ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1996, p. 2243–2247 Vol. 40, No. 10
0066-4804/96/$04.00+0

Efficacy of ER-30346, a Novel Oral Triazole Antifungal Agent, in Experimental Models of Aspergillosis, Candidiasis, and Cryptococcosis

KATSURA HATA,* JUNKO KIMURA, HIKARI MIKI, TOSHIO TOYOSAWA, MEGUMI MORIYAMA, AND KANEMASA KATSU

Department of Microbiology and Infectious Disease, Tsukuba Research Laboratories, Eisai Co., Ltd., Tsukuba, Ibaraki 300-26, Japan

Received 18 April 1996/Returned for modification 13 May 1996/Accepted 12 July 1996

ER-30346 is a novel oral triazole with a broad spectrum of potent activity against a wide range of fungi. In the present study, we investigated the therapeutic effects of oral ER-30346 on experimental local infections caused by *Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans* and compared them with those of itraconazole and fluconazole. In experimental murine models of pulmonary aspergillosis, candidiasis, and cryptococcosis, ER-30346 reduced the numbers of CFU in the lungs significantly compared with the numbers of CFU in the lungs of the controls ($P < 0.05$). ER-30346 was as effective as or more effective than itraconazole against pulmonary aspergillosis. Against pulmonary candidiasis and cryptococcosis, ER-30346 was more effective than itraconazole and was as effective as fluconazole. ER-30346 was also effective against pulmonary candidiasis caused by fluconazole-resistant *C. albicans*. In mice with intracranial cryptococcosis, ER-30346 reduced the numbers of CFU in the brains significantly compared with the numbers of CFU in the brains of the controls ($P < 0.05$) and was more effective than itraconazole and as effective as fluconazole. In an experimental model of oral candidiasis in rats, ER-30346 reduced the numbers of CFU in oral swabs significantly compared with the numbers of CFU in oral swabs from the controls ($P < 0.05$) and was more effective than itraconazole and as effective as fluconazole. Thus, ER-30346 shows efficacy in murine aspergillosis, candidiasis, and cryptococcosis models. Further studies are needed to determine the potential of ER-30346 for use in the treatment of these infections.

Invasive pulmonary aspergillosis is a life-threatening infection recognized increasingly in immunocompromised patients undergoing immunosuppressive or anticancer therapy. Pulmonary candidiasis also occurs in such immunocompromised patients (4, 8). Oral candidiasis and cryptococcal meningitis occur most frequently in human immunodeficiency virus-infected patients (18, 22). Thus, despite advances in antifungal therapy, fungal infections are increasingly frequent in immunocompromised patients (8).

The clinical efficacies of such new antifungal agents as fluconazole and itraconazole (9, 11) are still limited, especially against invasive aspergillosis, and activity against *Aspergillus fumigatus* must be further improved. With this in mind, we have directed our research toward the development of new azoles. One such new oral triazole, ER-30346 {(2*R*,3*R*)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol} (Fig. 1), has a broad antifungal spectrum and potent activity against major pathogenic fungi such as *A. fumigatus*, *Cryptococcus neoformans*, *Candida* species, and dermatophytes (10). We developed experimental local infection models of aspergillosis, candidiasis, and cryptococcosis, using models of these infections, to evaluate the efficacies of antifungal compounds.

In the present study, we evaluated the efficacy of ER-30346 in experimental models of pulmonary, intracranial, and oral infections caused by *A. fumigatus*, *Candida albicans*, or *C. neoformans* in comparison with those of fluconazole (23) and itraconazole (7).

(This work was presented in part at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., 17 to 20 September 1995.)

MATERIALS AND METHODS

Antifungal agents. ER-30346 and fluconazole were synthesized at Eisai Co. Ltd., Tokyo, Japan. Itraconazole was obtained commercially (Janssen Kyowa Co., Tokyo, Japan). The azoles were dissolved individually in dimethyl sulfoxide (DMSO) and were uniformly suspended by sonication in a ninefold volume of 0.5% sodium carboxymethyl cellulose (CMC).

Organisms. Three strains of *A. fumigatus* (C.I.-8, C.I.-10, and N.H.K.-1) and *C. neoformans* No. 3 were kindly provided by Y. Niki, Kawasaki Medical College, Okayama, Japan. Two strains of *C. albicans* (E81022 and E81113) were distinct clinical isolates recently obtained from hospitals in Japan. All isolates were stored at −80°C in Sabouraud dextrose broth containing 15% glycerol in our laboratory. The strains of *C. albicans* and *C. neoformans* for each study were grown on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich.) plates at 30°C for 24 to 48 h, and challenge organisms were prepared in sterile saline. Strains of *A. fumigatus* were grown on potato dextrose agar (PDA; Eiken Chemical Co., Tokyo, Japan) plates at 30°C for 1 week, and challenge organisms were prepared in sterile saline containing 0.05% Tween 80.

