

Efficacy of D0870 Compared with Those of Itraconazole and Amphotericin B in Two Murine Models of Invasive Aspergillosis

DAVID W. DENNING,^{1,2*} LINDA HALL,¹ MICHAEL JACKSON,¹ AND SALLY HOLLIS¹

*Department of Medicine, Section of Infectious Diseases, University of Manchester, Hope Hospital, Salford,¹
and Department of Infectious Disease & Tropical Medicine, North Manchester General
Hospital, Delaunays Road, Manchester M8 6RL,² United Kingdom*

Received 27 October 1994/Returned for modification 22 December 1994/Accepted 24 April 1995

D0870 is a novel azole antifungal compound. It was compared with conventional amphotericin B and itraconazole therapy in two murine models of invasive aspergillosis, one a systemic nonimmunocompromised mouse model and the other a temporarily neutropenic mouse respiratory model. D0870 was given orally and achieved measurable concentrations in serum approximately proportional to the daily dose with accumulation over time if it was given twice daily. Amphotericin B at 3.3 mg/kg of body weight was given intraperitoneally for four to six doses, and itraconazole was given orally in a cyclodextrin suspension at 5 to 50 mg/kg daily or twice daily (BID). The duration of therapy varied from 7 to 14 days. In the nonimmunocompromised mouse model, D0870 at 25 mg/kg BID was slightly inferior to amphotericin B and itraconazole with regard to mortality, with a median survival of 20 days for the three groups ($P = 0.03$ compared with amphotericin B). However, D0870 at 25 mg/kg BID was inferior to amphotericin B (but not itraconazole) with respect to renal culture ($P = 0.01$) and brain culture ($P = 0.0001$) results. Only amphotericin B was statistically superior to controls with regard to mortality. In the neutropenic mouse respiratory model, D0870 at 50 mg/kg/day was superior to amphotericin B, itraconazole, and controls with regard to mortality. D0870 at both 25 and 50 mg/kg/day was statistically superior to controls with regard to lung culture results ($P = 0.004$ to 0.04). A second experiment with a higher inoculum showed that no drug regimen was effective in that model. In all models low doses and concentrations of D0870 in serum were ineffective. D0870 has some efficacy for the treatment of invasive aspergillosis when it is given at modest doses.

Invasive aspergillosis represents a major threat to life, despite currently available therapy. Amphotericin B has been the standard therapy for many years, but the overall response rates are under 50% (4). In addition, amphotericin B is toxic, which has led to the development of alternative modes of administration, including lipid-associated preparations (8). The therapeutic ratios of these compounds for invasive aspergillosis may be superior, but clinical data do not suggest major improvements in efficacy. Itraconazole is the only licensed azole compound with useful clinical activity against invasive aspergillosis (4, 6). Results with this compound have also been mixed, particularly in patients in whom low concentrations are achieved in serum. There is therefore a substantial need for new compounds with anti-*Aspergillus* activity.

In two murine models of invasive aspergillosis in a total of five experiments, we compared amphotericin B and itraconazole with the new azole D0870.

MATERIALS AND METHODS

Both isolates used for the studies were typical *A. fumigatus* isolates from clinical sources. For the nonimmunocompromised mouse model, strain AF10 (5) was used, and for the neutropenic mouse respiratory model, strain AF15 was used. MICs and minimum fungicidal concentrations of all three drugs were determined in parallel by previously described methods (9). For isolate AF10, MICs and minimum fungicidal concentrations of D0870, itraconazole, and amphotericin B were 16, 2, and 2 $\mu\text{g/ml}$, respectively. For isolate AF15 MICs were 8, 2, and 0.5 $\mu\text{g/ml}$ (9) and minimum fungicidal concentrations were 8, 8, and 1 $\mu\text{g/ml}$ for D0870, itraconazole, and amphotericin B, respectively.

The inocula were prepared by culturing the organisms on potato glucose agar for 10 days at 35°C. Conidia were collected in sterile 0.9% phosphate-buffered saline containing 0.01% Tween 80 (PBS-Tween) and were stored at 4°C. The viability of the conidial suspension was determined by serial dilutions in PBS-Tween, and the suspension was subcultured onto horse blood agar plates. The inoculum was stored at 4°C until the day of infection (always a Tuesday for the nonimmunocompromised mouse model and a Monday for the respiratory model). Further adjustment of the inoculum was made, if necessary, just prior to infection of the mice.

Drugs. Amphotericin B, as a colloidal suspension in deoxycholate (Fungizone; Squibb), was given in 5% glucose intraperitoneally. The dose given to each mouse was 3.3 mg/kg of body weight on the basis of the mean weight of the mice 4 days before infection. Itraconazole was suspended in hydroxy- β -cyclodextrin, and D0870 was sonicated in Tween 80 (0.5%) with H₂O to produce a suspension. Both were given by gavage. D0870 doses were 5 mg/kg once daily and twice daily (BID) and 25 mg/kg BID in the first nonimmunocompromised mouse model and 10 mg/kg daily in the second and third experiments in the nonimmunocompromised mouse model. Itraconazole was given at the same doses as D0870 in experiment 1 and at 10 mg/kg BID in experiments 2 and 3. For the respiratory models (experiments 4 and 5), itraconazole was given at 50 mg/kg BID and D0870 was given at four doses from 10 to 100 mg/kg once daily. Controls were given 5% glucose intraperitoneally or by gavage.

