

## Treatment of Experimental Cryptococcal Meningitis and Disseminated Candidiasis with SCH39304

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Received 21 March 1989/Accepted 30 June 1989

**We studied the pharmacokinetics and in vivo antifungal action of SCH39304, a new antifungal azole compound, in rabbits. It crossed the blood-cerebrospinal fluid barrier in the presence or absence of meningeal inflammation, reaching approximately 60% of the simultaneous concentrations in serum. In the treatment of experimental cryptococcal meningitis, SCH39304 was as effective as fluconazole in reducing yeast counts in the subarachnoid space. SCH39304 and fluconazole both were highly effective against candida endophthalmitis, sterilizing the vitreous humor and the choroid and retina. SCH39304 suppressed candida endocarditis and reduced yeast counts in the kidney at all doses tested. SCH39304 was effective in the treatment of experimental cryptococcal meningitis and disseminated candidiasis. Further investigations in humans are warranted.**

As a result of the increasing number of immunocompromised hosts and the frequent use of potent, broad-spectrum antibacterial agents, fungal infections have become increasingly prevalent. The standard treatment for most systemic mycotic infections remains amphotericin B, despite its toxicity. The azole compounds have advanced the treatment of fungal infections. Clotrimazole and miconazole proved to be effective for treating superficial dermatophyte and yeast infections. Initially, the azoles were used topically, but an intravenous preparation of miconazole showed some success against disseminated mycoses. Later, ketoconazole was licensed for use in mucocutaneous (5, 6) and deep-seated fungal infections. Ketoconazole was eventually shown to be an effective oral therapy for some disseminated mycoses (2), notably paracoccidioidomycosis (11), blastomycosis (1), histoplasmosis (1), and coccidioidomycosis (14).

Despite the success of ketoconazole, the medical community continues to search for new agents with lower toxicities, broader spectra of activity, and better pharmacokinetic profiles. Two azole compounds, fluconazole and itraconazole, have recently emerged which have been studied successfully in animal models (10). Fluconazole and itraconazole are undergoing clinical trials. Another new azole compound, SCH39304, has favorable pharmacokinetics and potent, broad-spectrum antifungal activity. In this study we determined its pharmacokinetics in rabbits, evaluated its in vivo efficacy in two models of fungal infections, and compared its efficacy with that of fluconazole. The first model examined the efficacy of SCH39304 treatment of a central nervous system infection in immunocompromised rabbits with cryptococcal meningitis. The second model examined the effect of SCH39304 on infection in the kidney, eye, and heart during disseminated candidiasis.

### MATERIALS AND METHODS

**Animals.** New Zealand White rabbits (weight, 2 or 3 kg) were housed in separate cages and given rabbit chow (Purina) and water ad libitum. Intramuscular injections of 100 to 150 mg of ketamine (Ketaset; Bristol Laboratories, Syracuse, N.Y.) plus 15 to 25 mg of xylazine (Rompun; Mobay Corp., Shawnee, Kans.) were given for all procedures that

required anesthesia. Animals were sacrificed with an intravenous injection of sodium pentobarbital (Letalis; Barber Veterinary Supply, Fayetteville, N.C.).

**Antifungal agents.** SCH39304 (Schering-Plough Corp., Bloomfield, N.J.) was suspended in Cremaphor EL at 10 mg/ml. Fluconazole, which was prepared at Schering-Plough, was used in aqueous solution at 10 mg/ml. Both agents were administered by oral gavage to partially sedated rabbits. A commercial preparation of amphotericin B (5 mg/ml) containing deoxycholate (E. R. Squibb & Sons, Princeton, N.J.) was given intravenously.

**Organisms.** For pharmacokinetic analyses, the Eagan strain of *Haemophilus influenzae* type b was used to induce meningeal inflammation. In the efficacy studies, infection was induced with the following clinical yeast isolates: the H99 strain of *Cryptococcus neoformans* for fungal meningitis and the Carter strain of *Candida albicans* for disseminated candidiasis. All yeast strains were grown on Sabouraud agar plates containing 100 µg of chloramphenicol per ml.

