ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1990, p. 1560–1564
0066-4804/90/081560-05$02.00/0

Vol. 34, No. 8

SCH-39304 in Prevention and Treatment of Disseminated Candidiasis in Persistently Granulocytopenic Rabbits

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Received 27 December 1989/Accepted 31 May 1990

To investigate the potential use of SCH-39304 for the prevention and treatment of disseminated candidiasis in granulocytopenic patients, we studied its in vivo antifungal activity as preventive, early, and late treatments in three models (acute, subacute, and chronic) of disseminated candidiasis in persistently granulocytopenic rabbits. SCH-39304 was as effective as amphotericin B alone and fluconazole alone for the prevention of disseminated candidiasis. SCH-39304 alone and fluconazole alone were as effective as amphotericin B plus flucytosine for early treatment of subacute disseminated candidiasis. When treatment was delayed for 5 days to establish chronic disseminated candidiasis, SCH-39304 was less effective than amphotericin B plus flucytosine. In comparison with different treatment regimens, SCH-39304 was more effective in early and preventive treatment. Thus, SCH-39304 was comparable to treatment control regimens in prevention and early treatment of subacute disseminated candidiasis. SCH-39304 also was most effective in granulocytopenic rabbits with disseminated candidiasis when used for prevention or early treatment.

The treatment of systemic mycoses in granulocytopenic patients has been impeded by a lack of antifungal agents that have potent antifungal activity, good oral bioavailability, long half-life, good tissue penetration, and minimal toxicity (2, 14). The newly available triazole, fluconazole, as well as the investigational triazoles itraconazole and SCH-39304, offers potentially major advances in antifungal therapy. Although fluconazole and itraconazole have been studied extensively in animal models of experimental candidiasis and fluconazole is undergoing further clinical trials for use in disseminated candidiasis (2, 6, 7, 14), comparatively little is known about SCH-39304.

SCH-39304 is an N-substituted triazole antifungal compound that possesses potent activity against a broad spectrum of fungi, including *Candida* spp. and *Aspergillus* spp. It has a long plasma half-life and extensive penetration into multiple tissues, including the central nervous system (3, 5; T. J. Walsh, C. McCully, M. Rinaldi, F. Bayliss, J. Lee, P. A. Pizzo, and D. Poplack, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1361, 1989).

SCH-39304 has been shown to be effective in treatment of experimental *Candida* pyelonephritis and endophthalmitis in immunocompetent rabbits (5) and against disseminated candidiasis in noncompromised rats (4). Because little is known about the efficacy of SCH-39304 in the prevention and treatment of disseminated fungal infections in granulocytopenic hosts, we studied its activity in several models of experimental disseminated candidiasis in persistently granulocytopenic rabbits.

MATERIALS AND METHODS

Animals. Pathogen-free female New Zealand White rabbits (Hazleton, Rockville, Md.) weighing 2.0 to 3.0 kg each were used. A Silastic central venous catheter was inserted into each rabbit by using sterile operating room procedures, as previously described (11). The Silastic catheter permitted continuous nontraumatic venous access, supportive care, and monitoring throughout the period of profound granulocytopenia. Supportive care and monitoring included administration of intravenous (i.v.) fluids, parenteral antibiotics, and cytotoxic chemotherapy, as well as drawing of blood for daily evaluation of leukocyte counts, platelet counts, biochemical screenings, and plasma pharmacokinetics. Animals were housed in individual cages, provided with food and water ad libitum, and maintained in accordance with National Institutes of Health guidelines on care and use of laboratory animals (1).

Immunosuppression. To simulate the conditions of protracted granulocytopenia, we produced a 1- to 3-week period of granulocytopenia (granulocyte count of <500/μl) in our rabbits, depending on the model of experimental disseminated candidiasis. Profound granulocytopenia (granulocyte count of <100/μl) was caused by i.v. administration of 1-β-D-arabinofuranosylcytosine (kindly provided by The Upjohn Co., Kalamazoo, Mich.): 400 mg/m^2 per day i.v. on days 1 to 5 (induction) and 400 mg/m^2 per day i.v. every other 2 days (maintenance).