Animals. Female ICR mice (age, 5 weeks; weight, approximately 24 g; Charles River Japan Inc., Kanagawa, Japan) or male Sprague-Dawley rats (age, 5 weeks; weight, approximately 200 g; Japan SLC Inc., Shizuoka, Japan) were used throughout the experiments. They were housed in cages of 20 or 5 animals per group and had access to food and water ad libitum.

In vitro susceptibility tests. MICs were determined by the broth microdilution method on the basis of the standard method for antifungal susceptibility testing proposed by the National Committee for Clinical Laboratory Standards (21) with RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). Sterile, flat-well microtiter plates (Nunc A/S, Roskilde, Denmark) were used for the tests. The drug solutions were serially diluted twofold to give a range of final drug concentrations from 100 to 0.006 μg/ml; these were prepared with RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinopropanesulfonic acid (MOPS; Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 1% DMSO. Yeasts were grown on SDA (Difco Laboratories) at 30°C for 24 to 48 h,

* Corresponding author. Mailing address: Department of Microbiology and Infectious Disease, Tsukuba Research Laboratories, Eisai Co., Ltd., 1-3 Tokodai 5-chome, Tsukuba, Ibaraki 300-26, Japan. Phone: 81-298-475742. Fax: 81-298-475392.

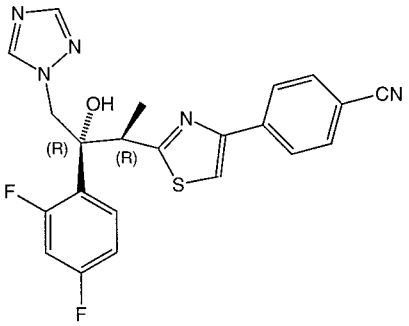

FIG. 1. Chemical structure of ER-30346.

TABLE 1. In vitro activities of ER-30346 and the other azoles against strains causing experimental local infections[a]

Strains	MIC (μg/ml)		
	ER-30346	Itraconazole	Fluconazole
A. fumigatus C.I.-8	0.39	0.78	>100
A. fumigatus C.I.-10	0.20	0.78	>100
A. fumigatus N.H.K.-1	0.20	0.78	>100
C. albicans E81022	≤0.006	0.05	0.39
C. albicans E81113	0.78	1.56	50
C. neoformans No. 3	0.025	0.10	3.13

[a] MICs were determined by the broth microdilution method with RPMI 1640 medium, the inoculum size was 2.5×10^3 cells or conidia per ml, and incubation was at 35°C for 48 to 72 h.

and filamentous fungi were grown on PDA (Eiken Chemical Co.) at 30°C for 1 to 2 weeks. The wells were inoculated with 100 μl of the culture suspension diluted to a final inoculum of 2.5×10^3 cells or conidia per ml with RPMI 1640 medium buffered to pH 7.0 with 0.165 M MOPS. Fungal growth was observed 48 h (72 h for *C. neoformans* and 96 h for dermatophytes) after incubation at 35°C. The MICs of the azoles were the lowest drug concentration which resulted in a visual turbidity less than or equal to 80% inhibition compared with the control fungal growth. The MICs of amphotericin B were the lowest drug concentration at which there was an absence of visible fungal growth.

Pulmonary infection in mice. (i) Aspergillosis. The mice were immunosuppressed by the subcutaneous injection of 5-fluorouracil (5-FU Kyowa; Kyowa Hakko Kogyo Co., Tokyo, Japan) at a dose of 200 mg/kg of body weight 7 days before infection and at a dose of 100 mg/kg of body weight 4 days before infection. On the day of infection, the mice were anesthetized intravenously with ketamine hydrochloride (Sankyo Co., Tokyo, Japan) at 0.5 mg/kg of body weight. The mice ($n = 10$) were infected intranasally with 1.0×10^5 conidia, in a 50-μl droplet, of *A. fumigatus* C.I.-8, *A. fumigatus* C.I.-10, and *A. fumigatus* N.H.K.-1 in the respective experiments. Drugs were orally administered, in a volume of 0.2 ml per dose, twice daily for 3 consecutive days starting 1 h after infection. Control groups received 10% DMSO in 0.5% CMC. The drugs were administered at doses of 2, 8, and 32 mg/kg. The mice were sacrificed 24 h after administration of the last dose, and the lungs were removed and homogenized in 3 ml of sterile saline. Homogenates were serially diluted 10-fold, dilutions were plated onto SDA plates containing 100 μg of ampicillin (Meiji Seika Co., Tokyo, Japan) per ml, and the plates were incubated at 37°C for 24 h. The number of CFU was counted, and the number of CFU per total tissue was calculated as reported previously (1).

(ii) Candidiasis. The mice were immunosuppressed and anesthetized on the day of infection in the same way as described above for the pulmonary aspergillosis model. The mice ($n = 10$) were infected intranasally with 1.8×10^5 cells, in a 50-μl droplet, of *C. albicans* E81022 (fluconazole-susceptible strain) and *C. albicans* E81113 (fluconazole-resistant strain) in the respective experiments. Drugs were orally administered, in a volume of 0.2 ml per dose, twice daily for 2 or 3 consecutive days starting 1 h after infection. Controls received 10% DMSO in 0.5% CMC. The drugs were administered at doses of 0.625, 2.5, and 10 mg/kg in the model of infection caused by a fluconazole-susceptible strain and 40 mg/kg in the model of infection caused by a fluconazole-resistant strain. Drug efficacy was assessed in the same way as described above for the pulmonary aspergillosis model.

