ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1989, p. 1932–1935
0066-4804/89/111932-04$02.00/0

Vol. 33, No. 11

Pharmacokinetics and Tissue Penetration of Sch 39304 in Granulocytopenic and Nongranulocytopenic Rabbits

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Received 8 February 1989/Accepted 28 July 1989

We studied the plasma pharmacokinetics and tissue penetration of Sch 39304 (SCH), a new antifungal triazole, in granulocytopenic [G(+)] and nongranulocytopenic [G(−)] rabbits. Five female New Zealand White G(−) rabbits were given a single oral dose of 2 mg of SCH per kg of body weight. Levels in plasma, determined by gas-liquid chromatography–electron capture, were obtained for 6 days. This procedure was repeated 2 weeks later with the same rabbits, which were induced and maintained G(+) with cytosine arabinoside. There were no significant differences between the pharmacokinetic parameters of G(+) and G(−) rabbits. Among all animals studied, the maximum concentration of the drug in plasma was 1.4 ± 0.11 μg/ml at 4 ± 0.5 h, the half-life was 25 ± 1.4 h, the volume of distribution at steady state was 3.8 ± 0.3 liters, and the area under the concentration-time curve was 44 ± 3.4 μg · h/ml. SCH was detectable in plasma up to day 6. Levels of SCH in tissue were studied at steady state in six G(+) and six G(−) rabbits receiving 2 mg of the drug orally per kg per day for experimental disseminated candidiasis. Tissue SCH levels equalled or exceeded those in plasma (at steady state) at all sites examined, and these ratios were similar in both G(+) and G(−) rabbits. Thus, plasma pharmacokinetics of orally administered SCH were similar for G(+) and G(−) rabbits, and SCH achieved high levels of penetration into multiple tissues, including the liver and the central nervous system.

Despite advances in antibacterial therapy, the treatment of systemic mycoses in immunocompromised patients remains difficult because of a lack of effective antifungal agents with favorable pharmacokinetic and pharmacologic properties. Agents which have good oral bioavailability, a long half-life, high tissue penetration, minimal toxicity, and broad antifungal activity in immunocompromised patients are needed. Currently available drugs are either toxic and available only in intravenous form (amphotericin B) or are erratically absorbed and variably effective in immunocompromised patients (ketoconazole) (2).

Sch 39304 (SCH) is a new N-substituted triazole antifungal compound that shows good pharmacokinetic properties in mice, rats, and humans (C. Lin, H. Kim, A. Lapguera, D. Loebenberg, G. H. Miller, and S. Symchowicz, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 163, 1988; W. Kramer, H. Kim, S. Symchowicz, G. Perentesis, M. Affrime, and C. Lin, 28th ICAAC, abstr. no. 165, 1988) and promising antifungal activity in mice and rabbits (M. M. Hobbs, K. A. Wright, J. R. Perfect, C. Y. Tso, and D. T. Durack, 28th ICAAC, abstr. no. 166, 1988; B. I. Restrepo, J. Ahrens, and J. R. Graybill, 28th ICAAC, abstr. no. 170, 1988). In order to study the plasma pharmacokinetic properties and tissue penetration of SCH and to determine whether granulocytopenia alters these properties, we studied the plasma pharmacokinetic properties of both granulocytopenic and nongranulocytopenic rabbits, and then we obtained tissues for determining drug levels at steady state from both granulocytopenic and nongranulocytopenic rabbits with experimental disseminated candidiasis.

MATERIALS AND METHODS

Drug. SCH was provided by Schering Corp. (Kenilworth, N.J.) as a lyophilized powder. A 5-mg/ml suspension was made in Emulphor EL-719P (GAF Corp., Pittsburgh, Pa.) and deionized water.

Rabbits. Seventeen female New Zealand White rabbits weighing between 2.5 and 3.5 kg were used in these studies. Animals were individually housed and provided food and water ad libitum, according to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication no. 85-23). In order to provide continued nontraumatic venous access, silastic central venous catheters were surgically placed under sterile technique and general anesthesia as previously described (5).

Plasma pharmacokinetics. Each of five nongranulocytopenic rabbits was given a single oral dose of 2 mg of SCH per kg of body weight. Plasma samples were obtained before and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 18, and 24 h after each dose and at 24-h intervals for the next 6 days. After a 2-week washout period, these same five rabbits were made and maintained granulocytopenic (<500/μl) with 400 mg of cytosine arabinoside (kindly provided by The Upjohn Co., Kalamazoo, Mich.) per m^2 per day, given intravenously. SCH was given after 5 days of cytosine arabinoside, and the plasma sampling procedure used for the nongranulocytopenic rabbits was repeated for the five granulocytopenic rabbits.