Antibiotic therapy. To prevent mortality and morbidity from bacterial infections, ceftazidime (Glaxo, Research Triangle Park, N.C.), 150 mg/kg of body weight per day i.v., and vancomycin (Eli Lilly & Co., Indianapolis, Ind.), 15 mg/kg per day i.v., were initiated on day 4 of 1-β-D-arabinofuranosylcytosine cytotoxic chemotherapy induction and were continued throughout the period of granulocytopenia.

Fungus and preparation of inoculum. *Candida albicans* NIH 86-21B from a granulocytopenic patient with autopsy-proven disseminated candidiasis was used for all experiments. Cultures of the isolate were maintained at −40°C in skim milk suspension. To prepare the inoculum, the organism was thawed, inoculated onto Sabouraud glucose agar plates, and incubated at 37°C. Twenty-four hours before inoculation, approximately 10 colonies were sampled, inoculated into 50 ml of Emmons modified Sabouraud glucose medium (pH 7.0) in a 250-ml Erlenmeyer flask, and incu-

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TABLE 1. SCH-39304 in prevention and treatment of disseminated candidiasis in persistently granulocytopenic rabbits

Animal models				Treatment regimens			
Model	Inoculum size (CFU)[a]	Survival endpoint[b] (days)	Histopathological features	Regimen	Time of first dose	Purpose	Animal models studied
Acute	1.0×10^6	4	Extensive visceral dissemination, myocutaneous lesions, mycelia without inflammatory reaction	Preventive	3 days before inoculation	Prevention of infection (evaluation of prophylaxis)	Subacute
Subacute	2.5×10^5	8	Pattern intermediate between acute and chronic models in the liver, spleen, and kidney; no myocutaneous lesions	Early	24 h after inoculation	Early treatment of infection (evaluation of empirical therapy)	Acute, subacute
Chronic	5.0×10^4	14	Confluent, fibrotic, calcified, necrotic lesions in the liver, spleen, and kidney; no myocutaneous lesions	Delayed	5 days after inoculation	Treatment of established infection	Chronic

[a] *C. albicans* NIH 86-21B.
[b] Untreated animals.

bated in a gyratory incubator at 80 oscillations per min at 37°C for 2 h. Approximately 10^5 organisms (counted by hemacytometer and observed to be predominantly blastoconidia in singlets and doublets) were then transferred into fresh 250-ml Erlenmeyer flasks containing 50 ml of Emmons modified Sabouraud glucose medium and incubated at 80 oscillations per min overnight for 16 h. The *Candida* suspension was centrifuged at 4,000 × *g* for 10 min, and the *Candida* pellet was suspended and then washed three times with 0.9% NaCl. The inoculum was adjusted by hemacytometer and by serial dilutions to a concentration of 10^4 organisms per ml (predominantly blastoconidia in singlets and doublets). Hemacytometer counts were verified by 10-fold serial dilutions of the suspension onto Sabouraud glucose agar plates. The total inoculum for each rabbit was suspended in 5 ml of sterile 0.9% NaCl and administered slowly via the indwelling Silastic i.v. catheter. Rabbits were inoculated on day 6 of induction chemotherapy, for a total granulocyte count of <500/μl.