(iii) Cryptococcosis. Healthy mice were used for the cryptococcosis model. On the day of infection, the mice ($n = 5$) were anesthetized in the same way as described above for the pulmonary aspergillosis model. The mice were infected intranasally with 1.0×10^5 cells, in a 50-μl droplet, of *C. neoformans* No. 3. Drugs were orally administered in the same way as described above for the pulmonary aspergillosis model. The drugs were administered at doses of 8 and 32 mg/kg. Drug efficacy was assessed in the same way as described above for the pulmonary aspergillosis model. The number of CFU was counted after 48 h of incubation at 30°C, and the number of CFU per total tissue was calculated as described above.

Intracranial cryptococcosis in mice. An animal model of intracranial cryptococcosis was induced basically as reported by Hector and Yee (13), with some modifications. Healthy mice were used. On the day of infection, the mice ($n = 5$) were anesthetized intravenously with ketamine hydrochloride at 0.5 mg/kg of body weight. The inoculum was delivered intracranially by puncturing the cranium with a 28-gauge needle and delivering 10-μl portions of the fungal suspension containing 1.0×10^4 cells of *C. neoformans* No. 3 as shallowly as possible. Drugs were orally administered on the same schedule as described above for the pulmonary cryptococcosis model. The mice were sacrificed 24 h after administration of the last dose, and the brains were removed and homogenized in 3 ml of sterile saline. Homogenates were serially diluted 10-fold, dilutions were plated onto SDA plates containing 100 μg of ampicillin per ml, and the plates were incubated at 30°C for 48 h. The number of CFU was counted, and the number of CFU per total tissue was calculated as described above.

Oral candidiasis in rats. An animal model of oral candidiasis was induced basically as reported by Jones and Russell (17), with some modifications. Namely, a 0.1% aqueous solution of tetracycline hydrochloride (Sigma Chemical Co., St. Louis, Mo.) was given to rats beginning 7 days before infection, and the concentration of tetracycline hydrochloride was reduced to 0.01% after infection. The rats ($n = 8$) were orally infected three times at 48-h intervals with 0.1 ml of a saline suspension containing 10^8 cells of *C. albicans* E81022. Drugs were orally administered, in a volume of 0.5 ml per dose, once daily for 3 consecutive days starting 2 days after the last infection. Control groups received 10% DMSO in 0.5% CMC. Drugs were administered at doses of 1 and 4 mg/kg. Drug efficacy was assessed 5 days after the last infection by measuring the number of *C. albicans* organisms in oral swabs. The oral swabs were suspended in 2 ml of sterile saline, the resulting suspension was serially diluted 10-fold, dilutions were plated onto SDA plates containing 100 μg of tetracycline hydrochloride per ml, and the plates were incubated at 37°C for 48 h. The number of CFU was counted, and the number of CFU per total swab was calculated.

Statistical analysis. Data for animals with pulmonary, intracranial, and oral infections were analyzed by the Kruskal-Wallis test; this was followed by the Tukey type comparison test. Statistical significance was defined as $P < 0.05$.

RESULTS

In vitro antifungal activity. The MICs for the strains of *A. fumigatus*, *C. albicans*, and *C. neoformans* used throughout the studies are presented in Table 1. The activity of ER-30346 was two to four times higher than that of itraconazole against the three strains of *A. fumigatus* and four to more than eight times higher than that of itraconazole against *C. albicans* E81022 and *C. neoformans* No. 3. Fluconazole demonstrated less in vitro activity than the other two drugs against the strains tested. *C. albicans* E81113 is fluconazole resistant, and the activity of fluconazole against this strain was 128 times less than that against fluconazole-susceptible strain E81022. The activity of ER-30346 against strain E81113 was >128 times less than that against strain E81022, and the activity of itraconazole was 32 times less.

Efficacy in the pulmonary infection model. (i) Aspergillosis. The efficacy of ER-30346 against pulmonary aspergillosis in mice is presented in Table 2. ER-30346 was the most effective of the drugs tested against *A. fumigatus* C.I.-8. The lungs of untreated mice contained 2.9 ± 0.5 log CFU 3 days after infection. ER-30346 at a dose of 32 mg/kg significantly reduced the log number of CFU to 1.1 ± 0.7 compared with numbers in the lungs of mice receiving the control treatment and treatment with itraconazole and fluconazole at 32 mg/kg. Itraconazole at 32 mg/kg significantly reduced the log number of CFU to 1.9 ± 0.4 compared with the numbers in mice receiving the control treatment and fluconazole at 32 mg/kg.

