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In Vitro and In Vivo Antifungal Activities of ER-30346, a Novel Oral Triazole with a Broad Antifungal Spectrum

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ER-30346 is a novel oral triazole with a broad spectrum of potent activity against a wide range of fungi. ER-30346, with MICs at which 90% of the strains tested are inhibited (MIC_{90}s) ranging from 0.025 to 0.78 μg/ml, was 4 to 32 times more active than itraconazole, fluconazole, and amphotericin B against *Candida albicans*, *Candida parapsilosis*, and *Candida glabrata*. Against *Candida tropicalis*, ER-30346, with an MIC_{90} of 12.5 μg/ml, was 2 to >8 times more active than itraconazole and fluconazole, but was 16 times less active than amphotericin B. ER-30346 (MIC_{90}, 0.78 μg/ml) was four to eight times more active than fluconazole and amphotericin B and had activity comparable to that of itraconazole against *Trichosporon beigelli*. The MIC_{90}s of ER-30346 were 0.10 μg/ml for *Cryptococcus neoformans* and 0.39 μg/ml for *Aspergillus fumigatus*. ER-30346 was 2 to 8 times more active than itraconazole and amphotericin B and 32 to >256 times more active than fluconazole. ER-30346 also showed good activity against dermatophytes, with MICs ranging from 0.05 to 0.39 μg/ml, and its activity was comparable to or 2 to 16 times higher than those of itraconazole and amphotericin B and >32 times higher than that of fluconazole. In vivo activity was evaluated with systemic infections in mice. Against systemic candidiasis and cryptococcosis, ER-30346 was comparable in efficacy to fluconazole and was more effective than itraconazole. Of the drugs tested, ER-30346 was the most effective drug against systemic aspergillosis. We studied the levels of ER-30346 in mouse plasma. The maximum concentration of drug in plasma and the area under the concentration-time curve for ER-30346 showed good linearity over a range of doses from 2 to 40 mg/kg of body weight.

The frequency of fungal infections has increased in immunocompromised patients receiving immunosuppressive or anticancer therapy. The major opportunistic pathogen has been *Candida albicans*. The occurrence of infections with non-*C. albicans Candida* species is increasing in frequency, and invasive pulmonary aspergillosis is a leading cause of mortality in bone marrow transplant recipients (3, 7). Human immunodeficiency virus-infected patients are particularly susceptible to mucosal candidiasis, cryptococcal meningitis, and diseases caused by other pathogens (14, 18).

Newer azole derivatives active against experimental fungal infections have recently been reported (1, 5, 6, 10, 19, 20, 22), and two of these, fluconazole and itraconazole, are in clinical use (8, 9). While these agents offer potential advantages over amphotericin B, such as reduced toxicity and a broad therapeutic index (8, 9), their activities are still limited, especially against *Aspergillus fumigatus*, and drugs with greater activity against *A. fumigatus* are needed. With this in mind, we have directed our research toward the development of new azoles. One such new 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* spp., and dermatophytes. In the present study, we compared the in vitro and in vivo antifungal activities of ER-30346 with those of fluconazole (19), itraconazole (5), and other antifungal agents.

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MATERIALS AND METHODS

Antifungal agents. ER-30346 and fluconazole were synthesized at Eisai Co. Ltd., Tokyo, Japan. The other antifungal agents were obtained commercially: itraconazole was from Janssen Kyowa Co., Tokyo, Japan, and amphotericin B was from Squibb Japan Inc., Tokyo, Japan. The drugs were dissolved individually in dimethyl sulfoxide (DMSO) for in vitro studies. For in vivo and pharmacokinetic studies, the drugs were dissolved individually in DMSO and were uniformly suspended by sonication in a ninefold volume of 0.5% sodium carboxymethyl cellulose (CMC).

Organisms. Two strains of *C. albicans* (MCY8622 and M1012) and two strains of *C. neoformans* (AJ4290 and Duke) were kindly provided by Y. Fukazawa, Yamanashi Medical College, Yamanashi, Japan. *C. neoformans* No. 3 was kindly provided by Y. Niki, Kawasaki Medical College, Okayama, Japan. *A. fumigatus* Tsukuba and *Trichophyton mentagrophytes* 40904 were kindly provided by Y. Mikami, Chiba University, Chiba, Japan. Two strains of *A. fumigatus* (TIMM0069 and TIMM0070), *T. mentagrophytes* TIMM1188, two strains of *Trichophyton rubrum* (TIMM1216 and TIMM1217), *Microsporum canis* TIMM0760, and *Microsporum gypseum* TIMM0776 were kindly provided by H. Yamaguchi, Teikyo University School of Medicine, Tokyo, Japan. The other fungal strains used in the study 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.

