

T-8581, a New Orally and Parenterally Active Triazole Antifungal Agent: In Vitro and In Vivo Evaluations

AKIRA YOTSUJI,* KATSUMI SHIMIZU, HARUMI ARAKI, KAZUO FUJIMAKI, NAGAKO NISHIDA, RITSUKO HORI, NAOKO ANNEN, SEIJI YAMAMOTO, HIROYOSHI HAYAKAWA, HIROYUKI IMAIZUMI, YASUO WATANABE, AND HIROKAZU NARITA

Research Laboratories, Toyama Chemical Co., Ltd., Toyama 930, Japan

Received 19 June 1996/Returned for modification 12 August 1996/Accepted 11 October 1996

T-8581 is a new water-soluble triazole antifungal agent. The geometric mean IC_{80S} ($GM-IC_{80S}$; where the IC_{80} is the lowest drug concentration which reduced the optical density at 630 nm by 80% compared with the optical density at 630 nm of the drug-free control) for *Candida albicans* were as follows: T-8581, 0.218 μ g/ml; fluconazole, 0.148 μ g/ml; and itraconazole, 0.0170 μ g/ml. For *Cryptococcus neoformans* the $GM-IC_{80S}$ were as follows: T-8581, 9.28 μ g/ml; fluconazole, 4.00 μ g/ml; and itraconazole, 0.119 μ g/ml. For *Aspergillus fumigatus* the $GM-IC_{80S}$ were as follows: T-8581, 71.0 μ g/ml; fluconazole, 239 μ g/ml; and itraconazole, 0.379 μ g/ml. Against systemic candidiasis in mice, the 50% effective doses (ED_{50S}) of T-8581, fluconazole, and itraconazole (given orally) were 0.412, 0.392, and >320 mg/kg of body weight, respectively. Against systemic aspergillosis in mice, the ED_{50S} of T-8581, fluconazole, and itraconazole (given orally) were 50.5, 138, and >320 mg/kg, respectively. T-8581 was also efficacious when it was given parenterally (ED_{50} , 59.2 mg/kg), while the ED_{50} of fluconazole given parenterally was >20 mg/kg. Against systemic aspergillosis in rabbits, T-8581 was more effective than fluconazole and itraconazole in prolonging the life span. The high concentrations of T-8581 were observed in the sera of mice, rats, rabbits, and dogs. Species differences in half-lives and areas under the concentration-time curves were observed, with the values for mice, rats, rabbits, and dogs increasing in that order. These results suggest that T-8581 would be a potentially effective antifungal drug for oral and parenteral use.

It has been reported that serious infections caused by fungi are an increasing problem because of factors such as intensive care practices, human immunodeficiency virus infections, organ transplantation, and other immunosuppressive conditions (7). Treatments for these infections are still limited to a few agents. Five systemic antifungal agents, amphotericin B, flucytosine, miconazole, fluconazole, and itraconazole, have been developed so far for clinical use. However, the clinical values of these agents have been limited by their relatively high risks of toxicity, the emergence of drug resistance, pharmacokinetic deficiencies, and/or insufficiencies in their antifungal activities (4, 8, 15, 17). Thus, much effort to develop novel antifungal agents which are more safe and efficacious are still being made.

T-8581, (*R*)-(-)-3-(2,4-difluorophenyl)-2,2-difluoro-3-hydroxy-4-(1*H*-1,2,4-triazole-1-yl)butanamide (Fig. 1), is a new water-soluble triazole antifungal agent. Maximum solubilities of T-8581 and fluconazole in water are 41.8 and 2.6 mg/ml, respectively. In this report, we describe the in vitro and in vivo antifungal activities and pharmacokinetic properties of T-8581.

(This work was presented in part at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., 17 to 20 September 1995 [23].)

MATERIALS AND METHODS

Antifungal agents. T-8581, fluconazole, and itraconazole were synthesized at the Research Laboratories, Toyama Chemical Co., Ltd., Toyama, Japan.

Fungi. A total of 160 strains from eight fungal species were used in the study. Almost all of the strains were clinical isolates; the rest were obtained from the American Type Culture Collection, Rockville, Md.; the Institute of Fermentation, Osaka, Japan; and the Research Center for Medical Mycology, Teikyo University School of Medicine, Tokyo, Japan.

