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In Vitro and In Vivo Antifungal Activities of D0870, a New Triazole Agent

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In vitro and in vivo antifungal activities of D0870 were evaluated in comparison with those of fluconazole. D0870, which is the *R*-enantiomer of ICI195,739, was found to be the mycologically active enantiomer by comparing the activities of D0870 with those of M16355 (*S*-enantiomer of ICI195,739). D0870 showed a broad spectrum of antifungal activity and MICs and minimum antibiotic concentrations 4- to 2,000-fold lower in synthetic amino acid medium (fungal) agar than those of fluconazole for various fungi. Although MICs of D0870 were affected by variation of the test conditions, such as type of medium, inoculum size of fungi, supplementation with fetal bovine serum, and pH of medium, they were consistently much lower than those of fluconazole under any condition. In vivo activities of D0870 in the systemic infection models with *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* in normal mice and in the mice immunosuppressed with cyclophosphamide or cortisone acetate were 2- to 7-fold and 3- to 89-fold greater than those of fluconazole, respectively. In these infection models in immunosuppressed mice, the therapeutic efficacy of D0870 was almost equivalent to that in normal mice, whereas the efficacy of fluconazole was 2- to 50-fold lower than that in normal mice.

It has been reported that the recent rise in the number of patients with systemic fungal infections closely relates to therapy using immunosuppressive or anticancer agents (6, 7). These kinds of agents reportedly cause the destruction of the cellular or humoral host defense system, and that destruction inevitably raises the frequency of opportunistic infections caused by fungi such as *Candida*, *Aspergillus*, or *Cryptococcus* species. Under these circumstances, the need for potent systemic antifungal agents has been increasing recently. Six systemic antifungal agents, amphotericin B (AMPH), flucytosine, miconazole (MCZ), ketoconazole, fluconazole (FCZ), and itraconazole, have been developed so far for clinical use. However, the clinical values of these agents have been limited primarily by their relatively high risks of toxicity, emergence of drug resistance, pharmacokinetic deficiencies, and/or insufficiency of their antifungal activities (7, 8, 14). Thus, much effort still has been made to develop novel potent antifungal agents which are safe and systemically effective against various deep-seated mycoses. D0870 (Fig. 1) is the *R*-enantiomer of ICI195,739, which has excellent in vitro and in vivo antifungal activities (4, 16). In this study, we found that the potent activity of ICI195,739 is attributable to D0870 and evaluated in vitro and in vivo activities of D0870 in comparison with those of FCZ and other antifungal agents.

MATERIALS AND METHODS

Antifungal agents. D0870, M16355, and FCZ were synthesized at Fuji Central Research Laboratory, Mochida Pharmaceutical Co., Ltd., Shizuoka, Japan. MCZ was obtained from Janssen Pharmaceutica, Beerse, Belgium. AMPH was purchased from Squibb Japan Inc., Tokyo, Japan.

Fungi. Twenty-nine fungal isolates were used for in vitro and in vivo studies. Four strains of *Candida albicans* (IFO 0579, IFO 1060, IFO 1269, and IFO 1594) and *Penicillium notatum* IFO 4640 were obtained from the Institute of Fermentation, Osaka, Japan. *C. albicans* IFM 40009 and *Aspergillus fumigatus* IFM 4942 were kindly provided by Kazuko Nishimura, Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan. The other strains were kindly provided by Hideyo Yamaguchi, Research Center for Medical Mycology, Teikyo University School of Medicine, Tokyo, Japan. Isolates were stored at −196°C in Sabouraud dextrose broth containing 5 or 10% dimethyl sulfoxide.