In vitro susceptibility tests. MICs were determined by the broth microdilution method based on the standard method for antifungal susceptibility testing proposed by the National Committee for Clinical Laboratory Standards (17) 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 Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich) at 30°C for 24 to 48 h, and filamentous fungi were grown on potato dextrose agar (PDA; Eiken Chemical Co., Tokyo, Japan) 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

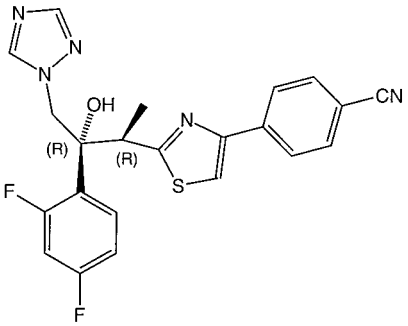

FIG. 1. Chemical structure of ER-30346.

48 h (72 h for *C. neoformans* and 120 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.

Animals. Female ICR mice (age, 4.5 to 5 weeks; weight, approximately 22 g; Charles River Japan Inc., Kanagawa, Japan) were used throughout the experiments. They were housed in cages of 20 per group and had access to food and water ad libitum.

Systemic infection in mice. (i) Candidiasis. *C. albicans* E81022 was grown on an SDA plate at 30°C for 24 h, and challenge organisms were prepared in sterile saline. Mice (age, 4.5 weeks; $n = 10$) were infected via the tail vein with 3.7×10^6 cells. Drugs were orally administered, in a volume of 0.2 ml per dose, 1 h after infection. Control groups received 10% DMSO in 0.5% CMC. Drugs were administered at doses of 2.5 and 10 mg/kg of body weight. Mortality was recorded daily for 14 days of infection. Drug efficacy was assessed by determining the delay in mortality.

(ii) Cryptococcosis. *C. neoformans* No. 3 was grown on an SDA plate at 30°C for 48 h, and challenge organisms were prepared in sterile saline. Mice (age, 5 weeks; $n = 10$) were infected via the tail vein with 2.0×10^6 cells. Drugs were orally administered, in a volume of 0.2 ml per dose, twice daily for 5 consecutive days starting 1 h after infection. Controls received 10% DMSO in 0.5% CMC. Drugs were administered at doses of 8 and 32 mg/kg. Mortality was recorded daily for 21 days of infection. Drug efficacy was assessed by determining the delay in mortality.

(iii) Aspergillosis. *A. fumigatus* Tsukuba was grown on a PDA plate at 30°C for 1 week, and challenge organisms were prepared in sterile saline containing 0.05% Tween 80. Mice (age, 5 weeks; $n = 10$) were infected via the tail vein with 1.0×10^7 conidia. Drugs were orally administered the same way they were administered in the cryptococcosis model. Mortality was recorded daily for 14 days of infection. Drug efficacy was assessed by determining the delay in mortality.

ER-30346 measurement in plasma. Blood samples were obtained from three female mice (age, 4.5 weeks) at 0.5, 1, 2, 6, 12, and 24 h. ER-30346 and itraconazole were orally administered in a volume of 0.2 ml per dose. These levels in plasma were determined by a high-pressure liquid chromatography (HPLC)-UV method. To determine the concentrations in plasma, 0.1 ml of internal standard solution, 1 ml of saline, and 1 ml of 1 N NaOH were added to each plasma sample. After adding 5 ml of diethyl ether, these samples were shaken for 10 min to extract ER-30346 and itraconazole. After centrifugation at 3,000 rpm for 10 min, the organic phase was collected. The organic phase was evaporated off, its residue was dissolved in 0.2 ml of 50% acetonitrile–50% water, and 50 μl of the sample was subjected to HPLC. HPLC conditions were as follows: column, YMC-Pack AM312 (6 mm [inner diameter] by 150 mm in length); mobile phase, acetonitrile-water (58:42; vol/vol); flow rate, 1 ml/min; detection, UV at 315 nm. This method detects 0.01 μg of ER-30346 and itraconazole per ml in plasma.