Antifungal susceptibility testing. The in vitro activities of the antifungal agents were estimated by a broth microdilution method with RPMI 1640 broth (Sigma Chemical Co., St. Louis, Mo.) buffered to pH 7 with morpholinepropanesulfonic

acid (3, 18, 20). Microdilution trays containing serial twofold dilutions of the antifungal agents (100 μ l) were prepared, and disposable multiple-well plates (96-well flat-bottom plates, Corning Laboratory Sciences Company, Corning, N.Y.) were used. Yeast inocula were prepared as followed. *Candida* strains were grown at 30°C on Sabouraud dextrose agar (Difco, Detroit, Mich.) for 24 h; *Cryptococcus* strains were grown at 30°C for 48 h. Yeast suspensions were made in sterile 0.85% saline and were adjusted to suspensions containing 1×10^4 to 5×10^4 yeast cells/ml. For *Aspergillus fumigatus*, suspensions were prepared from a mature culture grown at 30°C for 7 to 10 days on potato dextrose agar (Nissui, Tokyo, Japan). Fungal slants were covered with 5 ml of sterile 0.85% saline containing 0.05% Tween 80 and were gently probed with a sterile loop. Heavy particles were allowed to settle, and the upper homogeneous suspensions were removed and were diluted with RPMI 1640 broth and were then adjusted to suspensions containing 1×10^4 to 5×10^4 cells/ml. Each well was inoculated with 100 μ l of the inoculum suspension (final inoculum, 0.5×10^3 to 2.5×10^3 cells/ml). The trays were incubated at 35°C. Broth microdilution endpoints were determined after 1 to 4 days of incubation with the aid of a Wellreader SME3400 (IWAKI Glass Co., Ltd., Tokyo, Japan). The IC_{80} was the lowest drug concentration which reduced the optical density at 630 nm by 80% compared with the optical density at 630 nm of the drug-free control.

Murine model of systemic infection. Male mice of the ICR strain (Nihon SLC Inc., Shizuoka, Japan), each weighing 19 to 21 g, were used for studies of systemic infection. Mice ($n = 15$) were inoculated intravenously with yeast of *Candida albicans* TIMM1623 (8×10^6 cells/mouse) or conidia of *A. fumigatus* IFO8868 (5×10^6 cells/mouse). Antifungal agents were suspended in 0.5% methylcellulose solution for oral use and were dissolved in physiological saline solution for parenteral use. Therapy was administered orally (p.o.) or intraperitoneally (i.p.) and was initiated 2 h after infection and was continued once daily for 6 days. The volume administered p.o. and i.p. was 0.01 ml/g of body weight. Mortality was assessed over a period of 14 days. The 50% effective dose (ED_{50}) was calculated by the method of Litchfield and Wilcoxon (16). The significances of differences between the treatment groups were also determined by the method of Litchfield and Wilcoxon (16).

Rabbit model of systemic infection. Male rabbits of the JW strain (Sankyo Labo Service Co., Inc., Tokyo, Japan), each weighing 2 kg, were used for studies of systemic infection (19). Rabbits ($n = 6$ to 8) were inoculated intravenously with conidia of *A. fumigatus* IFO8868 (6×10^7 cells/rabbit). Antifungal agents were suspended in 0.5% methylcellulose solution for p.o. use and were dissolved in physiological saline solution for parenteral use. Therapy was initiated p.o. or intravenously 2 h after infection and was continued once daily for 9 days. Mortality was assessed over a period of 20 days. The significances of differences between the treatment groups were determined by the log-rank test.

Pharmacokinetic studies. Male mice of the ICR strain (Nihon SLC Inc.), each weighing 23 to 30 g, were each given a single p.o. or intravenous dose of T-8581.

* Corresponding author.

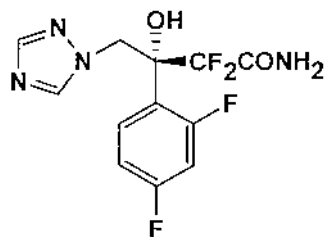


FIG. 1. Chemical structure of T-8581.