Tissue penetration. Six nongranulocytopenic and six granulocytopenic rabbits were inoculated intravenously with 1×10^6 or 2×10^5 CFU of *Candida albicans*. These rabbits were treated for 14 days with SCH at 2 mg/kg per day orally, beginning 24 h postinoculum. Twenty-four hours after the last dose of SCH, plasma samples were obtained, the animals were sacrificed, and tissue samples from multiple sites were collected at autopsy. Tissues were carefully dissected to avoid blood contamination, and the bladders were drained prior to dissection to avoid contamination by urine. Since the half-life of SCH in rabbits turned out to be about 24 h, these levels in plasma and tissue were essentially at steady

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state. All plasma and tissue samples were stored at −70°C until time of assay.

Assay. Levels of SCH in plasma were determined by gas-liquid chromatography (GLC)–electron capture. Plasma (1 ml) was diluted with 0.5 ml of water and extracted with 5 ml of ethyl acetate by being mixed on a Vortex mixer for 1 min. After centrifugation, 4 ml of the organic layer was transferred to a clean tube and evaporated to dryness under nitrogen at 50°C. The residue was then dissolved in 1.5 ml of water and applied to a 1-ml disposable octadecyl extraction column (J. T. Baker Chemical Co., Phillipsburg, N.J.) which had been prewashed successively with 2 ml of methanol and 2 ml of water under a 5- to 10-mm Hg vacuum (Speed Mate-30; Applied Separations). The extraction column was then eluted with 2 ml of water followed by 1 ml of 10% methanol and 1.5 ml of methanol. The methanol fraction was collected and vortexed with 2.5 ml of hexane. After centrifugation, the methanol was evaporated to dryness under nitrogen. The residue was dissolved in 200 μl of ethyl acetate which contained 0.05 μg of Sch 18778 (internal standard). One microliter of the mixture was injected onto the GLC column.

The GLC unit (model 3700; Varian, Palo Alto, Calif.) was equipped with a ^{63}Ni electron capture detector and a DB-17 fused-silica megabore column (30 cm by 0.53 mm inside diameter; J & W Scientific, Rancho Cordova, Calif.) with a film thickness of 0.1 μm. The carrier and make-up gases were nitrogen with flow rates of 5 and 25 ml/min, respectively. The temperatures for injector, column, and detector were 210, 245, and 290°C, respectively. Electrometer attenuation was set at 16 to 64, and the range was set at 12.

Peak height ratio, retention times, and concentration were calculated by an HP-3357 Laboratory Automation System (Hewlett-Packard Co., Palo Alto, Calif.). Calculations of SCH concentrations were based on the ratio of the peak height of SCH to the peak height of the internal standard. The calibration was done by an unweighted average of triplicate plasma standards (0.5 μg of SCH and 0.05 μg of internal standard per ml). The GLC method is specific for SCH, since the retention time of this drug was clearly different from those of several analogs (fluconazole, econazole, ketoconazole, miconazole, terconazole, and sulconazole). There was a good linear relationship between the peak height ratios and the concentrations. Regression analysis of the data revealed the following equation: $y = 0.999x + 0.0024$; $r = 0.999$. The sensitivity of the method was 0.025 μg/ml (coefficient of variation, 8.9%). Two concentrations (0.2 and 1.0 μg/ml) of SCH in plasma with six replications were prepared and analyzed in a single run. The mean observed concentrations were 0.205 μg/ml (coefficient of variation, 4.1%) and 0.993 μg/ml (coefficient of variation, 3.2%), respectively, which indicated that the GLC method was accurate and reproducible.

Levels of SCH in tissue were determined as described above, using a 1:1 dilution with water of tissue homogenized in a blender. Tissues from untreated rabbits were used as controls. The sensitivity, linearity, and reproducibility of GLC analysis of SCH in tissues were similar to results with plasma.

Pharmacokinetic calculations. The areas under the plasma concentration-time curves (AUC) were calculated by using the linear trapezoidal rule up to the final measured concentration and then extrapolated to infinity (1). The terminal half-life in the postdistributive phase was determined by regression analysis with an unweighted least-linear-squares method. The volume of distribution at steady state (V_{ss}) was determined by using the area under the moment curve (3). The pharmacokinetic parameters for each rabbit were individually determined. Differences in the means of each group were evaluated by Student's unpaired t test; a P value of <0.05 was considered significant.