**In vitro susceptibility testing.** 50% Inhibitory concentrations were determined in microtiter plates (Falcon; Becton Dickinson Labware, Oxnard, Calif.) by using serial twofold dilutions of SCH39304 in a modified preparation of synthetic amino acid medium for fungi (pH 7.4) (4). The inoculum size was  $5 \times 10^3$  CFU of *Candida albicans* or *Cryptococcus neoformans* per ml. Cultures were incubated in air for 24 h at 37°C. The  $A_{540}$  was read on a Titertek Multiscan and converted to percent transmission. The 50% inhibitory concentration was calculated to be the lowest concentration giving %T < [1%T control + 1/2(100 - %T control)], where %T control was the transmission recorded in drug-free wells (3). Portions of 100 µl from wells without growth were plated onto Sabouraud agar plates and incubated for 3 days to measure fungicidal activity. The minimal fungicidal concentration was defined as the lowest drug concentration resulting in a greater than 99% kill of the inoculum.

**CSF pharmacokinetics.** A dozen rabbits were given a single dose of SCH39304 (20 mg/kg). Serum and cerebrospinal fluid (CSF) samples were drawn 2, 4, 6, and 8 h later. Half of these animals had inflamed meninges induced by a nonlethal intracisternal inoculum of approximately  $10^6$  CFU of *H. influenzae* 18 h prior to dosing.

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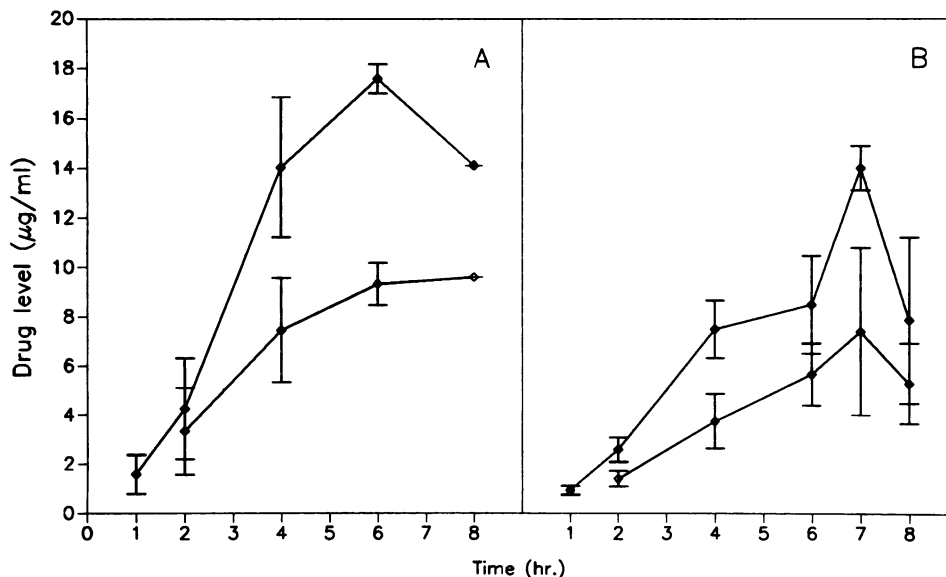


FIG. 1. Mean concentrations of SCH39304 in serum (◆) and CSF (◇) after a single oral dose of 20 mg/kg in normal rabbits (A) and in rabbits with meningeal inflammation (B).

**Antimicrobial assay.** Levels of SCH39304 in serum, CSF, and urine were measured by high-pressure liquid chromatography at Schering-Plough. The procedure involved ether extraction in alkaline pH, reversed-phase column separation, and quantitation by determination of the  $A_{205}$ . The limit of sensitivity of the method was approximately 0.5 µg of SCH39304 per ml of serum.

