits treated with SCH or amphotericin B begun 24 h after challenge is shown in Fig. 1. Survival was significantly prolonged by day 6 of the model with SCH 10 and SCH 15 therapy ($P = 0.008$ and 0.005, respectively, versus controls) and with amphotericin B at 1.5 mg/kg/day ($P = 0.005$ versus controls). By day 9, mortality occurred in 10 of 10 untreated controls compared with 1 of 10 rabbits treated with amphotericin B, 0 of 8 rabbits treated with SCH 10, and 0 of 10 rabbits treated with SCH 15 (for all three treatments, $P < 0.00001$).

Semiquantitative results of organ cultures are shown in Table 1. Extensive infection occurred in the liver, lung, and kidney tissues of all untreated controls. SCH 10 and SCH 15 significantly reduced the tissue burden in livers, lungs, and kidneys (100- to more than 1,000-fold) compared with untreated controls. These results were not statistically different from those seen with amphotericin B, although colony counts from rabbits receiving SCH 15 or amphotericin B were lower than those from rabbits receiving SCH 10.

The numbers of infected organs in treated animals and untreated controls are shown in Table 2. Although SCH 10 significantly reduced the tissue burden of *A. fumigatus*, liver tissue in three of eight rabbits ($P < 0.001$ versus controls), lung tissue in seven of eight rabbits, and kidney tissue in five of eight rabbits remained infected, as did organs in untreated controls. In contrast, SCH 15 effectively reduced levels of the organism in most tissues below the limits of detec-

TABLE 2. Organ cultures of temporarily immunosuppressed rabbits

Treatment group (*n*)[a]	No. of positive cultures/no. of rabbits tested[b]			
	Liver	Lung	Kidney	Brain
Control (10)	10/10	9/10	10/10	5/10
SCH 10 (8)	3/8†	7/8	5/8§	0/8§
SCH 15 (10)	0/10*	2/10‡,‖	0/10*,‖	0/10§
AmB 1.5 (10)	1/10*	3/10‡	0/10*,‖	2/10

[a] *n*, number of rabbits per group; AmB, amphotericin B at 1.5 mg/kg/day.
[b] *, $P < 0.0001$; †, $P < 0.001$; ‡, $P < 0.01$; §, $P < 0.02$ (all compared with controls, calculated by the Fisher exact test); ‖, $P < 0.05$ (compared with SCH 10 values, calculated by the Fisher exact test).

TABLE 3. Final aspergillus antigen levels in serum of temporarily immunosuppressed rabbits[a]

Treatment group (n)[b]	No. of rabbits with >50 ng of antigen per ml/no. of rabbits tested[c]	Mean concn of antigen (ng/ml) ± SE (range)[d]
Control (10)	10/10	4,200 ± 533 (1,000–5,000)
SCH 10 (8)	6/8	122 ± 32‡ (15–290)
SCH 15 (10)	3/10*	40 ± 18‡,§ (<10–145)
AmB 1.5 (10)	0/10†	13 ± 5‡,‖ (<10–50)

[a] Antigen levels in serum were detected on day of sacrifice or in final sample obtained prior to death.

[b] n, number of rabbits per group; AmB, amphotericin B at 1.5 mg/kg/day.

[c] *, $P < 0.0015$; †, $P < 0.001$ (both compared with controls, calculated by the Fisher exact test).

[d] ‡, $P < 0.001$ (compared with controls, calculated by the Wilcoxon rank sum test); §, $P < 0.05$; ‖, $P < 0.001$ (both compared with SCH 10, calculated by the Wilcoxon rank sum test).

tion. Liver tissue in 0 of 10 rabbits ($P < 0.0001$ versus controls), lung tissue in 2 of 10 rabbits ($P < 0.01$ versus controls), kidney tissue in 0 of 10 rabbits ($P < 0.0001$ versus controls), and brain tissue in 0 of 10 rabbits ($P < 0.0001$ versus controls) remained positive for *A. fumigatus*. SCH 15 was more effective in eradicating the organism from lung and kidney tissues than SCH 10 ($P < 0.05$). Amphotericin B also reduced detection in most tissues, although lung tissue in 3 of 10 rabbits and brain tissue in 2 of 10 rabbits remained positive for *A. fumigatus*. None of the rabbits receiving either dosage of SCH had positive brain tissues compared with the untreated controls, half of which were infected ($P < 0.02$).