Calculation of pharmacokinetic parameters. The values of the maximum concentration of drug in plasma (C_{max}) for ER-30346 and itraconazole were read directly from concentration data, and the area under the concentration-time curve from time zero to 24 h (AUC_{0-24}) for ER-30346 was calculated by the trapezoidal rule.

Statistical analysis. Data from mortality studies were analyzed by the log-rank test; this was followed by the Tukey type comparison test. Statistical significance was defined as $P < 0.05$.

RESULTS

In vitro antifungal activities. The antifungal activities of ER-30346, itraconazole, fluconazole, and amphotericin B against the clinical isolates are presented in Table 1. ER-30346 showed a broad spectrum of activity against a wide range of fungi covering *Candida* spp., *Trichosporon beigelii*, *C. neoformans*, and *A. fumigatus.*

Against *C. albicans*, *Candida parapsilosis*, and *Candida glabrata*, the activity of ER-30346, with the MICs at which 90% of the strains tested are inhibited (MIC_{90}s) ranging from 0.025 to 0.39 μg/ml, was 4 to 64 times higher than those of itraconazole, fluconazole, and amphotericin B. Against *Candida tropicalis*, ER-30346, with an MIC_{90} of 12.5 μg/ml, was 16 times less active than amphotericin B, although the MIC_{50} of ER-30346 (0.10 μg/ml) was 4 to 8 times lower than those of the other drugs tested. ER-30346 showed relatively higher levels of activity against three strains of *Candida krusei*, with MICs ranging from 0.05 to 0.39 μg/ml, compared with the activities of itraconazole (MICs, 0.39 to 0.78 μg/ml), fluconazole (MICs, 25 to 50 μg/ml), and amphotericin B (MICs, 0.78 to 1.56 μg/ml). The MIC_{90} of ER-30346 for *T. beigelii* was 0.78 μg/ml. ER-30346 was four to eight times more active than fluconazole and amphotericin B and had activity comparable to that of itraconazole. Against *C. neoformans*, ER-30346 also showed good activity, with an MIC_{90} of 0.10 μg/ml. ER-30346 was 4 to 32 times more active than the other drugs tested. Another antifungal feature of ER-30346 was its potency against *A. fumigatus*. ER-30346, with an MIC_{90} of 0.39 μg/ml, was 2 to 4 times more active than itraconazole and amphotericin B against *A. fumigatus* and was >256 times more active than fluconazole.

The antifungal activities of ER-30346, itraconazole, fluconazole, and amphotericin B against dermatophytes are presented in Table 2. ER-30346 showed good activity against *T. mentagrophytes*, *T. rubrum*, *M. gypseum*, and *M. canis*, with MICs ranging from 0.05 to 0.39 μg/ml. The activity of ER-30346 was 2 to 8 times higher than that of itraconazole against dermatophytes tested and was >32 times higher than that of fluconazole. ER-30346 was 8 to 16 times more active than amphotericin B against *T. mentagrophytes*, *T. rubrum*, and *M. gypseum*, and had activity comparable to that of amphotericin B against *M. canis.*

In vivo efficacy in mice. The MICs for the *C. albicans*, *C. neoformans*, and *A. fumigatus* strains used throughout the studies are presented in Table 3. The activity of ER-30346 was four to more than eight times higher than that of itraconazole against *C. albicans* and *C. neoformans* and was similar to that of itraconazole against *A. fumigatus*. Fluconazole demonstrated less in vitro activity than the other two drugs against the three strains tested.

We studied the therapeutic effects of orally administered ER-30346 on experimental systemic infections caused by *C. albicans*, *C. neoformans*, and *A. fumigatus*, comparing the efficacies of two doses of ER-30346, itraconazole, and fluconazole with each experimental model. Groups of 10 mice each were used for mortality studies. In the systemic candidiasis model, the control mice in the experiment (Fig. 2) were all dead by day 8. ER-30346 at a dose of 2.5 mg/kg delayed mortality significantly compared with the control treatment, but not compared with treatment with fluconazole and itraconazole at 2.5 mg/kg (Fig. 2A). Fluconazole at a dose of 2.5 mg/kg delayed mortality significantly compared with that for itraconazole at 2.5 mg/kg. ER-30346 and fluconazole at 10 mg/kg delayed mortality significantly compared with that for itraconazole at 10 mg/kg (Fig. 2B). No difference was demonstrated when ER-30346 and fluconazole at 10 mg/kg were compared. ER-30346 was as effective as fluconazole and was more effective than itraconazole against systemic candidiasis in mice.