In vitro antifungal activity. MICs and minimum antibiotic concentrations (MACs) were determined by the twofold agar dilution method with synthetic amino acid medium (fungal) (SAAMF; Nippon Bio-Supply Center Co., Ltd., Tokyo, Japan) agar which was constituted with morpholinepropanesulfonic acid (MOPS), Tris, and sodium phosphate buffers adjusted to pH 7.4 (9). Yeasts were cultured overnight at 30°C in Sabouraud dextrose broth (Difco Laboratories, Detroit, Mich.) and diluted to a final concentration of 10^5 cells per ml with sterilized saline. Filamentous fungi were maintained at 30°C on Sabouraud dextrose agar (SDA; Eiken Chemical Co., Ltd., Tokyo, Japan) for 2 to 3 weeks, and inocula (10^5 conidia per ml), which were diluted with sterilized saline containing 0.05% Tween 80, were prepared from the cultures. Five microliters of the diluted broth culture was inoculated onto the agar plates containing drugs with a Microplanter (Sakuma Seisakusho, Ltd., Tokyo, Japan), and fungal growth was observed 24 to 120 h after incubation at 30°C. The MIC was determined as the lowest drug concentration which prevented visible fungal growth. The MAC was determined as the lowest drug concentration which showed the partial inhibition of fungal growth compared with the control fungal growth (2, 13).

Effects of various culture conditions on MICs. The influences of culture medium, variation in inoculum size of fungi from 10^4 to 10^7 cells or conidia per ml, supplementation with

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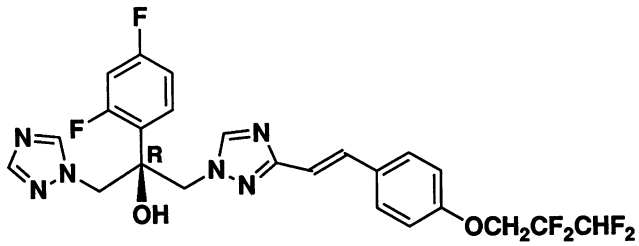

FIG. 1. Structure of D0870.

fetal bovine serum (FBS; Flow Laboratories, Inc., Irvine, Scotland) at a concentration of 20% (vol/vol), and pH of medium on MICs were examined by the twofold agar dilution method with two strains each of *C. albicans*, *Cryptococcus neoformans*, and *A. fumigatus* at an inoculum size of 10^5 cells or conidia per ml, except in the experiment with the changing inoculum sizes of fungi. Incubation times were 24 and 48 h for *C. albicans*, 48 h for *C. neoformans*, and 72 h for *A. fumigatus*, respectively. The mean MIC was determined as a geometric mean of MICs for the two strains except in the case that either MIC for the two could not be determined. Brain heart infusion agar (BHI), Casitone complex agar (CC), yeast morphology agar (YMA), and SDA were used for examining the effect of test media on MICs. Except in this experiment, SAAMF agar was used as a test medium, and the pH of the medium was adjusted with 10 N sodium hydroxide or 12 N hydrochloric acid.

In vivo antifungal activity. (i) Systemic fungal infections in normal mice. Male ICR strain mice (Japan SLC, Inc., Shizuoka, Japan), each weighing 18 to 20 g, were used for the experimental systemic infections in normal mice. Each fungal cell or conidial suspension was inoculated intravenously into each of 10 mice per group at inoculum sizes shown in Table 5. Appropriate doses of antifungal agents in 0.2 ml of 0.5% Tween 80 were administered orally 1 h postinfection or once a day for 5 days starting 1 h postinfection. The 50% effective doses (ED_{50}s) were calculated by the Litchfield-Wilcoxon method (12) from the number of mice surviving on days 7, 14, 21, and 28 after infection.

(ii) Systemic infections in immunosuppressed mice. Systemic infections in the mice immunosuppressed with cyclophosphamide (CPA) (ENDOXAN; Shionogi & Co., Ltd., Osaka, Japan) were produced as follows. CPA at a dose of 100 mg/kg of body weight was intraperitoneally administered to mice 4 days or 0 and 4 days before infection. The methods of infection, administration of drugs, and evaluation of ED_{50}s were the same as described above except for the challenge dose of each fungus. Systemic infections in the mice immunosuppressed with cortisone acetate (CA; Sigma Chemical Company, St. Louis, Mo.) were produced by a method basically identical to that for CPA except that CA at a dose of 4 mg per mouse was subcutaneously administered to mice 2 days before infection. The challenge dose of each fungus is shown in Table 5.