Groups of mice were sacrificed at various times up to 24 h after administration of the dose. Male rats of the SD strain (Nihon SLC Inc.), each weighing 240 to 290 g, were each given a single p.o. or intravenous dose of T-8581. Blood samples were drawn from the cervical vein for p.o. administration and from the saphenous artery for intravenous administration at various times up to 24 h after administration of the dose. Male rabbits of the JW strain (SKL Co., Inc., Tokyo, Japan), each weighing 1.45 to 2.20 kg, were each given a single p.o. or intravenous dose of T-8581. Blood samples were drawn from a lateral ear vein for p.o. administration and from the saphenous artery for intravenous administration at various times up to 24 h after administration of the dose. Male beagle dogs (CSK Research Park Co., Ltd., Nagano, Japan), each weighing 12.4 to 13.2 kg, were each given a single p.o. or intravenous dose of T-8581. Blood samples were drawn from the cephalic vein at various times up to 24 h after administration of the dose.

T-8581 was dissolved in distilled water for p.o. use and was dissolved in physiological saline solution for intravenous use. The dose was 10 mg/kg in the pharmacokinetic studies.

Blood from all studies was collected in SEPACLEAN A-5 tubes (Eiken Kizai Co., Ltd., Tokyo, Japan) and was centrifuged to separate the serum, which was stored at -20°C . The concentration of T-8581 in serum was assayed by the high-pressure liquid chromatography method.

Pharmacokinetic parameters. Pharmacokinetic parameters for T-8581 were estimated as follows. The maximum concentration of drug in serum (C_{max}) was determined from the observed serum drug concentration-time data. The apparent elimination constant k was calculated by linear least-squares regression analysis of the respective log-linear serum concentration-time data. The half-life ($t_{1/2}$) was calculated as $0.693/k$. The area under the concentration-time curve (AUC) was calculated by the trapezoidal method with extrapolation to infinity by using the k value.

RESULTS

In vitro antifungal activities. The antifungal activities of T-8581, fluconazole, and itraconazole against various pathogenic fungi are presented in Table 1. The geometric mean IC_{80}s (GM- IC_{80}s) of T-8581 were 0.218, 0.358, and 0.401 $\mu\text{g}/\text{ml}$ for *C. albicans*, *Candida tropicalis*, and *Candida parapsilosis*, respectively, which were similar to those of fluconazole. For *Candida guilliermondii* and *Candida krusei*, the GM- IC_{80}s of T-8581 were 1.19 and 10.2 $\mu\text{g}/\text{ml}$, respectively, which were 3.4- and 1.8-fold more potent than those of fluconazole, respectively. For *Candida glabrata*, the GM- IC_{80} of T-8581 was 11.8 $\mu\text{g}/\text{ml}$, which was 3.6-fold less potent than that of fluconazole. For *Cryptococcus neoformans*, the GM- IC_{80} of T-8581 was 9.28 $\mu\text{g}/\text{ml}$, which was 2.3-fold less than that of fluconazole. For *A. fumigatus*, the GM- IC_{80} of T-8581 was 71.0 $\mu\text{g}/\text{ml}$, which was 3.4-fold more than that of fluconazole. The GM- IC_{80}s of T-8581 and fluconazole were statistically inferior to those of itraconazole ($P < 0.01$ by the Kruskal-Wallis test) for six *Candida* species, *C. neoformans*, and *A. fumigatus*.

In vivo antifungal activity. (i) Systemic candidiasis in mice. The therapeutic effect of T-8581 compared with those of fluconazole and itraconazole against systemic infection with *C. albicans* TIMM1623 is described in Table 2. The control mice in the experiment were dead by days 1 to 9. T-8581 showed the greatest efficacy, with ED_{50}s of 0.412 mg/kg (p.o.) and 0.438 mg/kg (i.p.), which were similar to those of fluconazole (0.392 mg/kg [p.o.] and 0.396 mg/kg [i.p.]) and >700-fold superior to that of itraconazole (>320 mg/kg [p.o.]). The activities of