ER-30346 also showed a substantial therapeutic effect against systemic cryptococcosis. In the systemic cryptococcosis model, the control mice in the experiment (Fig. 3) were all

TABLE 1. Comparative in vitro activities of ER-30346 and other antifungal agents against clinical isolates[a]

Organism (no. of strains)	Antifungal agent	MIC (μg/ml)		
		Range	50%	90%
C. albicans (13)	ER-30346	≤0.006–0.20	≤0.006	0.025
	Itraconazole	0.05–0.39	0.10	0.39
	Fluconazole	0.20–3.13	0.39	0.78
	Amphotericin B	0.39–0.78	0.78	0.78
C. tropicalis (12)	ER-30346	0.006–>25	0.10	12.5
	Itraconazole	0.05–>12.5	0.39	>12.5
	Fluconazole	0.20–>100	0.78	>100
	Amphotericin B	0.39–1.56	0.78	0.78
C. parapsilosis (10)	ER-30346	≤0.006–25	0.012	0.05
	Itraconazole	0.012–>12.5	0.20	0.39
	Fluconazole	0.20–>100	0.39	3.13
	Amphotericin B	0.78	0.78	0.78
C. glabrata (13)	ER-30346	0.10–0.78	0.20	0.39
	Itraconazole	0.39–1.56	0.78	1.56
	Fluconazole	0.78–50	3.13	12.5
	Amphotericin B	0.78–3.13	0.78	1.56
T. beigelii (10)	ER-30346	0.10–0.78	0.39	0.78
	Itraconazole	0.39–1.56	0.78	0.78
	Fluconazole	1.56–6.25	3.13	6.25
	Amphotericin B	0.39–12.5	1.56	3.13
C. neoformans (13)	ER-30346	≤0.006–0.10	0.025	0.10
	Itraconazole	0.025–0.78	0.10	0.39
	Fluconazole	0.20–6.25	1.56	3.13
	Amphotericin B	0.39–0.78	0.78	0.78
A. fumigatus (17)	ER-302346	0.20–0.78	0.39	0.39
	Itraconazole	0.78	0.78	0.78
	Fluconazole	>100	>100	>100
	Amphotericin B	0.78–1.56	1.56	1.56

[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.

dead by day 12. ER-30346 and fluconazole at doses of 8 mg/kg delayed mortality significantly compared with that for itraconazole at 8 mg/kg (Fig. 3A). ER-30346 and fluconazole at 32 mg/kg delayed mortality significantly compared with that for itraconazole at 32 mg/kg (Fig. 3B). No difference was demonstrated when ER-30346 and fluconazole were compared. ER-30346 was as effective as fluconazole and was more effective than itraconazole against systemic cryptococcosis in mice.

ER-30346 was the most effective of the drugs tested against systemic aspergillosis. In the systemic aspergillosis model, the control mice in the experiment (Fig. 4) were all dead by day 7. ER-30346 at a dose of 10 mg/kg delayed mortality significantly compared with those for itraconazole and fluconazole at 10 mg/kg (Fig. 4A). ER-30346 and itraconazole at 40 mg/kg delayed mortality significantly compared with that for fluconazole at 40 mg/kg (Fig. 4B). No difference was demonstrated when ER-30346 and itraconazole at 40 mg/kg were compared. ER-30346 was more effective than itraconazole and fluconazole against systemic aspergillosis in mice.

Pharmacokinetics. The concentrations of ER-30346 and itraconazole in plasma after the administration of a single dose are presented in Fig. 5. The mean peak level of ER-30346 in plasma (± standard deviation) 30 min after the oral administration of a single dose of 2 mg/kg to three mice was 0.16 ±

TABLE 2. Antifungal activities of ER-30346 and other azoles against dermatophytes in RPMI 1640[a]

Organisms	MIC (μg/ml)			
	ER-30346	Itraconazole	Fluconazole	Amphotericin B
T. mentagrophytes 40904	0.10	0.39	100	1.56
T. mentagrophytes TIMM1188	0.05	0.20	6.25	0.78
T. rubrum TIMM1216	0.05	0.10	1.56	0.39
T. rubrum TIMM1217	0.05	0.20	3.13	0.78
M. canis TIMM0760	0.39	0.78	>100	0.39
M. gypseum TIMM0776	0.05	0.39	6.25	0.39

[a] MICs were determined by the broth microdilution method with RPMI 1640 medium, the inoculum size was 2.5×10^3 conidia per ml, and incubation was at 35°C for 120 h.