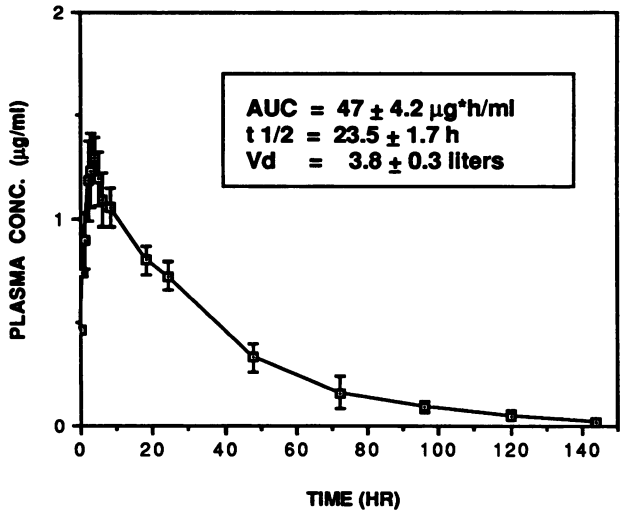

FIG. 1. Plasma SCH concentration versus time curve for five granulocytopenic rabbits after a 2-mg/kg oral dose. Each point plots the mean and standard error of level in plasma at that time. Mean calculated pharmacokinetic values for the five rabbits are shown in the box.

RESULTS

Single-dose plasma pharmacokinetics. Composite graphs representing the average concentration in plasma over time for granulocytopenic and nongranulocytopenic rabbits are shown in Fig. 1 and 2, respectively. The mean calculated pharmacokinetic values for the five rabbits in each group are also shown. The mean peak level of SCH for both groups was 1.4 μg/ml (range, 1.05 to 2.02 μg/ml), and the mean

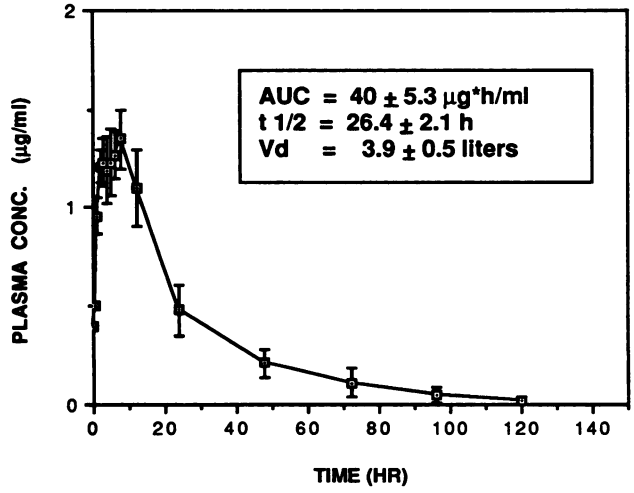

FIG. 2. Plasma SCH concentration versus time curve for five nongranulocytopenic rabbits after a 2-mg/kg oral dose. Each point plots the mean and standard error for level in plasma at that time. Mean calculated pharmacokinetic values for the five rabbits are shown in the box.

TABLE 1. Mean tissue concentrations of SCH and mean ratios of levels in tissue and plasma in granulocytopenic and nongranulocytopenic rabbits

Tissue site (n = 6)	Results (mean ± SD)			
	Nongranulocytopenic		Granulocytopenic	
	Concn of drug	Tissue/plasma ratio	Concn of drug	Tissue/plasma ratio
Cerebrum	1.6 ± 1.0 μg/g	2.3 ± 1.0	2.7 ± 0.87 μg/g	1.1 ± 0.22
Cerebellum	1.4 ± 0.16 μg/g	1.7 ± 0.54	2.8 ± 0.63 μg/g	1.3 ± 0.21
Choroid	0.9 ± 0.24 μg/g	1.3 ± 0.43	3.5 ± 1.2 μg/g	1.4 ± 0.30
Lung	1.2 ± 0.26 μg/g	1.4 ± 0.22	3.5 ± 0.89 μg/g	1.6 ± 0.22
Liver	11.9 ± 1.6 μg/g	15.0 ± 2.2	13.3 ± 1.7 μg/g	8.5 ± 1.8
Spleen	2.0 ± 0.54 μg/g	3.0 ± 1.1	3.6 ± 1.1 μg/g	1.5 ± 0.30
Kidney	1.3 ± 0.24 μg/g	1.6 ± 0.23	4.5 ± 1.4 μg/g	1.9 ± 0.26
Quadriceps	0.9 ± 0.21 μg/g	1.0 ± 0.13	2.4 ± 0.73 μg/g	1.1 ± 0.15
Vitreous	1.1 ± 0.24 μg/ml	1.3 ± 0.20	3.1 ± 1.0 μg/ml	2.0 ± 0.80
Cerebrospinal fluid	0.9 ± 0.22 μg/ml	1.0 ± 0.30		
Bile	1.7 ± 0.26 μg/ml	2.2 ± 0.67		
Plasma	0.94 ± 0.21 μg/ml		2.28 ± 0.60 μg/ml	

times of peak levels (T_{max}) were not significantly different for granulocytopenic (T_{max} = 3.6 ± 0.5 h) and nongranulocytopenic (T_{max} = 4.5 ± 0.96 h) rabbits, with an overall mean T_{max} of 4.0 ± 0.5 h. Similarly, the AUC, half-life, and V_{ss} were not significantly different between groups, since the overall means were 43.8 ± 3.4 μg · h/ml for AUC, 25 ± 1.4 h for half-life, and 3.8 ± 0.3 liters for V_{ss}.