These dosages of SCH and amphotericin B significantly reduced *Aspergillus* antigenemia compared with untreated controls. The final antigen levels measured in serum samples drawn at the time of sacrifice and in the last serum sample drawn prior to death are shown in Table 3. Higher antigen levels were measured in rabbits receiving SCH 10 than in those receiving SCH 15 ($P < 0.05$) or amphotericin B ($P < 0.001$). Antigen levels of >50 ng/ml were detected in all 10 controls, with a maximum level of 5,000 ng/ml. In contrast, a maximum antigen level of 290 ng/ml was seen in rabbits treated with SCH 10, compared with 145 ng/ml for SCH 15 and 50 ng/ml for amphotericin B. Final antigen levels of >50 ng/ml were seen in only 3 of 10 rabbits treated with SCH 15 ($P = 0.0015$ versus controls) and in none of the 10 rabbits receiving amphotericin B ($P < 0.0001$ versus controls).

Low levels of circulating antigen remained in the treated rabbits, and these levels correlated with persistence of infection. Antigen levels in the final samples drawn prior to autopsy or sacrifice were less than 50 ng/ml for 14 of 15 rabbits with no detectable *A. fumigatus* in organ cultures. In contrast, rabbits with one or two positive organs had 94 ± 20 ng of antigen per ml, and those with three or four positive organs had antigen levels of 4,200 ± 533 ng/ml.

DISCUSSION

Antifungal agents with increased efficacy and decreased toxicity are needed to improve the management of invasive aspergillosis (2). Newer azoles offer several potential advantages in the treatment of invasive fungal infection. SCH is one of the newer azoles which demonstrates several of these characteristics, including a broad spectrum of antifungal activity, excellent absorption following oral administration, minimal acute toxicity, and solubility characteristics which could permit intravenous administration (3, 7). While the potential for long-term toxicity may prevent further clinical investigation of SCH (8), this study demonstrates that in addition to the favorable pharmacokinetic characteristics of these compounds, excellent activity against invasive pathogens including molds such as *A. fumigatus* can be demonstrated.

Many of the newer antifungal compounds have recently been shown to possess activity in vivo with animal models of infection even when little or no activity can be demonstrated in vitro by some techniques (5). These differences have been seen particularly in the evaluation of molds and emphasize the difficulty of predicting in vivo efficacy on the basis of some in vitro results.

SCH has been shown to have inhibitory activity against *A. fumigatus*, but the azoles, including SCH, are not usually considered fungicidal against molds (4, 5). In a previous study with a murine model of invasive aspergillosis, SCH significantly reduced the tissue burden of infection but did not sterilize tissues (3). However, results achieved with our rabbit model demonstrated that this compound markedly reduces the tissue burden, as significant numbers of organs had no detectable *Aspergillus* colonies (according to an assay system that detects as few as two colonies per g of tissue. The success of this compound in rabbits may be due at least in part to the prolonged half-life of this compound (approximately 25 h), which results in high levels of this drug in serum (7).

This model, like all models of lethal infection, is limited by the fact that organs from untreated controls were not cultured at the same time as organs from the treated rabbits. Fungal burden in surviving rabbits may be reduced, in part, by the return of circulating granulocytes. However, in other studies using a sublethal challenge, we have shown that untreated controls surviving until sacrifice have a tissue burden virtually identical to that in controls cultured at autopsy (9). It is important to note that SCH not only reduced tissue burden but also improved survival and reduced antigen levels in serum, a fact which correlated with the reduced tissue burden of *A. fumigatus*. SCH 10, SCH 15, and amphotericin B markedly reduced circulating aspergillus antigen, compared with untreated controls. In contrast, all untreated controls developed significant antigenemia with maximum values ranging from 1,000 to 5,000 ng/ml compared with a range of <10 to 290 ng/ml for treated animals. SCH 15 was more effective in reducing or eliminating antigenemia than SCH 10, which correlated with elimination of *A. fumigatus* in tissues of rabbits receiving the higher dosages of drug.