TABLE 1. In vitro susceptibilities of T-8581, fluconazole, and itraconazole

Organism (no. of isolates)	Drug	IC_{80} ($\mu\text{g}/\text{ml}$)	
		Geometric mean	Range
<i>Candida albicans</i> (25)	T-8581	0.218	0.125–0.5
	Fluconazole	0.148	0.0625–0.5
	Itraconazole	0.0170	0.0039–0.0313
<i>Candida tropicalis</i> (25)	T-8581	0.358	0.25–1
	Fluconazole	0.312	0.125–1
	Itraconazole	0.0272	0.0078–0.0625
<i>Candida parapsilosis</i> (25)	T-8581	0.401	0.25–1
	Fluconazole	0.349	0.125–1
	Itraconazole	0.0661	0.0313–0.125
<i>Candida guilliermondii</i> (8)	T-8581	1.19	0.5–4
	Fluconazole	4.00	1–16
	Itraconazole	0.354	0.0625–1
<i>Candida krusei</i> (17)	T-8581	10.2	4–32
	Fluconazole	18.8	8–64
	Itraconazole	0.240	0.125–0.5
<i>Candida glabrata</i> (25)	T-8581	11.8	8–16
	Fluconazole	3.29	2–8
	Itraconazole	0.212	0.0625–0.5
<i>Cryptococcus neoformans</i> (14)	T-8581	9.28	2–16
	Fluconazole	4.00	1–8
	Itraconazole	0.119	0.0313–0.5
<i>Aspergillus fumigatus</i> (20)	T-8581	71.0	16–128
	Fluconazole	239	128–512
	Itraconazole	0.379	0.25–1

T-8581 and fluconazole were statistically superior to that of itraconazole by the method of Litchfield and Wilcoxon (16).

(ii) Systemic aspergillosis in mice. The therapeutic effect of T-8581 compared with those of fluconazole and itraconazole against systemic infection with *A. fumigatus* IFO8868 is described in Table 3. The control mice in the experiment were dead by days 2 to 9. T-8581 showed the greatest efficacy, with an ED_{50} of 50.5 mg/kg (p.o.), which was 2.7-fold superior to that of fluconazole (138 mg/kg [p.o.]) and >6.3-fold superior to that of itraconazole (>320 mg/kg [p.o.]). The activity of T-8581 was statistically superior to those of fluconazole and itraconazole by the method of Litchfield and Wilcoxon (16). T-8581 also showed the greatest efficacy by parenteral use (ED_{50} , 59.2 mg/kg [i.p.]), while that of fluconazole was >20 mg/kg (fluconazole has limited solubility in physiological saline solution).

(iii) Systemic aspergillosis in rabbits. The survival rates for rabbits treated orally are presented in Fig. 2. The control rabbits in the experiment were all dead by day 5. In contrast, 14.3%, 42.9%, and 85.7% of rabbits treated with 10, 20, and 40 mg of T-8581 per kg, respectively, survived to the end of the

TABLE 2. Therapeutic effects of T-8581, fluconazole, and itraconazole against systemic *C. albicans* TIMM1623 infection in mice

Drug	IC_{80} ($\mu\text{g}/\text{ml}$)	Route	ED_{50} (mg/kg)	95% Confidence limit
T-8581	0.125	p.o.	0.412	0.312–0.544
		i.p.	0.438	0.353–0.543
Fluconazole	0.125	p.o.	0.392	0.300–0.514
		i.p.	0.396	0.300–0.523
Itraconazole	0.0156	p.o.	>320	

TABLE 3. Therapeutic effects of T-8581, fluconazole, and itraconazole against systemic *A. fumigatus* IFO8868 infection in mice

Drug	IC ₈₀ (μg/ml)	Route	ED ₅₀ (mg/kg)	95% Confidence limit
T-8581	64	p.o. i.p.	50.5 59.2	37.4–68.2 44.5–78.8
Fluconazole	256	p.o. i.p.	138 >20.0	106–180
Itraconazole	0.5	p.o.	>320	

experiment (day 20). In comparison with T-8581, 0% and 14.3% of rabbits treated with 20 and 40 mg of fluconazole per kg, respectively, and 16.7% and 37.5% of rabbits treated with 20 and 40 mg of itraconazole per kg, respectively, survived. The death of rabbits treated with T-8581 at 20 and 40 mg/kg was prevented to a statistically significantly greater extent compared with the death rate for control rabbits ($P < 0.05$ by the log-rank test). With itraconazole and fluconazole at those doses, no statistically significant differences were observed compared with the control. T-8581 was more effective at prolonging the life span than fluconazole and itraconazole.