The lungs of untreated mice infected with *A. fumigatus* C.I.-10 contained 3.4 ± 0.4 log CFU 3 days after infection. ER-30346 at a dose of 2 mg/kg significantly reduced the log number of CFU to 2.5 ± 0.7, ER-30346 at 8 mg/kg significantly reduced the log number of CFU to 2.3 ± 0.7, itraconazole at 8 mg/kg significantly reduced the log number of CFU to 2.4 ±

TABLE 2. Efficacies of ER-30346, itraconazole, and fluconazole against pulmonary aspergillosis in mice[a]

Drug	Dose (mg/kg)	Log CFU/lung (mean ± SD)		
		C.I.-8	C.I.-10	N.H.K.-1
Control		2.9 ± 0.5	3.4 ± 0.4	3.4 ± 0.6
ER-30346	2	3.1 ± 0.3	2.5 ± 0.7[b]	2.9 ± 0.5
	8	2.6 ± 0.3	2.3 ± 0.7[b]	1.8 ± 0.5[b,c]
	32	1.1 ± 0.7[b,c,d]	0.9 ± 0.4[b,c]	0.5 ± 0.5[b,c]
Itraconazole	2	2.9 ± 0.6	2.9 ± 0.4	3.0 ± 0.8
	8	2.8 ± 0.2	2.4 ± 0.7[b]	2.3 ± 0.7[b]
	32	1.9 ± 0.4[b,c]	1.7 ± 0.5[b,c]	1.2 ± 0.7[b,c]
Fluconazole	32	2.8 ± 0.3	2.6 ± 0.7[b]	2.8 ± 0.9

[a] The mice ($n = 10$) were immunosuppressed by the subcutaneous injection of 5-fluorouracil and were infected intranasally with 10^5 conidia of *A. fumigatus* C.I.-8, C.I.-10, or N.H.K.-1 in the respective experiments. Drugs were orally administered twice daily for 3 consecutive days starting 1 h after infection.
[b] $P < 0.05$ compared with control treatment.
[c] $P < 0.05$ compared with treatment with fluconazole at 32 mg/kg.
[d] $P < 0.05$ compared with treatment with itraconazole at 32 mg/kg.

0.7, and fluconazole at 32 mg/kg significantly reduced the log number of CFU to 2.6 ± 0.7 compared with the numbers in the lungs of mice receiving the control treatment. ER-30346 at 32 mg/kg significantly reduced the log number of CFU in the lungs to 0.9 ± 0.4 compared with the numbers in mice receiving the control treatment and fluconazole at 32 mg/kg. Itraconazole at 32 mg/kg significantly reduced the log number of CFU to 1.7 ± 0.5 compared with the numbers in mice receiving the control treatment and fluconazole at 32 mg/kg.

The lungs of untreated mice infected with *A. fumigatus* N.H.K.-1 contained 3.4 ± 0.6 log CFU 3 days after infection. ER-30346 at a dose of 8 mg/kg significantly reduced the log number of CFU to 1.8 ± 0.5 and at 32 mg/kg to 0.5 ± 0.5 compared with the numbers in the lungs of mice receiving the control treatment and fluconazole at 32 mg/kg. Itraconazole at 8 mg/kg significantly reduced the log number of CFU to 2.3 ± 0.7 compared with the number in mice receiving the control treatment. Itraconazole at 32 mg/kg significantly reduced the log number of CFU to 1.2 ± 0.7 compared with the numbers in mice receiving the control treatment and fluconazole at 32 mg/kg.

ER-30346 and itraconazole thus demonstrated therapeutic effects dose dependently. In general, ER-30346 was as effective as or more effective than itraconazole against pulmonary aspergillosis and was more effective than fluconazole.

(ii) Candidiasis. The therapeutic effects of ER-30346 and the reference drugs against pulmonary candidiasis are presented in Table 3. The lungs of untreated mice infected with fluconazole-susceptible *C. albicans* E81022 contained 5.4 ± 0.2 log CFU 2 days after infection. ER-30346 at a dose of 2.5 mg/kg significantly reduced the log number of CFU to 4.3 ± 0.5 and at 10 mg/kg to 3.9 ± 0.1 compared with the numbers in the lungs of mice receiving the control treatment; ER-30346 at 2.5 mg/kg significantly reduced the log number of CFU compared with the numbers in mice receiving itraconazole at 2.5 mg/kg. Itraconazole at 10 mg/kg significantly reduced the log number of CFU to 4.6 ± 0.1 compared with the numbers in mice receiving the control treatment but not itraconazole at 2.5 mg/kg (5.45 ± 0.15 log CFU). Fluconazole at 2.5 mg/kg significantly reduced the log number of CFU to 4.0 ± 0.6 and at 10 mg/kg to 3.9 ± 0.4 compared with numbers in mice receiving the control treatment; fluconazole at 2.5 mg/kg significantly reduced the log number of CFU compared with the numbers in mice receiving itraconazole at 2.5 mg/kg. ER-30346 thus demonstrated therapeutic effects dose dependently, as did the other two drugs tested, and had efficacy comparable to that of fluconazole and was more effective than itraconazole against pulmonary candidiasis caused by fluconazole-susceptible *C. albicans.*