TABLE 3. In vitro activities of ER-30346 and other azoles against strains causing experimental systemic infections[a]

Strain	MIC (μg/ml)		
	ER-30346	Itraconazole	Fluconazole
C. albicans E81022	≤0.006	0.05	0.39
C. neoformans no. 3	0.025	0.10	3.13
A. fumigatus Tsukuba	0.39	0.78	>100

[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.

0.05 μg/ml. At 6 h, the concentration of ER-30346 in plasma had fallen to 0.09 ± 0.04 μg/ml, approximately 60% of the 30-min peak level. The mean peak level of ER-30346 1 h after the oral administration of a single dose of 10 mg/kg to three mice was 1.00 ± 0.08 μg/ml and was similar to that of itraconazole (0.98 ± 0.25 μg/ml). At 6 h, the concentration of ER-30346 in plasma had fallen to 0.50 ± 0.01 μg/ml, approximately 50% of the 1-h peak level. The peak level of ER-30346 6 h after the oral administration of a single dose of 40 mg/kg to a single mouse was 2.39 μg/ml. In each experiment, ER-30346 was detectable in all three mice at 12 h, but none of the three mice had a detectable level of 0.01 μg/ml at 24 h. Itraconazole was not detectable in any of the three mice at 12 h.

The half-life of ER-30346 (4.0 h) was about three times longer than that of itraconazole (1.4 h). The AUC of ER-30346 was 1.10 μg · h/ml at a dose of 2 mg/kg, 6.81 μg · h/ml at a dose of 10 mg/kg, and 28.8 μg · h/ml at a dose of 40 mg/kg. The AUC of itraconazole, 2.20 μg · h/ml at 10 mg/kg, was three times lower than that of ER-30346 at 10 mg/kg. The C_{max} and AUC of ER-30346 showed good linearity over the range of doses from 2 to 40 mg/kg in mice.

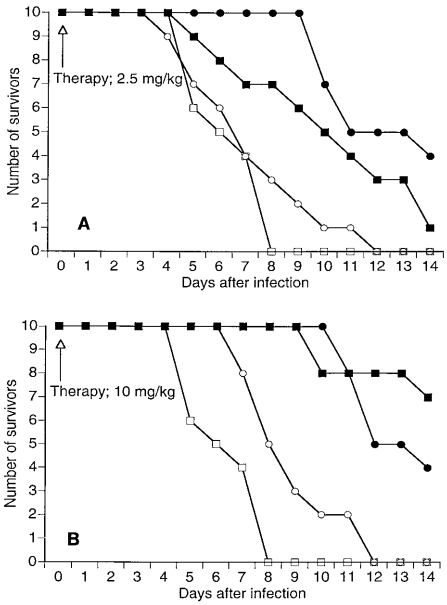

FIG. 2. Efficacies of ER-30346 and the other azoles tested against systemic infection caused by *C. albicans* E81022. Mice ($n = 10$) were infected with 3.7×10^6 cells and were orally treated with the tested drugs 1 h after infection. (A) The drugs were administered at doses of 2.5 mg/kg. $P < 0.05$, comparing ER-30346 treatment with control treatment; $P < 0.05$, comparing fluconazole treatment with control and itraconazole treatments. (B) Drugs were administered at doses of 10 mg/kg. $P < 0.05$, comparing ER-30346 treatment with control and itraconazole treatments; $P < 0.05$, comparing fluconazole treatment with control and itraconazole treatments. Symbols: □, control; ■, ER-30346; ○, itraconazole; ●, fluconazole.