The survival rates for rabbits treated parenterally are presented in Fig. 3. The control rabbits in the experiment were all dead by day 7. In contrast, 37.5% and 87.5% of rabbits treated with 20 and 40 mg of T-8581 per kg, respectively, survived to the end of the experiment (day 20). In comparison the survival

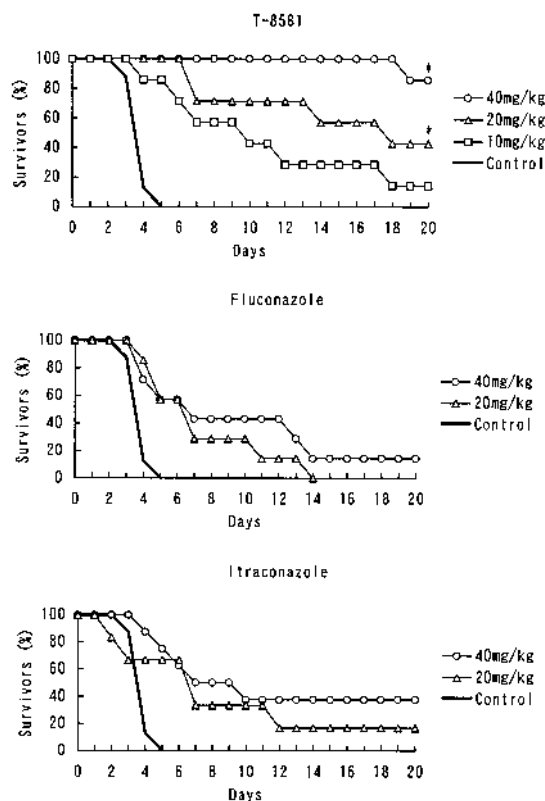


FIG. 2. Therapeutic effects of orally administered T-8581, fluconazole, and itraconazole against systemic *A. fumigatus* IFO8868 infection in rabbits. *, $P < 0.05$ versus control (log-rank test).

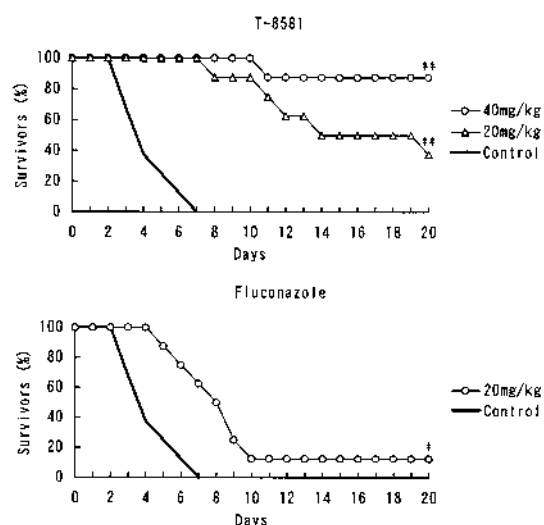


FIG. 3. Therapeutic effect of intravenously administered T-8581 and fluconazole against systemic *A. fumigatus* IFO8868 infection in rabbits. **, $P < 0.01$ versus control (log-rank test); *, $P < 0.05$ versus control (log-rank test).

of rabbits treated with T-8581, 12.5% of rabbits treated with 20 mg of fluconazole per kg (fluconazole has limited solubility in physiological saline solution) survived. The death of rabbits treated with T-8581 at 20 and 40 mg/kg was prevented to a statistically significantly greater extent compared with the death rate for control rabbits ($P < 0.01$ by the log-rank test). The death of rabbits with 20 mg of fluconazole per kg was prevented to a statistically significantly greater extent compared with the death rate for control rabbits ($P < 0.05$ by the log-rank test). T-8581 was more effective at prolonging the life span than fluconazole.