ER-30346 was also effective against pulmonary candidiasis caused by fluconazole-resistant *C. albicans*. The lungs of untreated mice contained 5.4 ± 0.7 log CFU 3 days after infection. Only ER-30346 at a dose of 40 mg/kg significantly reduced the log number of CFU to 4.1 ± 0.4 compared with the numbers in the lungs of mice receiving the control treatment, whereas itraconazole (4.8 ± 0.8 log CFU) and fluconazole (4.8 ± 0.9 log CFU) did not. ER-30346 thus was effective against pulmonary candidiasis caused by fluconazole-resistant *C. albicans.*

(iii) Cryptococcosis. The therapeutic effects of ER-30346 and the reference drugs against pulmonary cryptococcosis are presented in Table 4. In the first experiment, we compared the therapeutic effect of ER-30346 with those of the two other drugs at a dose of 8 mg/kg. The lungs of untreated mice contained 5.9 ± 0.1 log CFU 3 days after infection. ER-30346 significantly reduced the log number of CFU to 4.1 ± 0.4 and fluconazole significantly reduced the log number of CFU to 3.7 ± 0.3 compared with the numbers in the lungs of mice receiving the control treatment and itraconazole; itraconazole did not significantly reduce the log number of CFU (5.5 ± 0.2 log CFU) compared with the numbers in mice receiving the control treatment. In the next experiment we used the drugs at a dose of 32 mg/kg. The lungs of untreated mice contained 5.3 ± 0.2 log CFU 3 days after infection. ER-30346 significantly reduced the log number of CFU to 3.5 ± 0.2 and fluconazole significantly reduced the log number of CFU to 3.6 ± 0.2 compared with the numbers in mice receiving the control treatment and itraconazole; itraconazole significantly reduced the log number of CFU to 4.1 ± 0.2 compared with the num-

TABLE 3. Efficacies of ER-30346, itraconazole, and fluconazole against pulmonary candidiasis in mice[a]

Drug	Dose (mg/kg)	Log CFU/lung (mean ± SD)	
		E81022	E81113[b]
Control		5.4 ± 0.2	5.4 ± 0.7
ER-30346	0.625	5.3 ± 0.2	
	2.5	4.3 ± 0.5[c,d]	
	10	3.9 ± 0.1[c]	
	40		4.1 ± 0.4[c]
Itraconazole	0.625	5.2 ± 0.6	
	2.5	5.5 ± 0.2	
	10	4.6 ± 0.1[c]	
	40		4.8 ± 0.8
Fluconazole	0.625	5.1 ± 0.4	
	2.5	4.0 ± 0.6[c,d]	
	10	3.9 ± 0.4[c]	
	40		4.8 ± 0.9

[a] The mice ($n = 5$) were immunosuppressed by the subcutaneous injection of 5-fluorouracil and were infected intranasally with 1.8×10^5 cells of *C. albicans* E81022 or *C. albicans* E81113 in the respective experiments. The drugs were orally administered twice daily for 2 consecutive days starting 1 h after infection.
[b] Fluconazole-resistant strain.
[c] $P < 0.05$ compared with control treatment.
[d] $P < 0.05$ compared with treatment with itraconazole at 2.5 mg/kg.

TABLE 4. Efficacies of ER-30346, itraconazole, and fluconazole against pulmonary cryptococcosis in mice[a]

Drug	Dose (mg/kg)	Log CFU/lung (mean ± SD)
Control		5.9 ± 0.1
ER-30346	8	4.1 ± 0.4[b]
Itraconazole	8	5.5 ± 0.2
Fluconazole	8	3.7 ± 0.3[b]
Control		5.3 ± 0.2
ER-30346	32	3.5 ± 0.2[b]
Itraconazole	32	4.1 ± 0.2[c]
Fluconazole	32	3.6 ± 0.2[b]

[a] The mice ($n = 10$) were infected intranasally with 1.0×10^5 cells of *C. neoformans* No. 3. Drugs were orally administered twice daily for 3 consecutive days starting 1 h after infection.
[b] $P < 0.05$ compared with control treatment and treatment with itraconazole.
[c] $P < 0.05$ compared with control treatment.

bers in mice receiving the control treatment. ER-30346 thus had efficacy comparable to that of fluconazole and was more effective than itraconazole against pulmonary cryptococcosis.

Efficacy in the intracranial cryptococcosis model. The efficacy of ER-30346 against intracranial cryptococcosis in mice is as follows. In this experiment, we compared the therapeutic effect of ER-30346 with those of the other two drugs at a dose of 8 mg/kg. The brains of untreated mice contained 6.4 ± 0.2 log CFU 3 days after infection. ER-30346 significantly ($P < 0.05$) reduced the log number of CFU to 3.9 ± 0.2 and fluconazole significantly ($P < 0.05$) reduced the log number of CFU to 3.9 ± 0.3 compared with the numbers in mice receiving the control treatment and itraconazole; itraconazole did not reduce the log number of CFU (5.7 ± 0.7) compared with the number in mice receiving the control treatment. ER-30346 and fluconazole were more effective than itraconazole against intracranial cryptococcosis.