**Production of cryptococcal meningitis.** Beginning 1 day prior to infection and for the duration of the experiment, all animals received an intramuscular injection of cortisone acetate (2.5 mg/kg; Merck Sharp and Dohme, West Point, Pa.). Four-day-old cultures of *Cryptococcus neoformans* were suspended in 0.015 M phosphate-buffered saline, counted, and adjusted to  $10^8$  CFU/ml. Rabbits were sedated and inoculated intracisternally with 0.3 ml of the yeast suspension. On days 4, 7, 11, and 14 after inoculation, intracisternal taps were performed and approximately 0.3 ml of CSF was aspirated. The CSF was diluted in phosphate-buffered saline and cultured on Sabouraud agar with chloramphenicol. The results were expressed as  $\log_{10}$  CFU per milliliter of CSF.

**Production of disseminated candidiasis.** For cardiac catheterization, rabbits were anesthetized and the right carotid artery was exposed. A polyethylene catheter (PE-50; Clay-Adams, Parsippany, N.J.) was inserted and threaded to a point just across the aortic valve. The catheter was secured and left in place. The wound was closed with reflex staples. At 24 h after catheter insertion,  $10^6$  CFU of *C. albicans* suspended in 1 ml of phosphate-buffered saline was injected into the marginal ear vein.

Twenty-four hours after the last treatment, rabbits were sacrificed and the organs of interest and urine samples were removed. The heart was dissected and cardiac vegetations were harvested from the valve and from around the catheter tip. Each kidney was dissected, and the renal pelvis was swabbed for culture. Sections of each renal cortex, as well as the vitreous body, and the choroid and retina from each eye were collected.

All samples were weighed, homogenized, and cultured on Sabouraud agar with chloramphenicol. The urine and pelvic

swab cultures were recorded as either positive or negative. Colony counts of tissue homogenates were adjusted and expressed as  $\log_{10}$  CFU per gram of tissue.

**Treatment regimens.** In the cryptococcal meningitis model, treatment was started on day 4 of infection and continued daily for 10 days. Rabbits were randomly assigned to receive either 100 mg of SCH39304 or fluconazole per day (approximately 40 mg/kg per day) orally or 1 mg of amphotericin B per kg per day intravenously. Rabbits with disseminated candidiasis were started on therapy 24 h after inoculation and treated daily for 1 week. Initial experiments in rabbits with *Candida* infection compared a dose of 10 mg of SCH39304 per kg per day with a dose of 20 mg of fluconazole per kg per day. In subsequent experiments, the doses were reduced to 5 and 2.5 mg/kg per day, respectively.

**Statistical analysis.** For the cryptococcal meningitis model, the pattern of infection over the course of treatment was plotted as the growth curve of cryptococci in each rabbit. The slope of the linear portion of each growth curve was estimated by using least-squares analysis. The mean slopes for different treatment groups were analyzed in a one-way analysis of variance. The Tukey method was used for multiple comparisons of the final reduction of colony counts with different treatments.

For the *Candida* model, the Kruskal-Wallis rank sum test was used to analyze the pyelonephritis data. The endophthalmitis data were analyzed by the Fisher exact test (two-sided). The endocarditis data were analyzed by using a one-way analysis of variance.

## RESULTS

The 50% inhibitory concentrations of SCH39304 were 0.2 µg/ml for *Candida albicans* Carter and 6.25 µg/ml for *Cryptococcus neoformans* H99. No fungicidal activity was found against the *Candida albicans* strain when SCH39304 was tested at concentrations up to 100 µg/ml, but for *Cryptococcus neoformans*, the minimal fungicidal concentration was 25 µg/ml.