Antifungal compounds. SCH-39304 (Schering Corp., Kenilworth, N.J.) was prepared as a suspension in lactated Emulfor EL-719P (GAF Corp, Pittsburgh, Pa.) and deionized water for a final concentration of 5 mg/ml and administered at 2 mg/kg per day per os. Fluconazole was kindly provided by Pfizer Central Research (Groton, Conn.) as the oral encapsulated formulation and administered in a saline suspension at 2 mg/kg per day per os. This dosage of SCH-39304 was selected to parallel the dosage and concentrations in plasma (1 to 2 μg/ml) anticipated for clinical trials. Amphotericin B (E. R. Squibb & Sons, Princeton, N.J.) and flucytosine (kindly provided by Hoffmann-La Roche Inc., Nutley, N.J.) were administered at 0.6 and 50 mg/kg per day i.v., respectively. MICs determined at 24 h by macrodilution in synthetic amino acid antifungal medium (American Biorganics Inc., North Tonawanda, N.Y.) against *C. albicans* 86-21B used in this study were 0.125 μg/ml for amphotericin B, 16 μg/ml for flucytosine, 8 μg/ml for fluconazole, and 4 μg/ml for SCH-39304.

Plasma pharmacokinetics and tissue penetration of SCH-39304 in our rabbit models were previously reported (3). This study revealed no differences between granulocytopenic and nongranulocytopenic rabbits in gastrointestinal absorption or plasma pharmacokinetics of SCH-39304 measured by gas-liquid chromatography. In both granulocytopenic and nongranulocytopenic rabbits, the mean peak concentration in plasma was 1.4 ± 0.11 μg/ml at 4 ± 0.5 h and the half-life in plasma was 25 ± 1.4 h.

Animal models and treatment regimens. We evaluated three models of disseminated candidiasis in persistently granulocytopenic rabbits: acute, subacute, and chronic disseminated candidiasis. Treatment regimens were preventive, early, and delayed. These models and treatment regimens are outlined in Table 1 and in greater detail elsewhere (8, 10–12). Early treatment was studied in acute and subacute models of disseminated candidiasis. Delayed treatment was evaluated in chronic disseminated candidiasis. Amphotericin B alone was used as a control in the preventive model, whereas the combination of amphotericin B plus flucytosine (A+FC), as well as fluconazole, was used as a control in the treatment models.

Surviving rabbits with persistent granulocytopenia were treated with antifungal therapy for 14 days. Rabbits were euthanized by pentobarbital anesthesia upon completion of the study for postmortem examinations. Six to eight rabbits were assigned to each treatment group, resulting in 18 to 24 rabbits per experiment.

Assessment of antifungal efficacy. Representative sections of cerebrum, lung, liver, spleen, kidney, and chorioretina were weighed and then homogenized in sterile, reinforced polyethylene bags (Tekmar Corp., Cincinnati, Ohio), as previously described (13). Each tissue homogenate was serially diluted 100-fold from 10^{-2} to 10^{-4} in sterile normal saline. A 0.1-ml quantity of undiluted homogenate and of each dilution was separately plated onto Sabouraud glucose agar containing chloramphenicol and gentamicin. Culture plates were incubated at 37°C for 24 h, after which CFU were counted. The number of CFU per gram of tissue was calculated for each organ. The method was sensitive to ≥10 CFU/g.

Microbiological response to antifungal therapy was evaluated by comparisons of mean tissue concentrations of *C. albicans* (measured in CFU per gram). Data were graphed as the mean of $\log_{10}$ (CFU per gram) ± standard error of the mean (SEM). Clinical response to antifungal therapy was

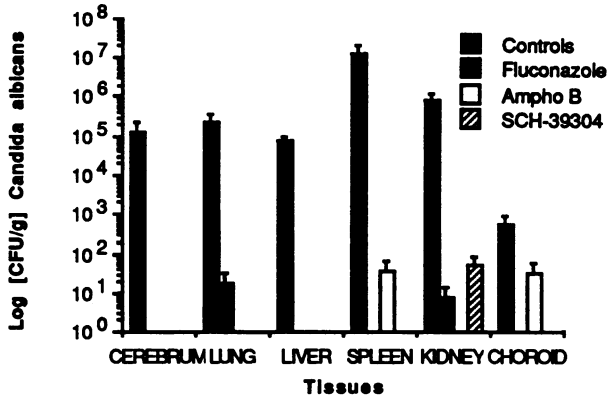

FIG. 1. Preventive treatment of subacute disseminated candidiasis. Bars represent mean *C. albicans* CFU (± SEM) recovered from each tissue. An untreated control group and three treatment groups were studied for each tissue; absence of bars indicates <10 CFU/g.