Efficacy in the oral candidiasis model. The therapeutic effects of ER-30346 and the reference drugs against oral candidiasis in rats are presented in Table 5. In the first experiment, we compared the therapeutic effect of ER-30346 with those of the other two drugs given at doses of 1 mg/kg. Oral swabs of untreated rats contained 3.5 ± 0.4 log CFU 5 days after the last infection. ER-30346 significantly reduced the log number of CFU in swabs to 1.9 ± 0.8 compared with the numbers in rats receiving the control treatment and itraconazole. Fluconazole significantly reduced the log number of CFU to 2.4 ± 0.4 compared with the numbers in rats receiving the control treatment. Itraconazole did not reduce the log number of CFU (3.0 ± 0.6) compared with the numbers in rats receiving the control treatment. In the next experiment we used the drugs at a dose of 4 mg/kg. The swabs of untreated mice contained 3.1 ± 0.3 log CFU 5 days after infection. ER-30346 significantly reduced the log number of CFU to 0.8 ± 1.0, itraconazole significantly reduced the log number of CFU to 1.0 ± 1.3, and fluconazole significantly reduced the log number of CFU to 0.3 ± 0.7 compared with the numbers in rats receiving the control treatment. In general, ER-30346 had efficacy comparable to that of fluconazole and was more effective than itraconazole against oral candidiasis.

TABLE 5. Efficacies of ER-30346, itraconazole, and fluconazole against oral candidiasis in rats[a]

Drug	Dose (mg/kg)	Log CFU/swab (mean ± SD)	
		1 day	5 days
Control		3.4 ± 0.4	3.5 ± 0.4
ER-30346	1	3.3 ± 0.3	1.9 ± 0.8[b,c]
Itraconazole	1	3.3 ± 0.3	3.0 ± 0.6
Fluconazole	1	3.3 ± 0.3	2.4 ± 0.4[b]
Control		3.5 ± 0.3	3.1 ± 0.3
ER-30346	4	3.3 ± 0.2	0.8 ± 1.0[b]
Itraconazole	4	3.4 ± 0.3	1.0 ± 1.3[b]
Fluconazole	4	3.4 ± 0.3	0.3 ± 0.7[b]

[a] The rats ($n = 8$) were orally infected three times at 48-h intervals with 1.0×10^8 cells of *C. albicans* E81022. Drugs were orally administered once daily for 3 consecutive days starting 2 days after the last infection.
[b] $P < 0.05$ compared with control treatment.
[c] $P < 0.05$ compared with treatment with itraconazole.

DISCUSSION

ER-30346 is a novel oral thiazole-containing triazole with a broad spectrum of potent activity against major pathogenic fungi, especially *A. fumigatus*, and shows good therapeutic efficacy against systemic infections caused by *A. fumigatus* (10). The expanding population of immunocompromised patients receiving immunosuppressive or anticancer therapy has resulted in an increased incidence of opportunistic mycoses. Local infections are becoming an increasing problem, as are systemic infections caused by *Candida* spp. Invasive pulmonary aspergillosis, for instance, is a life-threatening infection increasingly recognized in immunocompromised patients (3, 8, 19), and pulmonary candidiasis and cryptococcosis have also become problems in certain clinical settings (16, 20, 31). We therefore developed experimental models of pulmonary infections with *A. fumigatus* (1, 28) and *C. albicans* (24, 25) in immunosuppressed mice. We evaluated the efficacy of ER-30346 against pulmonary infections caused by *A. fumigatus*, *C. albicans*, and *C. neoformans* in comparison with those of itraconazole and fluconazole. 5-Fluorouracil treatment elicited great reduction in the number of leukocytes, especially neutrophils, in mice (29), and pulmonary infection caused by *Pseudomonas aeruginosa* was induced in 5-fluorouracil-treated mice (26). 5-Fluorouracil treatment was therefore applied to pulmonary aspergillosis and candidiasis models with a slightly modified treatment schedule. The neutropenic pulmonary model appears to more closely mimic the situation in clinical settings. A pulmonary cryptococcosis model could be induced in healthy mice (15, 27).

ER-30346 demonstrated good therapeutic effects dose dependently in an experimental pulmonary aspergillosis model in mice. Although itraconazole appears to be efficacious against invasive aspergillosis (5, 6), additional effective antifungal drugs against aspergillosis must also be developed. Interestingly, ER-30346 was as effective or was more effective than itraconazole in the pulmonary aspergillosis model. ER-30346 constantly reduced the log number of CFU more than itraconazole did against three strains of *A. fumigatus*, even though the difference in the numbers of CFU between the animals receiving ER-30346 and itraconazole was less than 1 log. Furthermore, we confirmed that ER-30346 was the most effective of the drugs tested against systemic infections caused by *A. fumigatus* (10).