The pharmacokinetics of SCH39304 were determined in

TABLE 1. SCH39304 concentrations in serum, CSF, and urine from rabbits with cryptococcal meningitis or disseminated candidiasis

Model (no. of rabbits)	Dose	No. of doses	Time (h) after last dose	Concn ( $\mu\text{g/ml}$ ; mean $\pm$ SE)		
				Serum	CSF	Urine
<i>Cryptococcus neoformans</i> (6)	100 mg/day <sup>a</sup>	1	2	20.02 $\pm$ 1.61	11.78 $\pm$ 1.62	ND <sup>b</sup>
			6	34.02 $\pm$ 3.09	22.05 $\pm$ 2.82	ND
			24	38.35 $\pm$ 2.07	27.52 $\pm$ 1.49	ND
<i>Candida albicans</i> (5)	10 mg/kg per day	1	2	7.10 $\pm$ 1.62	ND	ND
			6	8.92 $\pm$ 1.53	ND	ND
			24	6.44 $\pm$ 0.57	ND	ND
			7	24	7.74 $\pm$ 1.03	ND

<sup>a</sup> Approximately 40 mg/kg per day.

<sup>b</sup> ND, Not done.

early experiments by using a single dose of drug. Figure 1 illustrates the pharmacokinetics of SCH39304 after a single dose of 20 mg/kg. In all animals, levels in serum rose over the first 6 h following ingestion and reached peak levels of about 15  $\mu\text{g/ml}$ . Drugs could be measured in the CSF at the first time point sampled (2 h postdosing), regardless of the presence of meningeal inflammation. The median percent penetration from serum to CSF was 57% during the first 8 h after administration of a single dose. The presence of meningeal inflammation did not influence drug penetration into the CSF.

The pharmacokinetics of SCH39304 were also followed during the course of therapy in the experimental models of infection. Table 1 shows the drug concentrations in various body fluids after treatment with SCH39304. In all animals, oral administration produced measurable levels of SCH39304 in serum. In the *Candida* model, drug concentrations in serum 24 h after a single dose were not significantly different from the levels at 2 h. After a single dose or after seven daily doses, the concentrations in serum at 24 h also did not differ significantly. Drug levels in urine were determined 24 h after the last dose and showed high concentrations of SCH39304 (40 to 50  $\mu\text{g/ml}$ ) in all animals.

As shown in the bacterial meningitis model, the drug penetrated the CSF freely. SCH39304 concentrations were also measured in rabbits with fungal meningitis. High drug levels persisted in both the serum and CSF from 2 to 24 h after the administration of a single dose.

Figure 2 shows the effects of various treatments on experimental cryptococcal meningitis in immunosuppressed rabbits. Initially, a dose of 20 mg of SCH39304 per kg per day was used. There was no difference between colony counts from treated animals and those from controls. Therefore, we increased the dose to 100 mg per day, or approximately 40 mg/kg per day. Over a 10-day observation period, colony counts in CSF from rabbits that received no treatment did not change. Only animals treated with amphotericin B had colony counts which were significantly different ( $P < 0.05$ ) from those of untreated animals on day 7 of infection. By day 11, all treatment groups had colony counts which were statistically different ( $P < 0.05$ ) from those of untreated animals. On day 14, the final reduction in colony counts was greatest in rabbits with amphotericin B therapy. Colony counts from animals treated with SCH39304 or fluconazole were not statistically different from each other at any point during the observation period. The rate of reduction of colony counts is represented by the slope of the linear portion of the growth curve for the infection in each rabbit. The mean slopes of the growth curves for rabbits in all three treatment groups were not statistically different after day 11.

*Candida albicans* was eliminated from ocular tissue by both SCH39304 and fluconazole (Fig. 3). Treatment with either SCH39304 at 10 mg/kg per day or fluconazole at 20 mg/kg per day significantly ( $P < 0.05$ ) reduced colony counts in the vitreous body and choroid and retina when compared with the reductions in untreated animals. At our limits of detection, these ocular tissues were sterile after therapy, regardless of the dose administered. Thus, it was not possible to determine the relative efficacies of these agents against ocular infections.

In the *Candida* endocarditis model, the infection was measured and expressed as the  $\log_{10}$  CFU per gram of cardiac vegetations. Rabbits that received no treatment and that survived 1 week had counts of  $6.42 \pm 0.60$  (mean  $\pm$  standard error). Five animals treated with SCH39304 and four animals treated with fluconazole at equal doses of 10 mg/kg per day for 1 week had significantly ( $P < 0.05$ ) lower counts of  $3.02 \pm 0.79$  and  $3.55 \pm 0.72$ , respectively.