RESULTS

In vitro and in vivo antifungal activities of D0870 compared with those of M16355. MICs for 50% of strains tested (MIC_{50}s) of D0870 and M16355 for 10 strains of *C. albicans* at the incubation time of 48 h were 6.25 and 6.25 μg/ml, respectively. Thus, both drugs showed almost the same MICs against *C. albicans*. However, the MAC for 50% of strains tested (MAC_{50}) of D0870 under the same culture conditions was 0.0015 μg/ml and about 500-fold lower than the MAC_{50} (0.78 μg/ml) of M16355. Against systemic candidiasis in mice, a single oral dose of D0870 showed excellent therapeutic efficacy with ED_{50}s of <1.0 and 2.6 mg/kg on days 7 and 14, respectively, whereas M16355 at doses of up to 30 mg/kg was not active against the infection at all. Furthermore, MICs of D0870 determined in SDA were 4- to

TABLE 1. In vitro antifungal activities of D0870, FCZ, MCZ, and AMPH against yeasts in SAAMF agar

Organism	Incubation time (h)	Concn (μg/ml) of drug:							
		D0870		FCZ		MCZ		AMPH	
		MIC	MAC	MIC	MAC	MIC	MAC	MIC	MAC
C. albicans IFO 1269	24	0.05	0.0015	25	0.20	0.10	0.012	1.56	0.39
	48	3.13	0.0015	>100	0.39	3.13	0.012	3.13	0.78
C. albicans IFO 1594	24	0.024	0.0015	3.13	0.20	0.05	0.012	1.56	0.39
	48	0.39	0.003	>100	0.39	0.39	0.024	6.25	0.78
C. albicans IFM 40009	24	6.25	0.0015	>100	0.20	1.56	0.012	1.56	0.39
	48	12.5	0.006	>100	0.20	3.13	0.024	6.25	0.78
C. krusei TIMM 0269	24	0.39	0.05	50	12.5	0.78	0.05	12.5	3.13
	48	1.56	0.39	100	50	6.25	0.78	12.5	12.5
C. tropicalis TIMM 0313	24	0.024	0.003	6.25	0.78	3.13	0.012	3.13	3.13
	48	0.10	0.012	25	0.78	3.13	0.024	12.5	3.13
C. glabrata TIMM 1064	24	0.10	0.003	12.5	3.13	0.024	0.0015	3.13	3.13
	48	0.39	0.10	25	3.13	0.20	0.024	6.25	3.13
C. parapsilosis TIMM 0292	24	0.012	0.0015	3.13	1.56	0.39	0.024	3.13	3.13
	48	0.05	0.012	12.5	3.13	3.13	0.10	12.5	3.13
C. guilliermondii TIMM 0260	24	0.012	0.003	6.25	3.13	0.10	0.012	3.13	1.56
	48	0.024	0.012	6.25	3.13	0.78	0.10	6.25	3.13
C. pseudotropicalis TIMM 0302	24	0.003	0.0015	0.20	0.10	≦0.0002	≦0.0002	3.13	3.13
	48	0.006	0.0015	0.39	0.20	0.003	≦0.0002	12.5	3.13
C. stellatoidea TIMM 0310	48	0.0008	0.0008	0.39	0.20	≦0.0002	≦0.0002	3.13	1.56
C. neoformans TIMM 0354	48	0.39	0.10	50	6.25	0.78	0.05	3.13	1.56
C. neoformans TIMM 0362	48	0.39	0.10	50	6.25	0.78	0.05	3.13	1.56
T. cutaneum TIMM 1286	48	0.024	0.003	0.78	0.39	0.024	0.012	3.13	1.56

TABLE 2. In vitro antifungal activities of D0870, FCZ, MCZ, and AMPH against filamentous fungi in SAAMF agar