ER-30346 is further characterized by its efficacy against fluconazole-resistant *C. albicans* infections in mice. Fluconazole-resistant *Candida* spp. are reported to have emerged clinically (2, 3, 12, 14, 30). ER-30346 proved to be effective against pulmonary candidiasis caused by a fluconazole-resistant strain of *C. albicans* in mice. With the present model, despite in vitro

MIC and substantial levels in plasma, itraconazole was not effective in reducing the numbers of CFU. One of the reasons that ER-30346 but not itraconazole was effective may be the long half-life of ER-30346 in mice, as described in our previous report (10). The efficacy of ER-30346 against a *C. albicans*-resistant strain was lower, however, than that against a fluconazole-susceptible *C. albicans* strain. Further study is needed to confirm the efficacy of ER-30346 in other experimental models of infection, such as systemic and oral infections caused by fluconazole-resistant *C. albicans*. ER-30346 had efficacy comparable to that of fluconazole against pulmonary candidiasis caused by a fluconazole-susceptible strain of *C. albicans* and was more effective than itraconazole. ER-30346 also showed a good therapeutic effect against pulmonary cryptococcosis, had efficacy comparable to that of fluconazole, and was more effective than itraconazole.

Cryptococcal meningitis and oral candidiasis occur most frequently in human immunodeficiency virus-infected patients (8). Cryptococcosis, often occurring as relapsing meningitis, is most lethal (22). Although oral candidiasis is not life-threatening, the sustained immunosuppression in these patients facilitates the recurrence of infection (18). We developed experimental models of these two infections and evaluated the efficacy of ER-30346 against these infections in mice and rats. ER-30346 demonstrated a good therapeutic effect in the models of cryptococcal meningitis and oral candidiasis. ER-30346 had efficacy comparable to that of fluconazole and was more effective than itraconazole against intracranial cryptococcosis in mice and oral candidiasis in rats.

ER-30346 was therapeutically as effective as or more effective than itraconazole against pulmonary infections caused by *A. fumigatus* and fluconazole-resistant *C. albicans* in mice. Against other infections caused by *C. neoformans* and fluconazole-susceptible *C. albicans*, ER-30346 was as effective therapeutically as fluconazole and was more effective than itraconazole. These results suggest that ER-30346 may have potential efficacy as therapy against aspergillosis, cryptococcosis, and candidiasis.

ER-30346 is thus a very promising drug for the treatment of fungal infections, and therefore, further studies on its pharmacokinetic and toxicological behaviors are warranted.

ACKNOWLEDGMENT

We thank Yoshito Niki, Kawasaki Medical College, Okayama, Japan, for providing the fungal strains.