Pharmacokinetic studies with various animals. The concentrations of T-8581 in the sera of mice, rats, rabbits, and dogs following the administration of single p.o. doses are presented in Fig. 4. The pharmacokinetic parameters of T-8581 for each species following the administration of a single p.o. or intravenous dose are presented in Table 4. High concentrations of T-8581 were observed in the sera of various animals following

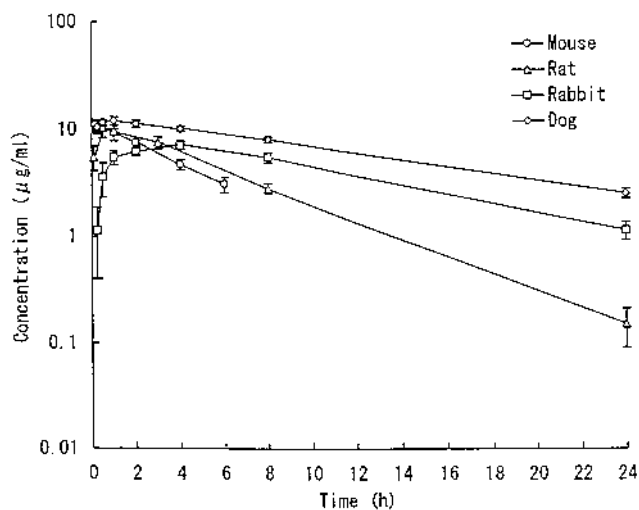


FIG. 4. Concentration of orally administered T-8581 (10 mg/kg) in serum. Values are means \pm standard deviations.

TABLE 4. Pharmacokinetic parameters of T-8581

Animal	Route	C_{\max} ($\mu\text{g}/\text{ml}$) ^a	$t_{1/2}$ (h)	AUC ($\mu\text{g} \cdot \text{h}/\text{ml}$)
Mouse	p.o.	10.9 ± 1.5	3.2	51.7
	i.v.		3.2	46.1
Rat	p.o.	10.5 ± 1.4	3.8	76.9
	i.v.		4.1	72.6
Rabbit	p.o.	7.14 ± 0.67	7.2	115
	i.v.		8.7	139
Dog	p.o.	12.0 ± 1.1	9.9	209
	i.v.		8.9	216

^a Values are means ± standard deviations.

the administration of single oral doses. C_{\max} s for mice, rats, rabbits, and dogs were 10.9, 10.5, 7.14, and 12.0 $\mu\text{g}/\text{ml}$, respectively. After 24 h, T-8581 was observed in the sera of the various animals except mice. Differences in $t_{1/2}$ s were observed among the animal species tested. $t_{1/2}$ s in mice, rats, rabbits, and dogs were 3.2, 3.8 to 4.1, 7.2 to 8.7, and 8.9 to 9.9 h, respectively. Differences in AUCs were also observed among the animal species tested. AUCs increased in mice (46.1 to 51.7 $\mu\text{g} \cdot \text{h}/\text{ml}$), rats (72.6 to 76.9 $\mu\text{g} \cdot \text{h}/\text{ml}$), rabbits (115 to 139 $\mu\text{g} \cdot \text{h}/\text{ml}$), and dogs (209 to 216 $\mu\text{g} \cdot \text{h}/\text{ml}$), in that order. Oral bioavailability, calculated by the ratio of the AUCs following p.o. and intravenous administrations, highly suggested almost complete absorption in each species.

DISCUSSION

With the increasing numbers of immunocompromised patients seen over the last decade, there have been concomitant increases in the incidence of opportunistic fungal infections (7). The search for systemically active antifungal agents is in progress, and two triazole agents, fluconazole and itraconazole, have been developed and used worldwide (21). Although the triazole antifungal agents, especially fluconazole, have shown few side effects coupled with good therapeutic activity, particularly against candidiasis and cryptococcosis, the clinical efficacy of fluconazole against aspergillosis is not always sufficient (6). Additionally, *C. albicans* strains resistant to fluconazole have also been isolated from AIDS patients (2, 15). Furthermore, it has been reported that infections caused by *C. glabrata* and *C. krusei*, which are intrinsically resistant to fluconazole, are increasing in patients treated with fluconazole (1, 12).