Organism	Incubation time (h)	Concn (μg/ml) of drug:							
		D0870		FCZ		MCZ		AMPH	
		MIC	MAC	MIC	MAC	MIC	MAC	MIC	MAC
A. fumigatus TIMM 0063	72	1.56	0.024	>100	0.78	1.56	0.024	12.5	1.56
A. fumigatus IFM 4942	72	1.56	0.024	>100	0.78	0.78	0.024	12.5	3.13
M. gypseum TIMM 0776	72	0.20	0.0015	50	0.78	0.39	0.0015	12.5	0.78
M. canis TIMM 0760	72	0.78	0.012	100	25	0.78	0.20	6.25	6.25
T. mentagrophytes TIMM 1188	72	0.39	0.10	50	12.5	0.39	0.05	6.25	6.25
T. mentagrophytes TIMM 1189	96	0.20	0.05	50	6.25	0.20	0.024	6.25	6.25
T. rubrum TIMM 1216	120	0.012	0.0008	1.56	0.39	0.006	0.0008	6.25	0.78
Phialophora verrucosa TIMM 0907	120	0.78	0.024	12.5	1.56	0.05	0.024	6.25	6.25
P. notatum IFO 4640	72	12.5	0.012	>100	12.5	0.78	0.003	50	3.13

over 1,000-fold lower than those of M16355 for other yeasts, such as *Candida krusei*, *Candida glabrata*, and *C. neoformans*, and filamentous fungi such as *A. fumigatus*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. Thus, in vitro and in vivo activities of D0870 were much greater than those of M16355.

In vitro antifungal activity of D0870 compared with those of FCZ, MCZ, and AMPH. Tables 1 and 2 summarize MICs and MACs of D0870 in comparison with those of other drugs for various pathogenic fungi. The data for a representative 3 of 10 strains of *C. albicans* are shown in Table 1. MICs of D0870 for 10 strains of *C. albicans* at the incubation time of 48 h ranged from 0.39 to 12.5 μg/ml (MIC_{50} = 6.25 μg/ml) and were much lower than those of FCZ (MIC_{50} = >100 μg/ml) and almost equal to those of MCZ (MIC_{50} = 3.13 μg/ml) and AMPH (MIC_{50} = 3.13 μg/ml). For the same strains and at the same incubation time, D0870 showed potent MACs, ranging from 0.0008 to 0.006 μg/ml (MAC_{50} = 0.0015 μg/ml), which were over 100-fold lower than those of FCZ (MAC_{50} = 0.2 μg/ml) and AMPH (MAC_{50} = 0.78 μg/ml) and about 8-fold lower than those of MCZ (MAC_{50} = 0.012 μg/ml). For two strains of *C. neoformans*, D0870 showed an excellent mean MIC of 0.39 μg/ml and a MAC of 0.1 μg/ml, which were much lower than those of FCZ and AMPH and almost equal to those of MCZ. MICs and MACs of D0870 against the yeasts other than *C. albicans* and *C. neoformans*, such as *C. krusei*, *C. glabrata*, *Candida pseudotropicalis*, *Candida stellatoidea*, and *Trichosporon cutaneum*, were comparable to those of MCZ and much lower than those of FCZ and AMPH. Furthermore, MICs and MACs of D0870 were much lower than those of FCZ, MCZ, and AMPH for *Candida tropicalis*, *Candida parapsilosis*, and *Candida guilliermondii* (Table 1). For two strains of *A. fumigatus*, D0870 also showed an excellent mean MIC of 1.56 μg/ml and a MAC of 0.024 μg/ml, which were much lower than those of FCZ and AMPH and almost equal to those of MCZ. MICs and MACs of D0870 for filamentous fungi other than *A. fumigatus*, such as *Microsporum gypseum*, *Microsporum canis*, two strains of *T. mentagrophytes*, and *T. rubrum*, were also equivalent to those of MCZ and much lower than those of FCZ and AMPH (Table 2).

Effects of various culture conditions on MICs of D0870. Table 3 summarizes the influence of test media on MICs of D0870 and other drugs. MICs of D0870 determined in BHI, CC, YMA, and SDA against two strains each of *C. albicans*, *C. neoformans*, and *A. fumigatus* were almost the same as those determined in SAAMF agar, and they were also much lower than those of FCZ.