The effects of SCH39304 and fluconazole on renal candidiasis are shown in Table 2. Initially, we compared treatment with SCH39304 (10 mg/kg per day) with treatment with twice the dose of fluconazole (20 mg/kg per day). Treatment with either azole at these doses significantly ( $P < 0.001$ ) reduced yeast counts in the renal cortex (Table 2) and urinary collecting system (Table 2) when compared with the counts

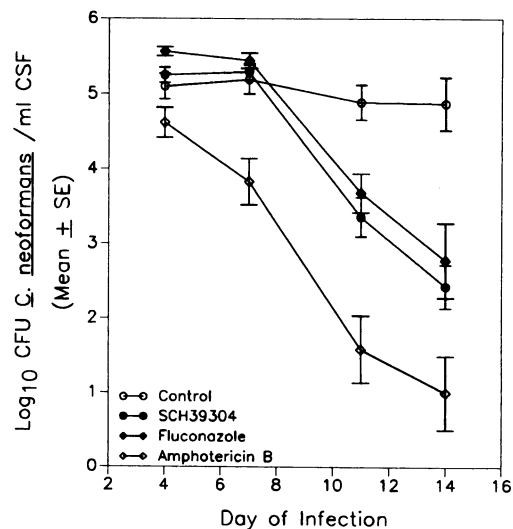


FIG. 2. Quantitative yeast counts from the CSF of rabbits with cryptococcal meningitis. The effects of four different treatment regimens are shown.

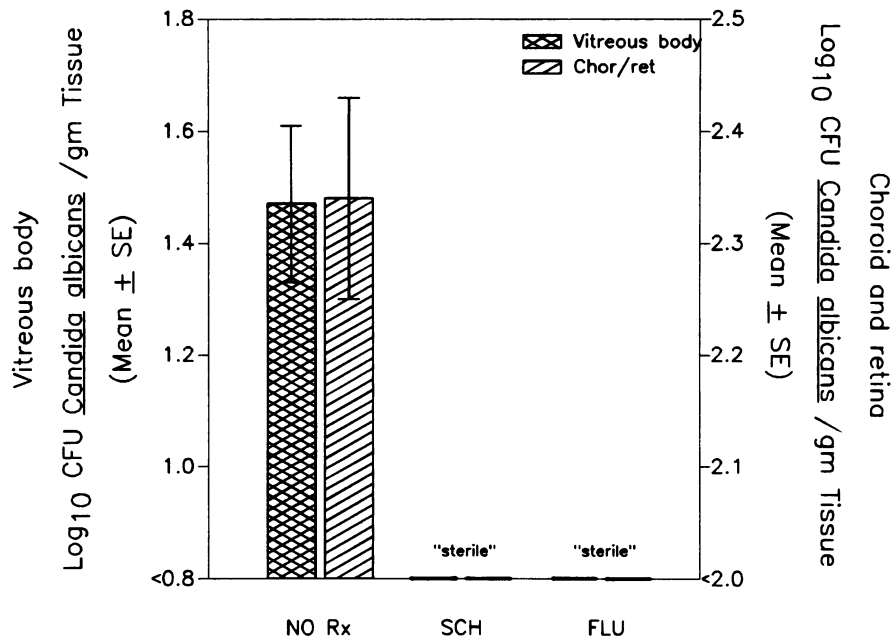


FIG. 3. Quantitative yeast counts in the vitreous body and choroid and retina of rabbits with *Candida* endophthalmitis. The effects of treatment with SCH39304 (10 mg/kg per day) and fluconazole (20 mg/kg per day) are shown.

in untreated animals. Subsequently, lower doses were used to treat rabbits with this infection. SCH39304 significantly reduced yeast counts at all doses tested, and no dose was significantly better. Fluconazole was most effective at a dose of 20 mg/kg per day. Reduction of yeast counts in the kidney was greater with SCH39304 than with fluconazole with dosage regimens of 10 and 2.5 mg/kg per day, respectively ( $P < 0.03$ ). For the different treatment regimens, the mean survivals were 93% for rabbits treated with SCH39304 and 83% for rabbits treated with fluconazole.