T-8581, a new water-soluble triazole antifungal agent, showed potent in vitro antifungal activity against various pathogenic fungi such as *Candida* spp., *C. neoformans*, and *A. fumigatus*. T-8581 had in vivo antifungal activity similar to that of fluconazole against systemic candidiasis in mice. T-8581 was also more efficacious than fluconazole against systemic aspergillosis in mice and rabbits. Troke and colleagues (22) showed that fluconazole has significantly more potent activity than ketoconazole against *A. fumigatus* in a murine model. The evaluation of fluconazole in a murine model of aspergillosis may be limited by the relatively rapid clearance of fluconazole in mice, because sustained levels are not readily achieved in the sera and tissues of mice (5). The $t_{1/2}$ s of fluconazole in serum are 22 h for humans, 8.9 h for rabbits, but only 4.8 h for mice after p.o. administration (14). The $t_{1/2}$ s of T-8581 in serum were 7.2 h for rabbits and 3.2 h for mice after p.o. administration.

Against systemic aspergillosis, T-8581 given p.o. at 20 mg/kg to rabbits and 50.5 mg/kg to mice was effective at prolonging the life span. The therapeutic dosage was less for rabbits compared with that for mice. Once-daily therapy was commonly used to treat systemic fungal infections in mice (9, 22). Once-daily administration of fluconazole as well as T-8581 was not sufficient for achieving optimal effects in mice because of its relatively short $t_{1/2}$ in mice. The lack of efficacy of itraconazole may be due to the vehicle (0.5% methylcellulose solution). When cyclodextrin was used as a carrier in mice, the concentrations of itraconazole were greatly enhanced, but the effect was negligible for fluconazole (13). Itraconazole appears to be much more active when cyclodextrin is used as a carrier. The pharmacokinetic profiles of imidazole antifungal agents such as miconazole and econazole were characterized by poor oral bioavailability and low concentrations in serum due to significant first-pass metabolism and a large volume of distribution (10, 11). T-8581 had a pharmacokinetic profile similar to that of fluconazole (14), unlike miconazole and econazole (10, 11). This pharmacokinetic profile undoubtedly contributed to the efficacy of T-8581 in vivo.

T-8581 is highly soluble. T-8581 given parenterally was efficacious ($\text{ED}_{50} = 59.2 \text{ mg}/\text{kg}$ [i.p.]) against systemic aspergillosis in mice, while the ED_{50} of fluconazole was $>20 \text{ mg}/\text{kg}$ (fluconazole has limited solubility in physiological saline solution). T-8581 given parenterally may be effective against aspergillosis and candidiasis caused by fluconazole-resistant strains. Maximum solubilities of T-8581 and fluconazole in water are 41.8 and 2.6 mg/ml, respectively, so the volume of a dose of T-8581 given parenterally is less than that for fluconazole. The high solubility of T-8581 may allow it to be used instead of fluconazole for high-dose therapy.

These results, coupled with its good pharmacokinetic properties, suggest that T-8581 may be an effective antifungal drug for oral and parenteral use and encourage us to perform further studies to assess its toxicity.