Tissue penetration. Table 1 shows the mean levels of SCH in tissue and the mean ratios of levels in tissue and plasma at steady state for multiple sites in granulocytopenic and nongranulocytopenic rabbits. Levels of SCH in all tissues examined equalled or exceeded simultaneous concentrations in plasma. The liver-to-plasma ratio was significantly ($P < 0.05$) greater than for other tissues, showing 8.5- to 15-fold-higher concentrations of drug in liver than in plasma. There was no significant difference ($P > 0.05$) between the granulocytopenic and nongranulocytopenic rabbits in steady-state levels in plasma and in the mean ratios of concentrations in tissue and plasma.

DISCUSSION

This study demonstrates that SCH has favorable pharmacokinetic properties in both granulocytopenic and nongranulocytopenic rabbits. The drug was well absorbed, reaching a mean peak level within 4 h, but had a long half-life that led to detectable levels in plasma even 6 days after the relatively small dose of 2 mg/kg. This long half-life exceeds those of ketoconazole, miconazole, itraconazole, fluconazole, and flucytosine (4, 6). This combination of good absorption and long half-life resulted in levels of SCH in plasma which exceeded the MICs for *C. albicans* (0.08 to 0.3 μg/ml) for as long as 48 h postdose (F. Meunier, C. Lambert, and P. Van der Auwera, 28th ICAAC, abstr. no. 162, 1988).

To assess the ability of SCH to penetrate tissues, the means of the ratios of levels in tissue and plasma (Table 1) were calculated by determining the ratios of the levels in tissue and plasma for each individual rabbit in each group and then by calculating the mean of these six ratios. Thus, each value is not the ratio of the mean level in tissue over the mean level in plasma. This first method appears to be more representative of the true degree of tissue penetration, because even if a particular rabbit has a low level in plasma, its level in tissue may be many times higher, and thus its ratio, or relative degree of tissue penetration, would be high.

Accordingly, tissue penetration at steady state was excellent, with drug levels that equalled or exceeded levels in plasma in multiple sites, including the central nervous system. The liver showed a nearly 10-fold-higher concentration and the spleen showed up to 3-fold-higher levels than were in plasma. These properties may be particularly favorable for treatment of hepatosplenic candidiasis and other mycoses which frequently infect the liver and spleen. Moreover, the ability of this drug to penetrate well into such tissues as the choroid, vitreous, bile, and kidney may permit effective therapy of fungal infections of these often inaccessible sites.

Neither granulocytopenia secondary to cytotoxic chemotherapy nor disseminated candidiasis inhibited the uptake of SCH into tissues. The trough levels in plasma after 14 daily doses were somewhat higher, though not significantly so, in the granulocytopenic rabbits ($P > 0.05$). Since granulocytopenic rabbits eat and drink less and are more docile than nongranulocytopenic ones, the trend toward higher levels after multidosing to steady state may be due to better compliance to drug feeding, diminished clearance or V_{ss} in these rabbits, or more complete absorption due to decreased binding of drug to gastrointestinal contents in the granulocytopenic rabbits. However, compliance in accepting SCH appeared to be similar for both groups. SCH may also bind to *Candida* lesions. *Candida* lesions in granulocytopenic rabbits are larger and more numerous than those in nongranulocytopenic rabbits and have a paucity of surrounding inflammatory cells. The absence of granulocytes around *Candida* lesions of granulocytopenic rabbits may permit better penetration of SCH into these foci. SCH may also be more slowly released from these lesions in granulocytopenic rabbits. Another possibility is that SCH may bind to circulating granulocytes. In granulocytopenic rabbits, more SCH may be present in the plasma than in the cellular fraction.

Clearly, no animal model can ever completely reproduce the complexity of a granulocytopenic patient. Granulocytopenic patients may differ somewhat from our granulocytopenic rabbits in that cytotoxic drugs other than cytosine arabinoside may cause greater mucosal damage, greater gut transit time, or other changes in these patients that may affect the absorption, distribution, and elimination of SCH. Nevertheless, the apparently favorable pharmacokinetic and pharmacological properties of SCH—good absorption, long half-life, excellent tissue penetration—make it a potentially useful antifungal drug.

ACKNOWLEDGMENTS

We express our gratitude to Vanessa Thomas and Joanne Peter for technical assistance and to the staff of the Office of Laboratory Animal Science of the National Cancer Institute for their excellent animal care.

This study was supported in part by a grant from the Schering Corp.

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