When the inoculum size of each fungus was varied from 10^4 to 10^7 cells or conidia per ml, the mean MIC of D0870 for two strains of each fungus increased from 1.57 to >100 μg/ml for *C. albicans* at the incubation time of 24 h, from 0.39 to >100 μg/ml for *C. neoformans*, and from 2.35 to 6.25 μg/ml for *A. fumigatus*, as did the MICs of FCZ and MCZ, which changed from 200 to >800 and 0.20 to >100 μg/ml (MIC for one strain) for *C. albicans*, from 37.5 to >800 and 0.20 to 100 μg/ml for *C. neoformans*, and from 400 to 800 (MIC for one strain) and 0.78 to 3.13 μg/ml for *A. fumigatus*, respectively. On the other hand, the mean MICs of AMPH changed from 1.56 to 2.35 μg/ml for *C. albicans*, 3.13 to 4.69 μg/ml for *C. neoformans*, and 7.82 to 25 μg/ml for *A. fumigatus* and were less affected, especially for the yeasts, by increases in the inoculum size of fungi.

By the addition of FBS to SAAMF agar at 20%, the mean

TABLE 3. Effect of culture medium on the antifungal activities of D0870, FCZ, MCZ, and AMPH

Organism	Incubation time (h)	MIC (μg/ml)															
		D0870				FCZ				MCZ				AMPH			
		BHI	CC	YMA	SDA	BHI	CC	YMA	SDA	BHI	CC	YMA	SDA	BHI	CC	YMA	SDA
C. albicans IFO 1269	24	1.56	0.78	1.56	6.25	>100	>100	>100	>100	0.78	0.78	0.78	1.56	0.20	NT[a]	NT	0.20
	48	12.5	3.13	6.25	6.25	>100	>100	>100	>100	3.13	1.56	6.25	1.56	0.39	NT	NT	0.39
C. albicans IFM 40009	24	3.13	1.56	1.56	6.25	>100	>100	>100	>100	1.56	1.56	1.56	3.13	0.20	NT	NT	0.78
	48	12.5	6.25	12.5	12.5	>100	>100	>100	>100	6.25	3.13	12.5	3.13	0.39	NT	NT	1.56
C. neoformans TIMM 0354	48	0.39	0.39	0.78	0.78	50	50	50	100	0.39	0.20	0.39	0.20	0.39	NT	NT	0.78
C. neoformans TIMM 0362	48	0.39	0.39	0.39	0.39	25	25	50	50	0.39	0.20	0.39	0.10	0.20	NT	NT	0.39
A. fumigatus TIMM 0063	72	3.13	3.13	12.5	6.25	>100	>100	>100	>100	1.56	3.13	3.13	1.56	0.78	NT	NT	3.13
A. fumigatus IFM 4942	72	3.13	3.13	12.5	6.25	>100	>100	>100	>100	1.56	3.13	6.25	1.56	1.56	NT	NT	3.13

[a] NT, not tested.

TABLE 4. Effect of pH of SAAMF agar on the antifungal activities of D0870, FCZ, MCZ, and AMPH

Organism	Incubation time (h)	MIC (μg/ml)											
		D0870			FCZ			MCZ			AMPH		
		pH 3	pH 5	pH 7.4	pH 3	pH 5	pH 7.4	pH 3	pH 5	pH 7.4	pH 3	pH 5	pH 7.4
C. albicans IFO 1269	24	0.024	3.13	0.10	25	>800	25	3.13	12.5	0.20	1.56	1.56	6.25
	48	0.39	12.5	6.25	200	>800	>800	6.25	12.5	1.56	1.56	1.56	6.25
C. albicans IFM 40009	24	0.39	6.25	6.25	400	>800	>800	3.13	12.5	1.56	1.56	1.56	6.25
	48	12.5	25	12.5	400	>800	>800	6.25	12.5	3.13	3.13	3.13	6.25
C. neoformans TIMM 0354	48	0.39	0.39	0.78	50	50	50	6.25	0.39	0.78	3.13	1.56	6.25
C. neoformans TIMM 0362	48	0.20	0.39	0.78	25	50	50	3.13	0.39	0.78	1.56	1.56	6.25
A. fumigatus TIMM 0063	72	1.56	6.25	6.25	800	800	800	6.25	3.13	1.56	3.13	12.5	6.25
A. fumigatus IFM 4942	72	0.78	6.25	3.13	400	>800	400	3.13	3.13	0.78	6.25	50	12.5