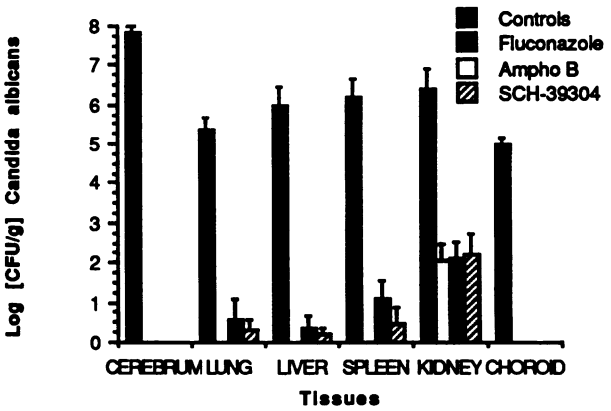

FIG. 2. Early treatment of subacute disseminated candidiasis. Bars represent mean *C. albicans* CFU (± SEM) recovered from each tissue. An untreated control group and three treatment groups were studied for each tissue; absence of bars indicates <10 CFU/g.

measured by comparing durations of survival after inoculation. The *Candida* concentrations (CFU/g) for each tissue and the durations of survival after inoculation among the antifungal groups and untreated controls were compared by unpaired Student's *t* test. The proportions of tissues cleared in all groups were compared by chi-square analysis and two-tailed Fisher's exact test.

RESULTS

Preventive treatment. Preventive treatment was evaluated in persistently granulocytopenic rabbits with subacute disseminated candidiasis (Fig. 1). Persistently granulocytopenic rabbits receiving preventive treatment with SCH-39304, fluconazole, and amphotericin B each exerted a $\geq 10^3$ reduction of *C. albicans* concentration (CFU per gram) in the cerebrum, lung, liver, spleen, and kidney in comparison with untreated controls ($P \leq 0.01$). There was no significant difference in *C. albicans* CFU per gram of kidneys between rabbits treated with amphotericin B and those treated with SCH-39304 ($P = 0.27$). All treated rabbits survived for 14 days of therapy.

Early treatment. Early antifungal therapy (24 h after inoculation) was evaluated in models of subacute and acute disseminated candidiasis. In comparison with no treatment (controls), SCH-39304, fluconazole, and A+FC exerted similar activities in all tissues for early treatment of subacute disseminated candidiasis (Fig. 2). A $\geq 10^4$-fold reduction was obtained in the cerebrum, lung, liver, spleen, and choroid ($P \leq 0.001$). Each of the antifungal compounds also exerted a $\geq 10^3$-fold reduction in CFU per gram in the kidney. All rabbits with subacute disseminated candidiasis receiving early treatment (24 h after inoculation) survived and were sacrificed at the end of 14 days of antifungal therapy. All untreated control rabbits died within 7 days following inoculation.

Early treatment with SCH-39304 and amphotericin B was also evaluated in a high-inoculum model of acute disseminated candidiasis with persistent granulocytopenia (Fig. 3). The combination A+FC was more active than SCH-39304 in all tissue sites in the model of acute disseminated candidiasis and was significantly more effective in the spleen and kidney ($P \leq 0.01$) (Fig. 3). In comparison with untreated controls with acute disseminated candidiasis (mean survival, 2.3 ± 0.3 days), rabbits treated with A+FC survived longer (6.5 ± 1.0 days; $P = 0.02$) than did those treated with SCH-39304 (4.5 ± 0.9 days; $P = 0.07$). There was no significant difference in survival between animals treated with A+FC and those treated with SCH-39304 ($P = 0.20$).