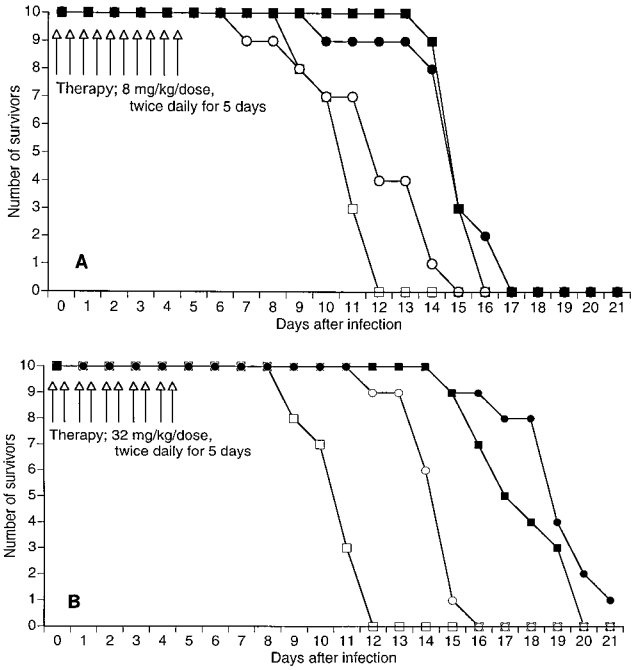

FIG. 3. Efficacies of ER-30346 and the other azoles tested against systemic infection caused by *C. neoformans* No. 3. Mice ($n = 10$) were infected with 2×10^6 cells and were orally treated with the tested drugs twice daily for 5 consecutive days starting 1 h after infection. (A) Drugs were administered at doses of 8 mg/kg. $P < 0.05$, comparing ER-30346 with control treatment and itraconazole treatments; $P < 0.05$, comparing fluconazole treatment with control and itraconazole treatments. (B) Drugs were administered at doses of 32 mg/kg. $P < 0.05$, comparing ER-30346 treatment with control and itraconazole treatments; $P < 0.05$, comparing fluconazole treatment with control and itraconazole treatments. Symbols: □, control; ■, ER-30346; ○, itraconazole; ●, fluconazole.

DISCUSSION

ER-30346 is a novel oral thiazole-containing triazole with a broad spectrum of potent antifungal activity against major pathogenic fungi, such as *Candida* species, *C. neoformans*, *A. fumigatus*, and dermatophytes. The most important advantage of ER-30346 is its potency against *A. fumigatus*, one of the major causes of life-threatening opportunistic fungal infections (7). ER-30346 was highly active against *A. fumigatus* (MIC_{90}, 0.39 μg/ml) and was two to four times more active than itraconazole and amphotericin B. The antifungal activity of ER-30346 against *A. fumigatus* seems enhanced by the introduction of one carbon chain between the benzylic *tert*-carbon and thiazole substituents and the cyano group on the aromatic ring attached to thiazole (16). In general, the electron withdrawal group enhanced the antifungal activity of ER-30346, and the enhancement of ER-30346 activity by the cyano group on the aromatic ring attached to thiazole (16). In general, the electron withdrawal group enhanced the antifungal activity of ER-30346, and the enhancement of ER-30346 activity by the cyano group seems to result from this effect. ER-30346 was also highly active against yeast fungi except for *C. tropicalis.* ER-30346 was 4 to 32 times more active than the other drugs tested against *C. albicans*, *C. parapsilosis*, and *C. glabrata* isolates resistant to fluconazole (2, 11) and *C. neoformans*. For *C. tropicalis*, the MIC_{50} and MIC_{90} of ER-30346 were 0.10 and 12.5 μg/ml, respectively. ER-30346 was 16 times less active

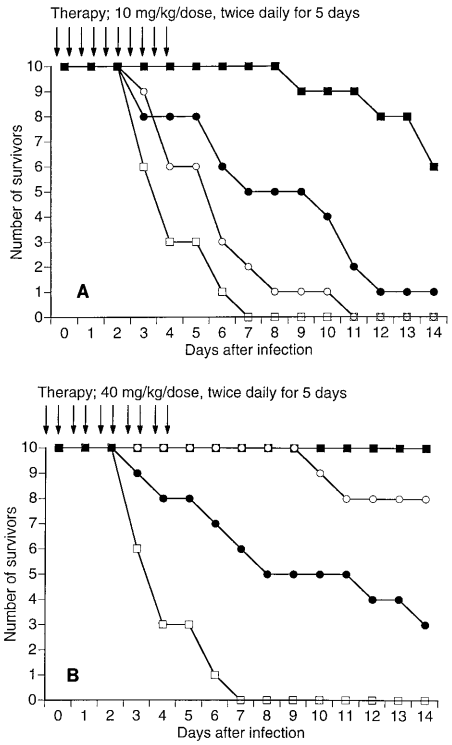

FIG. 4. Efficacies of ER-30346 and the other azoles tested against systemic infection caused by *A. fumigatus* Tsukuba. Mice ($n = 10$) were infected with 1.0×10^7 conidia and were orally treated with the tested drugs twice daily for 5 consecutive days starting 1 h after infection. (A) Drugs were administered at doses of 10 mg/kg. $P < 0.05$, comparing ER-30346 treatment with control, itraconazole, and fluconazole treatments. (B) Drugs were administered at doses of 40 mg/kg. $P < 0.05$, comparing ER-30346, itraconazole, and fluconazole treatments with control treatment; $P < 0.05$, comparing ER-30346 and itraconazole treatments with fluconazole treatment. Symbols: □, control; ■, ER-30346; ○, itraconazole; ●, fluconazole.