MIC of D0870 for two strains of each fungus increased from 1.59 to 14.1 μg/ml for *C. albicans* at the incubation time of 24 h and was less affected for *C. neoformans* (from 0.39 to 0.78 μg/ml) and *A. fumigatus* (from 4.69 to 9.38 μg/ml). These phenomena were also observed for FCZ, whose mean MIC changed from 12.5 to 50 μg/ml (MIC for one strain) for *C. albicans*, from 50 to 37.5 μg/ml for *C. neoformans*, and from 600 to 300 μg/ml for *A. fumigatus*. In the case of MCZ, the mean MIC increased from 0.44 to 31.3 μg/ml for *C. albicans*, from 0.39 to 3.13 μg/ml for *C. neoformans*, and from 0.78 to 12.5 μg/ml for *A. fumigatus*. On the other hand, the mean MIC of AMPH for *C. albicans* decreased slightly, from 3.13 to 0.78 μg/ml, and was less affected for *C. neoformans* (from 3.13 to 1.56 μg/ml) and *A. fumigatus* (from 15.6 to 9.38 μg/ml) by the addition of FBS.

By reduction of the pH of SAAMF agar from 7.4 to 3.0, MICs of D0870 for the same six strains of fungi became lower, as did those of AMPH, whereas MICs of FCZ were hardly affected and those of MCZ became higher concomitantly with the reduction of the pH of the medium (Table 4).

In vivo antifungal activity of D0870. Table 5 summarizes the therapeutic effects of oral D0870 on experimental systemic infections in normal mice and in the mice immunosuppressed with CPA or CA in comparison with those of FCZ or AMPH. D0870 was active against systemic candidiasis in normal mice with ED_{50}s of 0.6 and 3.4 mg/kg on days 7 and 14, respectively, which were about two- to sevenfold more potent than the 1.5 and 24.5 mg of FCZ per kg. On the other hand, against systemic candidiasis in the mice immunosuppressed by one injection of CPA (100 mg/kg), D0870 also showed excellent efficacy with ED_{50}s of 1.1 and 3.9 mg/kg on days 7 and 14, respectively, and ED_{50}s of D0870 were up to 26-fold more potent than the 9.2 and >100 mg of FCZ per kg. In the case that CPA was injected twice into mice, ED_{50}s of D0870 on days 7 and 14 were 0.9 and 2.0 mg/kg, respectively, and were up to 89-fold lower than the ED_{50}s of FCZ. Furthermore, for the mice immunosuppressed by an injection of 4 mg of CA per mouse, ED_{50}s of D0870 on day 7 were 1.5 mg/kg (one treatment) and <1.0 mg/kg (five treatments) and were over 30-fold lower than the ED_{50}s of FCZ. Thus, D0870 showed almost the same in vivo activity in both normal and immunosuppressed mice, whereas FCZ was much less active in immunosuppressed mice than in normal mice.

Similarly, in the systemic infections with *C. neoformans* TIMM 0362, the difference in efficacy between D0870 and FCZ was prominent in immunosuppressed mice rather than in normal mice. Namely, D0870 was up to 4.6-fold better than FCZ in normal mice, whereas D0870 was up to 9.5-fold more potent than FCZ in the mice immunosuppressed with CPA and 18.1-fold more potent than FCZ in those immunosuppressed with CA. Compared with those of AMPH in the mice immunosuppressed with CPA, ED_{50}s of D0870 on days 14, 21, and 28 were 1.7, 5.5, and 18.9 mg/kg, respectively, and higher than ED_{50}s of AMPH, which were 1.0, 1.9, and 2.8 mg/kg on the respective days.