DISCUSSION

The correlation between in vitro antifungal activity of a therapeutic agent and in vivo efficacy is often imprecise. However, many findings on humans and animals indicate the importance of obtaining drug concentrations that are at least above the MIC for the causative organism. The first step in

our evaluation was to examine the in vitro susceptibilities of the yeasts used in our model to SCH39304 and then to determine the pharmacokinetics of SCH39304 in rabbits. SCH39304 possessed reasonably good in vitro antifungal activity against our clinical isolates. The drug was reliably absorbed into the bloodstream of rabbits; rapid and persistent drug levels were also found in the CSF. The prolonged half-life of SCH39304 in serum has been demonstrated in species other than rabbits, including humans (C. Lin, H. Kim, A. Lapiguera, D. Loebenberg, G. H. Miller, and S. Sychowitz, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 163, 1988; W. Kramer, H. Kim, S. Sychowitz, G. Perentesis, M. Affrime, and C. Lin, 28th ICAAC, abstr. no. 165, 1988).

The pharmacokinetics of SCH39304 were comparable to those of fluconazole. Both compounds showed low protein binding (10 to 20% for fluconazole and 10 to 15% for SCH39304), similarity in size (a molecular weight of 306 for fluconazole and a molecular weight of 331 for SCH39304), and good penetration into the CSF. In contrast, other azoles, such as ketoconazole, vibunazole, and itraconazole, penetrate poorly into the CSF (8).

In vitro susceptibility testing of new antifungal agents is an important first step in the screening of antimicrobial agents, but the success of an agent depends on its in vivo activity. In this study we examined the azole compound SCH39304 in two established animal models of fungal infection. The cryptococcal meningitis model allows evaluation of drug activity in the central nervous system during significant immune suppression (9). The disseminated candidiasis model is used to evaluate drug activity at three important tissue sites.

SCH39304 and fluconazole significantly reduced yeast counts in the treatment of cryptococcal meningitis. It is interesting that even with high drug concentrations in the CSF, a reduction of colony counts in animals treated with azoles was not observed until day 11 of infection, after 1

TABLE 2. Effect of SCH39304 and fluconazole treatment on rabbits with renal candidiasis

Dose (mg/kg)	Log <sub>10</sub> CFU of <i>Candida albicans</i> /g of renal cortex (mean ± SE) <sup>a</sup>		Percent positive cultures <sup>b</sup>			
			Pelvic swab		Urine	
	SCH	FLU	SCH	FLU	SCH	FLU
20	ND <sup>c</sup> (0)	1.54 ± 0.10 (10)	ND	0	ND	29
10	1.63 ± 0.08 (26)	2.14 ± 0.14 (10)	11	20	0	20
5	1.71 ± 0.18 (5)	2.45 ± 0.27 (4)	17	50	0	0
2.5	1.92 ± 0.19 (6)	2.83 ± 0.20 (4)	0	0	0	50

<sup>a</sup> When no dose was given, the colony count was 3.44 ± 0.20 log<sub>10</sub> CFU of *Candida albicans* per g of renal cortex in 23 rabbits. Abbreviations: SCH, SCH39304; FLU, fluconazole. Numbers in parentheses are the numbers of organs.

<sup>b</sup> When no dose was given, samples from pelvic swabs had 71% positive cultures and samples from urine had 50% positive cultures.

<sup>c</sup> ND, Not done.

week of therapy. This finding is difficult to explain, considering the pharmacokinetics of these compounds. On the other hand, amphotericin B showed rapid fungicidal activity against *Cryptococcus neoformans* meningitis in this model, despite very low drug concentrations in CSF (7). This more rapid killing of yeasts in the CSF by amphotericin B compared with that by the azoles does not necessarily represent a clinical advantage. Indeed, our *in vivo* studies showed that despite initial superior antifungal effects, the rate of yeast killing in the CSF was similar for all three antifungal agents by the end of the treatment period.