REFERENCES

- Akova, M., H. E. Akalin, O. Uzun, and D. Gur. 1991. Emergence of *Candida krusei* infection after therapy of oropharyngeal candidiasis with fluconazole. *Eur. J. Clin. Microbiol. Infect. Dis.* 10:598-599.
- Baily, G. G., F. M. Perry, D. W. Denning, and B. K. Mandal. 1994. Fluconazole-resistant candidosis in an HIV cohort. *AIDS* 8:787-792.
- Espinel-Ingroff, A., K. Dawson, M. Pfaller, E. Anaissie, B. Breslin, D. Dixon, A. Fothergill, V. Peatznick, J. Peter, M. Rinaldi, and T. Walsh. 1995. Comparative and collaborative evaluation of standardization of antifungal susceptibility testing for filamentous fungi. *Antimicrob. Agents Chemother.* 39:314-319.
- Fan-Havard, P., D. Capano, S. M. Smith, A. Mangia, and R. H. K. Eng. 1991. Development of resistance in *Candida* isolates from patients receiving prolonged antifungal therapy. *Antimicrob. Agents Chemother.* 35:2302-2305.
- Fromtling, R. A. 1988. Overview of medically important antifungal azole derivatives. *Clin. Microbiol. Rev.* 1:187-217.
- Graybill, J. R. 1989. New antifungal agents. *Eur. J. Clin. Microbiol. Infect. Dis.* 8:402-412.
- Hay, R. J. 1991. Overview of the treatment of disseminated fungal infections. *J. Antimicrob. Chemother.* 28(Suppl. B):17-25.
- Hay, R. J. 1991. Antifungal therapy and the new azole compounds. *J. Antimicrob. Chemother.* 28(Suppl. B):36-46.
- Hector, R. F., and E. Yee. 1990. Evaluation of Bay R3783 in rodent models of superficial and systemic candidiasis, meningeal cryptococcosis, and pulmonary aspergillosis. *Antimicrob. Agents Chemother.* 34:448-454.
- Heel, R. C., R. N. Brogden, G. E. Pakes, T. M. Speight, and G. S. Avery. 1980. Miconazole: a preliminary review of its therapeutic efficacy in systemic infections. *Drugs* 19:7-30.
- Heel, R. C., R. N. Brogden, T. M. Speight, and G. S. Avery. 1978. Econazole: a review of its antifungal activity and therapeutic efficacy. *Drugs* 16:177-201.
- Hitchcock, C. A., G. W. Pye, P. F. Troke, E. M. Tohson, and D. W. Warnock. 1993. Fluconazole resistance in *Candida glabrata*. *Antimicrob. Agents Chemother.* 37:1962-1965.
- Hostetler, J. S., L. H. Hanson, and D. A. Stevens. 1992. Effect of cyclodextrin

- on the pharmacology of antifungal oral azole. *Antimicrob. Agents Chemother.* **36**:477–480.
14. **Humphrey, M. J., S. Jevons, and M. H. Tarbit.** 1985. Pharmacokinetic evaluation of UK-49, 858, a metabolically stable triazole antifungal drug, in animals and humans. *Antimicrob. Agents Chemother.* **28**:648–653.
 15. **Law, D., C. B. Moore, H. M. Wardle, L. A. Ganguli, M. G. L. Keaney, and D. W. Denning.** 1994. High prevalence of antifungal resistance in *Candida* in AIDS. *J. Antimicrob. Chemother.* **34**:659–668.
 16. **Litchfield, J. T., and F. Wilcoxon.** 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **96**:99–113.
 17. **Lyman, C. A., and T. J. Walsh.** 1992. Systemically administered antifungal agents: a review of their clinical pharmacology and therapeutic applications. *Drugs* **44**:9–35.
 18. **National Committee for Clinical Laboratory Standards.** 1994. Reference method for broth dilution antifungal susceptibility testing for yeasts. Proposed standard. Document M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 19. **Patterson, T. F., P. Minitier, and V. T. Andriole.** 1990. Efficacy of fluconazole in experimental invasive aspergillosis. *Rev. Infect. Dis.* **12**(Suppl. 3):S281–S285.
 20. **Rex, J. H., M. A. Pfaller, A. L. Barry, P. W. Nelson, and C. D. Webb for the NIAID Mycoses Study Group and the Candidemia Study Group.** 1995. Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B as treatment of nonneutropenic patients with candidemia. *Antimicrob. Agents Chemother.* **39**:40–44.
 21. **Saag, M. S., and W. E. Dismukes.** 1988. Azole antifungal agents: emphasis on new triazole. *Antimicrob. Agents Chemother.* **32**:1–8.
 22. **Troke, P. F., R. J. Andrews, M. S. Marriott, and K. Richardson.** 1987. Efficacy of fluconazole (UK-49, 858) against experimental aspergillosis and cryptococcosis in mice. *J. Antimicrob. Chemother.* **19**:663–670.
 23. **Yotsuji, A., K. Shimizu, N. Nishida, R. Hori, N. Ishii, S. Yamamoto, Y. Watanabe, H. Imaizumi, and H. Narita.** 1995. T-8581, a new orally and parenterally active triazole antifungal: *in vitro* and *in vivo* evaluation, abstr. F82, p. 127. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.