REFERENCES

1. **Allendoerfer, R., D. Loebenberg, M. G. Rinaldi, and J. R. Graybill.** 1995. Evaluation of SCH51048 in an experimental model of pulmonary aspergillosis. Antimicrob. Agents Chemother. **39:**1345–1348.
2. **Barchiesi, F., A. L. Colombo, D. A. Mcgough, A. W. Fothergill, and M. G. Rinaldi.** 1994. In vitro activity of itraconazole against fluconazole-susceptible and resistant *Candida albicans* isolates from oral cavities of patients infected with human immunodeficiency virus. Antimicrob. Agents Chemother. **38:** 1530–1533.
3. **Bossche, H. V., P. Marichal, F. C. Odds, L. Le Jeune, and M.-C. Coene.** 1992. Characterization of azole-resistant *Candida glabrata* isolate. Antimicrob. Agents Chemother. **36:**2602–2610.
4. **DeGregorio, M. W., W. M. F. Lee, C. A. Linker, R. A. Jacobs, and C. A. Ries.** 1982. Fungal infections in patients with acute leukemia. Am. J. Med. **73:** 543–548.
5. **Denning, D. W., J. Y. Lee, J. S. Hostetler, P. Pappas, C. A. Kauffman, D. H. Dewsnup, J. N. Galgiani, J. R. Graybill, A. M. Sugar, A. Catanzaro, H. Gallis, J. R. Perfect, B. Dockery, W. E. Dismukes, and D. A. Stevens.** 1994. NIAID mycoses study group multicenter trial of oral itraconazole therapy for invasive aspergillosis. Am. J. Med. **97:**135–144.
6. **Denning, D. W., R. M. Tucker, L. H. Hanson, and D. A. Stevens.** 1989. Treatment of invasive aspergillosis with itraconazole. Am. J. Med. **86:**791–800.
7. **Espinel-Ingroff, A., S. Shadomy, and R. J. Gebhart.** 1984. In vitro studies with R51,211 (itraconazole). Antimicrob. Agents Chemother. **26:**5–9.
8. **Georgopapadakou, N. H., and T. J. Walsh.** 1996. Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob. Agents Chemother. **40:**279–291.
9. **Graybill, J. R.** 1989. New antifungal agents. Eur. J. Clin. Microbiol. Infect. Dis. **5:**402–412.
10. **Hata, K., J. Kimura, H. Miki, T. Toyosawa, T. Nakamura, and K. Katsu.** 1996. In vitro and in vivo antifungal activities of ER-30346, a novel oral triazole with a broad antifungal spectrum. Antimicrob. Agents Chemother. **40:**2237–2242.
11. **Hay, R. J.** 1991. Antifungal therapy and new azole compound. J. Antimicrob. Chemother. **28**(Suppl. A)**:**35–46.
12. **He, X., R. N. Tiballi, L. T. Zarins, S. F. Bradley, J. A. Sangeorzan, and C. A. Kauffman.** 1994. Azole resistance in oropharyngeal *Candida albicans* strains isolated from patients infected with human immunodeficiency virus. Antimicrob. Agents Chemother. **38:**2495–2497.
13. **Hector, R. F., and E. Yee.** 1990. Evaluation of Bay R 3783 in rodent models of superficial and systemic candidiasis, meningeal cryptococcosis, and pulmonary aspergillosis. Antimicrob. Agents Chemother. **34:**448–454.
14. **Hitchcock, C. A., G. W. Pye, P. F. Troke, E. M. Johnson, and D. W. Warnock.** 1993. Fluconazole resistance in *Candida glabrata*. Antimicrob. Agents Chemother. **37:**1962–1965.
15. **Hoag, K. A., N. E. Street, G. B. Huffnagle, and M. F. Lipscomb.** 1995. Early cytokine production in pulmonary Cryptococcus neoformans infections distinguishes susceptible and resistant mice. Am. J. Respir. Cell. Mol. Biol. **13:**487–495.
16. **Huffnagle, G. B., R. M. Strieter, T. J. Standiford, R. A. Mcdonald, M. D. Burdick, S. L. Kunkel, and G. B. Toews.** 1995. The role of monocyte chemotactic protein-1 (MCP-1) in the recruitment of monocytes and $CD4^+$ T cells during a pulmonary Cryptococcus neoformans infection. J. Immunol. **155:**4790–4797.
17. **Jones, J. H., and C. Russell.** 1974. The histology of chronic candidal infection of rat's tongue. J. Pathol. **113:**97–100.
18. **Larsen, R. A.** 1990. AIDS commentary: azoles and AIDS. J. Infect. Dis. **162:**727–730.
19. **Meyer, R. D., L. S. Young, D. Armstrong, and B. Yu.** 1973. Aspergillosis complicating neoplastic disease. Am. J. Med. **54:**6–15.
20. **Myerowitz, R. L., G. J. Pazin, and C. M. Allen.** 1977. Disseminated candidiasis: changes in incidence, underlying disease, and pathology. Am. J. Clin. Pathol. **68:**29–38.
21. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing for yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
22. **Pietroski, N. A., D. Pharm, and J. Stern.** 1990. Cryptococcal meningitis and other fungal infections of the central nervous system. Curr. Opin. Infect. Dis. **3:**608–613.
23. **Richardson, K., K. W. Brammer, M. S. Marriott, and P. F. Troke.** 1985. Activity of UK-49,858, a bistriazole derivative, against experimental infection with *Candida albicans* and *Trichophyton mentagrophytes*. Antimicrob. Agents Chemother. **27:**832–835.
24. **Sawyer, R.** 1990. Experimental pulmonary candidiasis. Mycopathologia **109:** 99–109.
25. **Sawyer, R. T., and A. G. Harmsen.** 1989. The relative contribution of resident pulmonary alveolar macrophage and inflammatory polymorphonuclear neutrophils in host resistance to pulmonary infection by *Candida albicans*. Mycopathologia **108:**95–105.
26. **Toyosawa, T., K. Hata, J. Ueno, M. Moriyama, and K. Katsu.** 1994. Effects of cefclidin on experimental respiratory infection caused by *Pseudomonas aeruginosa* in immunosuppressed mice. Chemotherapy (Tokyo) **42:**1242–1246.
27. **Uchida, K., and H. Yamaguchi.** 1991. The therapeutic effects of itraconazole, a new triazole antifungal agent, for experimental fungal infections. Jpn. J. Antibiot. **44:**588–599.
28. **Van Cutsem, J., F. Van Gerven, M.-A. Van de Van, M. Borgers, and P. A. J. Janssen.** 1984. Itraconazole, a new triazole that is orally active in aspergillosis. Antimicrob. Agents Chemother. **26:**527–534.
29. **Van Zant, G.** 1984. Studies of hematopoietic stem cells spared by 5-fluorouracil. J. Exp. Med. **159:**679–690.
30. **Venkateswarlu, K., D. W. Denning, N. J. Manning, and S. L. Kelly.** 1995. Resistance of fluconazole in *Candida albicans* from AIDS patients correlated with reduced intracellular accumulation of drug. FEMS Microbiol. Lett. **131:**337–341.
31. **Yamaguchi, H., K. Makimura, H. Kume, and M. Okudaira.** 1993. Recent trend of deep-seated mycoses in Japan and related medical problems. Prog. Med. **13:**2675–2681.