If this animal model predicts the response of disease in humans to these agents, it is likely that in humans, amphotericin B treatment would sterilize the CSF faster than the azole compounds would, but azoles should have a positive influence on treatment of this infection. In fact, fluconazole alone has already been used successfully to control cryptococcal meningitis in humans (13, 15). Our data on SCH39304 show that its *in vitro* activity, pharmacokinetics, and *in vivo* efficacy are similar to those of fluconazole in rabbits with experimental cryptococcal meningitis. It is likely that SCH39304 will also be effective in the management of human cryptococcal meningitis.

In rabbits with *Candida* infections, SCH39304 was also effective. In early *Candida* endophthalmitis the infection was significantly reduced in both ocular tissues studied. We did not measure SCH39304 concentrations in eye structures, but the ocular pharmacokinetics for other azoles have been determined (12). Fluconazole penetrates well into several ocular tissues and fluids. A similar efficacy with fluconazole suggests that SCH39304 attains therapeutic drug levels in the eye. However, it should be mentioned that against a chronic *Candida* endophthalmitis model in rabbits, when therapy is delayed until 1 week after infection, the azoles are less successful (12). SCH39304 has not been tested in this chronic ocular infection to determine whether it has any therapeutic advantage, but our initial results with this azole should encourage further evaluation.

Both SCH39304 and fluconazole significantly suppressed intravascular infection in rabbits with *Candida* endocarditis. These results are consistent with the long half-lives of the two drugs in serum. Their inability to completely eradicate this infection was not surprising, however, since they had only *in vitro* fungistatic activity against the *Candida* isolate that we used. However, these results indicate the need for further investigation into the medical management of *Candida* endocarditis with these long-acting azoles. Extended courses of therapy with these azole compounds along with surgery are potentially useful therapeutic options for organisms with this very difficult infection.

*Candida* pyelonephritis in rabbits is a convenient model for examining antifungal activity at several points along the urinary tract. Since fungal infections of the urinary tract have become particularly common and their management is difficult, we looked for the presence of SCH39304 in urine during a fungal infection. The high concentrations of SCH39304 found in the urine of rabbits suggest that it would be effective in urinary tract infections. SCH39304 was particularly effective at reducing yeast counts at all urinary tract sites we examined. For the range of doses used, we could not see a dose response; a reduction of the dose did not significantly reduce efficacy. Oral agents like SCH39304 or fluconazole have great appeal for study in *Candida* urinary tract infections.

SCH39304 is a new azole with broad-spectrum antifungal activity and excellent pharmacokinetics. The recent *in vivo*

evaluation of this compound in other animal models has been very encouraging. Some investigators have found SCH39304 to be more potent than fluconazole in their *in vivo* systems (D. Loebenberg, R. Parmegiani, A. Cacciapuoti, B. Antonacci, C. Norris, F. Menzel, Jr., E. L. Moss, Jr., T. Yarosh-Tomaine, C. C. Lin, R. S. Hare, and G. H. Miller, 28th ICAAC, abstr. no. 167, 1988; B. I. Restrepo, J. Ahrens, and J. R. Graybill, 28th ICAAC, abstr. no. 170, 1988; H. J. Schmitt, E. M. Bernard, M. Hauser, and D. Armstrong, 28th ICAAC, abstr. no. 171, 1988; G. S. Kobayashi, S. J. Travis, and G. Medoff, 28th ICAAC, abstr. no. 172, 1988). We found this agent to be comparable to fluconazole in the treatment of cryptococcal meningitis and more potent at lower doses in the treatment of renal candidiasis. Its excellent *in vivo* activity in our animal models against *Candida* and *Cryptococcus* infections suggests that further clinical trials in humans with these mycoses should be encouraged.

#### ACKNOWLEDGMENTS

We thank Janet Routten and Mary Ann Howard for help in manuscript preparation.

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

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