Delayed treatment. Antifungal therapy with SCH-39304 and with A+FC was delayed by 5 days in a low-inoculum model of chronic disseminated candidiasis (Fig. 4). There was no difference in survival between the two groups (13.2 versus 14.1 days). The combination A+FC caused a $\geq 10^2$-fold reduction in *C. albicans* in the cerebrum, lung, and spleen in comparison with untreated controls ($P \leq 0.05$). SCH-39304 caused a comparable $\geq 10^2$-fold reduction in the lung in comparison with untreated controls ($P \leq 0.05$). Antifungal activity for both regimens was greater in the lung than in other sites. A ≥10-fold reduction in CFU per gram was found in 33 of 36 tissues treated with A+FC in comparison with 16 of 36 tissues treated with SCH-39304 ($P \leq 0.001$). The antifungal effects of SCH-39304 and A+FC in chronic disseminated candidiasis were less than that observed in the subacute model treated with preventive or early antifungal therapy.

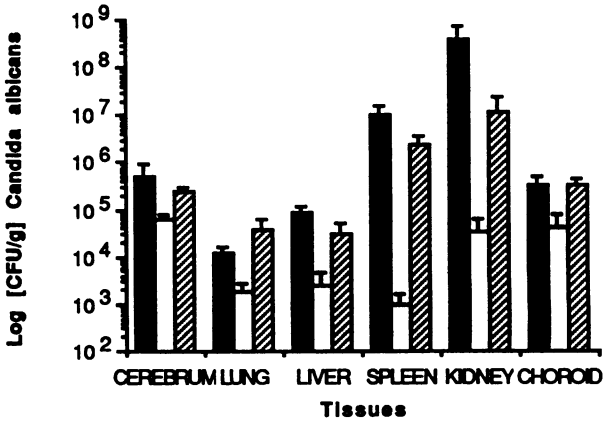

FIG. 3. Early treatment of acute disseminated candidiasis. Bars represent mean *C. albicans* CFU (± SEM) recovered from each tissue. An untreated control group (■) and groups treated with A+FC (□) or SCH-39304 (▨) were studied for each tissue.

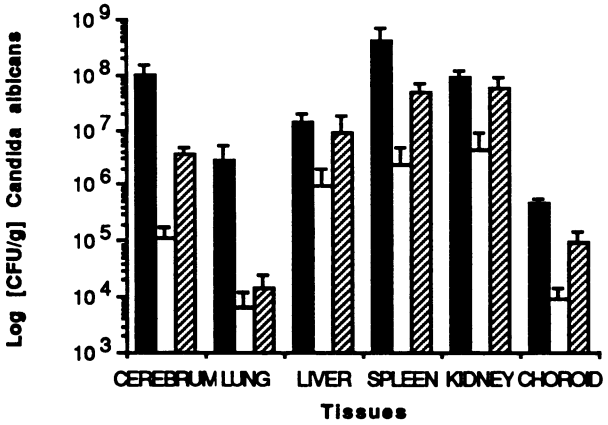

FIG. 4. Delayed treatment of chronic disseminated candidiasis. Bars represent mean *C. albicans* CFU (± SEM) recovered from each tissue. An untreated control group (■) and groups treated with A+FC (□) or SCH-39304 (▨) were studied for each tissue.

DISCUSSION

These studies showed that SCH-39304 was most active against systemic candidiasis in persistently granulocytopenic rabbits when used as either preventive or early antifungal therapy. By comparison, SCH-39304 was less effective against established chronic infection. Preventive treatment with SCH-39304 was as effective as amphotericin B alone and as fluconazole against subacute disseminated candidiasis. Early treatment with SCH-39304 against subacute disseminated candidiasis was as effective as fluconazole and as the combination A+FC. Previous studies demonstrated that fluconazole was less effective than A+FC in delayed treatment of chronic disseminated candidiasis (10).