In the case of the systemic infections with *A. fumigatus* IFM 4942, ED_{50}s of D0870 in normal mice on days 7, 14, 21, and 28 ranged from 20.9 to 50.6 mg/kg, and they were relatively higher than those for *C. albicans* and *C. neoformans*. However, compared with FCZ, whose ED_{50}s ranged from 76.7 to >100 mg/kg, D0870 was still more potent, though it did not exceed AMPH, whose ED_{50}s ranged from <1.0 to 10.2 mg/kg. On the other hand, ED_{50}s of D0870 in the mice immunosuppressed with CPA, which were also lower than those of FCZ, ranged from 12.2 to 41.8 mg/kg, and D0870 showed almost the same efficacy as that observed for the infection in normal mice, whereas AMPH was about 10-fold less active against the infection in immunosuppressed mice than against that in normal mice. With the mice immunosuppressed with CA, ED_{50}s of D0870 on days 7 to 28 ranged from 42.5 to 80.3 mg/kg, and D0870 was still effective, whereas FCZ was not active at all.

Thus, D0870 was much more potent than FCZ against any kind of infection, and the difference in efficacy between the two drugs was prominent especially in the infections in immunosuppressed mice.

DISCUSSION

The searches for novel, systemically active antifungal agents are now in progress all over the world, and two triazole agents, FCZ and itraconazole, have been developed in the last decade. Although the triazole antifungal agents, especially FCZ, can attain low incidences of side effects coupled with good therapeutic responses particularly in nonimmunocompromised patients, the clinical efficacy of those drugs is not always sufficient in immunocompromised patients, especially in those with persistent neutropenia (18). Additionally, strains resistant to FCZ have also been isolated (5, 11, 20) in cases of disseminated *C. albicans* infection in AIDS patients. Furthermore, it has been reported that the infections caused by other *Candida* species such as *C. glabrata* and *C. krusei*, which are resistant to FCZ, are increasing in the patients treated with FCZ (1, 3, 15, 21). Under these circumstances, more potent antifungal agents, which are safe and active against such resistant strains, are desired urgently. According to the comparative study of D0870 and M16355, which are *R*- and *S*-enantiomers of ICI195,739, respectively, we have reached the conclusion

TABLE 5. In vivo antifungal activities of D0870, FCZ, and AMPH in normal and immunosuppressed mice

Organism	Challenge dose (cells/mouse)	Immunosuppressive agent (dose)	No. of doses	Time (days)	ED_{50} (mg/kg) of drug:			Excess of ED_{50} of FCZ over ED_{50} of D0870 (fold)
					D0870	FCZ	AMPH	
C. albicans IFM 40009	5×10^6	None	1	7	0.6	1.5	NT[a]	2.5
				14	3.4	24.5	NT	7.2
				21	>100	>100	NT	ND[b]
C. albicans IFM 40009	1×10^6	CPA (100 mg/kg, 1×)	1	7	1.1	9.2	NT	8.4
				14	3.9	>100	NT	>25.6
				21	16.8	>100	NT	>6.0
				28	46.2	>100	NT	>2.2
C. albicans IFM 40009	1×10^6	CPA (100 mg/kg, 2×)	1	7	0.9	80.3	NT	89.2
				14	2.0	100	NT	50.0
				21	11.0	100	NT	9.1
				28	>100	>100	NT	ND
C. albicans IFM 40009	1×10^6	CA (4 mg/mouse, 1×)	1	7	1.5	69.7	NT	46.5
				14	>30	>100	NT	ND
C. albicans IFM 40009	1×10^6	CA (4 mg/mouse, 1×)	5	7	<1.0	>30	NT	>30
				14	2.2	>30	NT	>13.6
				21	>30	>30	NT	ND
C. neoformans TIMM 0362	1×10^7	None	5	14	3.7	17.0	NT	4.6
				21	12.2	>30	NT	>2.5
				28	27.0	>30	NT	>1.1
C. neoformans TIMM 0362	3×10^6	CPA (100 mg/kg, 2×)	5	14	1.7	16.1	1.0	9.5
				21	5.5	50.9	1.9	9.3
				28	18.9	65.3	2.8	3.5
C. neoformans TIMM 0362	3×10^6	CA (4 mg/mouse, 1×)	5	14	1.8	32.6	NT	18.1
				21	10.0	>100	NT	>10.0
				28	20.8	>100	NT	>4.8
A. fumigatus IFM 4942	2×10^7	None	5	7	20.9	76.7	<1.0	3.7
				14	28.1	>100	1.1	>3.6
				21	44.7	>100	2.9	>2.2
				28	50.6	>100	10.2	>2.0
A. fumigatus IFM 4942	3×10^6	CPA (100 mg/kg, 2×)	5	7	12.2	61.2	13.5	5.0
				14	32.1	84.3	13.5	2.6
				21	37.4	>100	19.4	>2.7
				28	41.8	>100	19.4	>2.4
A. fumigatus IFM 4942	5×10^6	CA (4 mg/mouse, 1×)	5	7	42.5	>100	NT	>2.4
				14	47.7	>100	NT	>2.1
				21	63.5	>100	NT	>1.6
				28	80.3	>100	NT	>1.2