In these studies, SCH 15 was as effective as amphotericin B in reducing the tissue burden of liver, lung, and kidney tissues. However, with amphotericin B, 3 of 10 rabbits continued to have *Aspergillus* organisms cultured from lung tissues, and 2 of 10 rabbits had positive brain cultures. None of the rabbits treated with either dosage of SCH had brain cultures positive for *A. fumigatus*, compared with positive brain cultures in half the untreated controls ($P < 0.02$). SCH has excellent central nervous system penetration (7), which may be an important advantage of azole compounds for the management of disseminated infection.

In conclusion, SCH effectively prolongs survival, significantly reduces antigenemia, and dramatically reduces the tissue burden in an immunosuppressed rabbit model of invasive aspergillosis. These studies show that the newer azoles may offer a significant improvement in the treatment of invasive aspergillosis.

ACKNOWLEDGMENTS

We thank David Loebenberg for helpful advice.

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

REFERENCES

1. **Aisner, J., S. C. Schimpff, and P. H. Wiernik.** 1977. Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. Ann. Intern. Med. **86**:539–543.
2. **Bodey, G. P., and S. Vartivarian.** 1989. Aspergillosis. Eur. J. Clin. Microbiol. Infect. Dis. **5**:413–437.
3. **Defaveri, J., M. H. Salazar, M. G. Rinaldi, and J. R. Graybill.** 1990. Pulmonary aspergillosis in mice: treatment with a new triazole SCH39304. Am. Rev. Respir. Dis. **142**:512–518.
4. **Fromtling, R. A.** 1988. Overview of medically important antifungal azole derivatives. Clin. Microbiol. Rev. **1**:187–217.
5. **Graybill, J. R.** 1989. New antifungal agents. Eur. J. Clin. Microbiol. Infect. Dis. **5**:402–412.
6. **Graybill, J. R., and S. R. Kaster.** 1984. Experimental murine aspergillosis: comparison of amphotericin B and a new polyene antifungal drug, SCH 28191. Am. Rev. Respir. Dis. **129**:292–295.
7. **Lee, J. W., C. Lin, D. Loebenberg, M. Rubin, P. A. Pizzo, and T. J. Walsh.** 1989. Pharmacokinetics and tissue penetration of Sch 39304 in granulocytopenic and nongranulocytopenic rabbits. Antimicrob. Agents Chemother. **33**:1932–1935.
8. **Loebenberg, D.** Personal communication.
9. **Patterson, T. F., D. George, P. Miniter, and V. T. Andriole.** 1991. The role of fluconazole in the early treatment and prophylaxis of experimental invasive aspergillosis. J. Infect. Dis. **164**:575–580.
10. **Patterson, T. F., P. Miniter, J. Dijkstra, F. C. Szoka, Jr., J. L. Ryan, and V. T. Andriole.** 1989. Treatment of experimental invasive aspergillosis with novel amphotericin B/cholesterol-sulfate complexes. J. Infect. Dis. **159**:717–724.
11. **Patterson, T. F., P. Miniter, J. L. Ryan, and V. T. Andriole.** 1988. Effect of immunosuppression and amphotericin B on aspergillus antigenemia in an experimental model. J. Infect. Dis. **158**:415–422.
12. **Sabetta, J. R., P. Miniter, and V. T. Andriole.** 1985. The diagnosis of invasive aspergillosis by an enzyme-linked immunosorbent assay for circulating antigen. J. Infect. Dis. **152**: 946–953.
13. **Shadomy, S., and A. Espinel-Ingroff.** 1988. Methods for bioassay of antifungal antimicrobics: laboratory evaluation, p. 327–337. *In* A. I. Laskin and H. A. Lechevalier (ed.), CRC handbook of microbiology, 2nd ed., vol. VI. CRC Press, Inc., Boca Raton, Fla.
14. **Young, R. C., J. E. Bennett, C. L. Vogel, P. P. Carbone, and V. T. DeVita.** 1970. Aspergillosis. The spectrum of disease in 98 patients. Medicine (Baltimore) **49**:147–173.