Several studies in our laboratory have demonstrated differences in treatment outcome as a function of the timing and stage of infection (12). The antifungal triazoles itraconazole, fluconazole, and SCH-39304 were most effective and as active as A+FC when administered as preventive or early treatment. Other investigators found similar properties of antifungal azoles in the treatment of experimental *Candida* endophthalmitis (7). Persistently granulocytopenic rabbits with chronic disseminated candidiasis were treated for approximately 14 days with SCH-39304 at a dosage of 2 mg/kg per day in this study. Other experiments conducted in our laboratory indicated that larger doses of 4 mg/kg per day for 2 weeks were no more effective than 2 mg/kg per day for 2 weeks. Perhaps a more protracted course (≥3 weeks) of SCH-39304, 2 mg/kg per day, would be more effective in the model of chronic disseminated candidiasis.

That the antifungal activity of investigational triazoles is dependent on timing of administration and pattern of infection may be a general property of this class of antifungal compounds. There are several possible mechanisms that may be hypothesized for these differences in the responsiveness of different phases of candidiasis to SCH-39304. The larger size of untreated lesions of chronic deep visceral candidiasis (e.g., hepatosplenic candidiasis) may prevent sufficient radial diffusion of antifungal compounds. Early fibrosis around untreated, established lesions may further impair antifungal drug penetration. Fungal elements in the center of the larger *Candida* lesions may have a reduced metabolic activity and less susceptibility to triazole antifungal agents, which inhibit cytochrome P-450-dependent enzymes involved in sterol synthesis. Exposure of fungi to subinhibitory concentrations of poorly diffusing antifungal drugs may lead to emergence of resistance. These possible mechanisms remain hypothetical and require further investigation.

The antifungal effect of the combination A+FC was greater than that of SCH-39304 in treatment of chronic disseminated candidiasis. However, A+FC in these studies also was less effective in the delayed treatment of chronic disseminated candidiasis than in the early treatment of disseminated candidiasis. Our earlier studies of chronic disseminated candidiasis showed that A+FC was more effective in treatment of this infection. However, in comparison with rabbits in previous experiments, rabbits in this study were more profoundly granulocytopenic (granulocyte counts of ≤100/μl) and more likely to have refractory infection.

Granulocytopenic rabbits receiving preventive or early treatment for subacute infection had comparable survival rates. These trends in survival correlated with effective antifungal activity in all tissues. However, concentrations of *C. albicans* in tissue may be a more sensitive indicator of antifungal activity in neutropenic animals than is mortality. For example, while there was only a marginal difference in survival rates between rabbits with acute disseminated candidiasis which were treated with A+FC and those treated with SCH-39304, there were significant differences observed in concentrations of *C. albicans* in spleen and kidney tissues. Granulocytopenic patients, by comparison, may survive their initial fungemia after a course of antifungal therapy but may subsequently be found to have persistent hepatic or other deep-tissue candidiasis that was not eradicated during the initial treatment (9).

Clearly, no experimental animal model completely simulates the complexities of individual granulocytopenic patients. Nevertheless, these experimental findings suggest that SCH-39304 might be best studied in preventive or early-treatment regimens against disseminated candidiasis in persistently granulocytopenic patients. Amphotericin B given early in the course of infection has been found to be effective in reducing the frequency and mortality of invasive fungal infections in profoundly granulocytopenic patients (14). The potent in vivo activity against *Candida* spp. and *Aspergillus* spp., a good pharmacokinetic profile, and the relative lack of toxicity of SCH-39304 warrant its further investigation for preventive or early empirical antifungal therapy in granulocytopenic patients.

ACKNOWLEDGMENTS

We thank the staffs of the Office of Laboratory Animal Science of the National Cancer Institute and of the Veterinary Resources Branch, Division of Research Services, National Institutes of Health, for their excellent laboratory animal care.

This study was supported in part by a grant from the Schering-Plough Corporation.

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