than amphotericin B on the basis of the MIC_{90}, although the MIC_{50} of ER-30346 was eight times lower than that of amphotericin B. Six of 12 *C. tropicalis* strains tested were resistant to ER-30346. These six strains of *C. tropicalis* were also resistant to itraconazole and fluconazole. Throughout the in vitro susceptibility study, ER-30346 MICs rose when itraconazole and fluconazole MICs rose. It seems that all azoles tested have very similar properties, and the mechanisms of resistance to these azoles are likely the same.

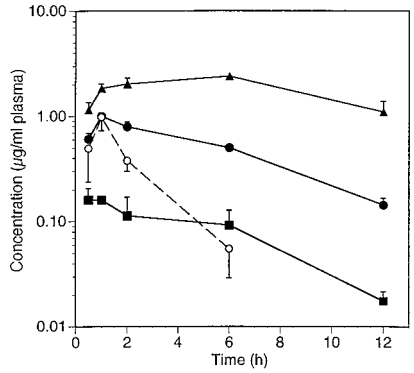

FIG. 5. Single-dose pharmacokinetics: mean ± standard deviation levels of ER-30346 in plasma after the administration of single dose of 2, 10, or 40 mg/kg and mean levels of itraconazole after the administration of a single dose of 10 mg/kg to three mice. Symbols: ■, ER-30346 at 2 mg/kg; ●, ER-30346 at 10 mg/kg; ▲, ER-30346 at 40 mg/kg; ○, itraconazole at 10 mg/kg.

ER-30346 also showed good activity against dermatophytes, and its activity was two to eight times higher than that of itraconazole, which was clinically used for the therapy of onychomycosis. Further study is needed to confirm the efficacy of ER-30346 in an experimental superficial infection model.

Like its in vitro activity, ER-30346 was found to have good in vivo characteristics. ER-30346 was more effective than itraconazole and fluconazole against experimental systemic aspergillosis in mice. Also against systemic candidiasis and cryptococcosis in mice, ER-30346 was more effective than itraconazole and had activity comparable to that of fluconazole. In the pharmacokinetic study, the C_{max} and AUC of ER-30346 showed good linearity over the range of doses from 2 to 40 mg/kg, and a substantial level of ER-30346, correlated with the in vivo efficacy of ER-30346, could be detected in the plasma of mice. The C_{max} of ER-30346 at a dose of 10 mg/kg (1.00 μg/ml) was similar to that of itraconazole at a dose of 10 mg/kg (0.98 μg/ml) and was about seven times lower than that of fluconazole at a dose of 10 mg/kg (12). This result suggested that ER-30346 is absorbed at levels similar to those for itraconazole. The half-life of ER-30346 (4.0 h) was about three times longer than that of itraconazole (1.4 h) and was similar to that of fluconazole (12). It is well known that in vivo activity is affected by in vitro activity, the levels of compounds in plasma, the binding rates of compounds to plasma proteins (13, 21), etc. In mice, the long half-life and high level of in vitro activity of ER-30346 seemed to result in in vivo efficacy higher than that of itraconazole. Further study is needed to confirm the efficacy of ER-30346 by using in vivo studies with other animal species such as rats, dogs, and monkeys.

Taken together, ER-30346 has a broad antifungal spectrum and a potent antifungal activity against major pathogenic fungi. ER-30346 also shows good therapeutic efficacy against systemic infections caused by *C. albicans*, *C. neoformans*, and *A. fumigatus*, which are causes of deep-seated mycoses in immunocompromised hosts (3, 4, 7, 15, 23). ER-30346 is thus a very promising drug for the treatment of fungal infections, and therefore, further studies on its in vivo efficacy in experimental local infection models and its pharmacokinetic and toxicological behaviors are warranted.

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