[a] NT, not tested.
[b] ND, not determined.

that D0870, the *R*-enantiomer, could be a candidate for treatment of deep-seated mycoses and have evaluated the in vitro and in vivo antifungal activities of D0870 in comparison with those of FCZ and other antifungal agents.

Comparing MICs between D0870 and M16355, we found that D0870 was much more active than M16355 against various fungi, except for *C. albicans*. For *C. albicans*, although D0870 showed MICs almost equivalent to those of M16355, MACs were found to be remarkably lower than those, and in vivo activity of D0870 was found to be remarkably superior to that of M16355. Thus, D0870 was confirmed to be the mycologically active compound of ICI195,739. Since the MAC was thought to be a relevant index as shown in the above-mentioned comparison study, we evaluated in vitro antifungal activity of D0870 precisely in comparison with those of FCZ, MCZ, and AMPH with both the MIC and the MAC. Although triazole agents are generally known to have poor activities in vitro (17), D0870 was found to be active against various fungi including *C. albicans*, *C. krusei*, *C. glabrata*, *C. neoformans*, and *A. fumigatus* and showed potent in vitro activity in various media, such as BHI, CC, YMA, and SDA, as well as in SAAMF agar, which was equivalent to that of MCZ and better than that of FCZ. The in vitro activity of D0870 was affected by increase of inoculum size of fungi and by addition of FBS to the culture medium, and the phenomenon was commonly observed for other azole compounds such as FCZ and MCZ, whereas the in vitro activity of AMPH was less affected. The difference between azole compounds and AMPH shown here may be attributable to their differences in antifungal mechanism. In contrast, by reduction of the pH of the culture medium, the MICs of D0870 became lower, as did those of AMPH, whereas the change in the MICs of FCZ was not obvious and the MICs of MCZ were contrastingly higher. Since the bacterial and fungal infection sites, especially the intraphagosomal environment of macrophages (10, 19), are thought to be rather acidic, the characteristic of D0870 that the antifungal activity becomes stronger under acidic conditions is expected to work effectively in vivo in terms of eradicating fungi.

As well as in vitro activity, D0870 was found to have a good in vivo characteristic. In the infections in immunosuppressed mice as well as in normal mice, D0870 showed almost the same therapeutic efficacy, whereas that of FCZ was remarkably attenuated in the infections in immunosuppressed mice. Although the reasons why D0870 was still

effective in the infections in immunosuppressed mice are not fully understood yet, the good in vivo activity of D0870 may be partly attributable to its good in vitro activity. Anyhow, considering that fungal infections occur predominantly in immunocompromised patients, D0870 is expected to be clinically more effective than FCZ.

Thus, we found that the antifungal activity of D0870 was much superior to that of FCZ in both in vitro and in vivo studies. These results strongly suggest that D0870 has considerable potential for the treatment of deep-seated mycoses in immunocompromised hosts and may be a valuable alternative to currently available antifungal agents.

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