Mice. Virus-free, female CD-1 mice (age, 5 weeks) were purchased from Charles River UK Ltd. In the nonimmunocompromised mouse model, only mice that were 5 to 6 weeks of age and that weighed 21 to 24.9 g 4 days prior to the experiment were used. For the respiratory model, mice of 4 to 5 weeks of age weighing 18 to 20 g were used. Ten mice per experimental group were used in the experiments. Mice were reassorted after weighing. Mice were housed together in cages and were allowed food and water *ad libitum*.

Immunosuppression. No immunosuppressants were given to those mice used in the nonimmunocompromised mouse model. In the pulmonary model, mice received cyclophosphamide at 200 mg/kg administered intravenously 3 days prior to challenge. A small group of uninfected mice were killed, then blood was collected in EDTA tubes, and leukocyte counts were determined with a Coulter Counter (and neutrophil counts were determined by manual counting with a hemocytometer) on several days after cyclophosphamide administration.

Mouse infection. In the nonimmunocompromised mouse model, the mice were infected by injection of a 0.15-ml inoculum into the tail vein. In the first experiment the inoculum was 6.8×10^7 conidia per ml, and in the second and

* Corresponding author. Mailing address: North Manchester General Hospital, Delaunays Road, Manchester M8 6RL, United Kingdom. Fax: 44 (161)-787-7432.

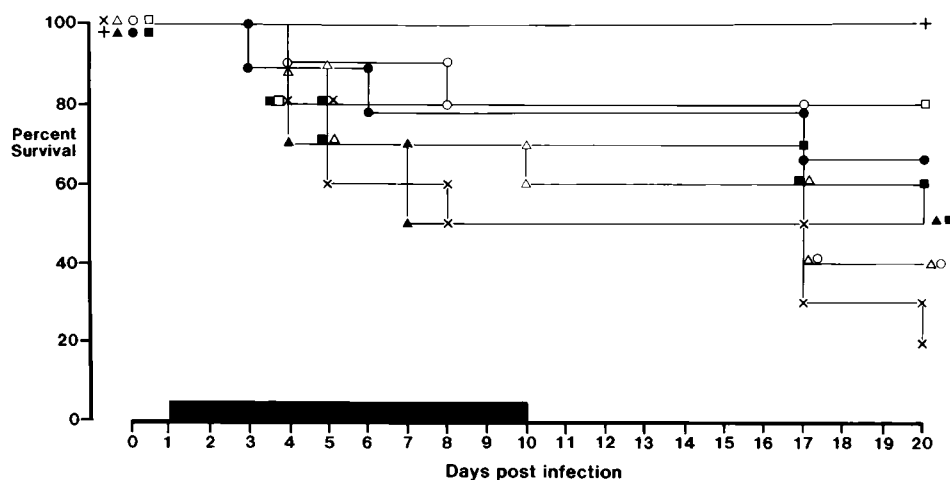


FIG. 1. Cumulative mortality against time in nonimmunocompromised mouse model (experiment 1; isolate AF10). +, amphotericin B at 3.3 mg/kg; □, itraconazole at 25 mg/kg BID; △, itraconazole at 5 mg/kg BID; ○, itraconazole at 5 mg/kg/day; ■, D0870 at 25 mg/kg BID; ▲, D0870 at 5 mg/kg BID; ●, D0870 at 5 mg/kg/day; ×, 5% glucose control.

third experiments it was 5.6×10^7 conidia per ml, as determined by viability counts.

In the respiratory model, mice were anaesthetized with ketamine given intramuscularly (0.07 ml per mouse, 4 mg/kg). They were kept warm on a heated table, and oxygen was administered to overcome any respiratory distress. The inoculum (30 μ l) was administered as a droplet on the nose with a Gilson pipetman. In experiment 4, the inoculum was 2.9×10^8 /ml, and in experiment 5 it was 5×10^8 /ml, as determined by viability counts.

Treatment. Cages were randomly assorted after infection and treatment groups were assigned by cage number. In the nonimmunocompromised mouse model, treatment was started 24 h after infection and was continued for 10 days in the first experiment, 7 days in the second experiment, and 14 days in the third experiment. The mice were observed for 10 days after treatment in the first experiment, 13 days after treatment in the second experiment, and 6 days after treatment in the third experiment. In the respiratory model, treatment commenced 18 h after infection and continued for 10 days, with 4 days of observation after the end of treatment.

Cultures. Surviving mice were sacrificed by cervical dislocation on day 21 in the nonimmunocompromised mouse model and on day 14 in the respiratory model. Brains and kidneys were removed for culture in the nonimmunocompromised mouse model (because these are the primary target organs) and lungs were removed for culture in the respiratory model. The organs were placed into 5 ml of sterile PBS containing penicillin (100 IU/ml) and streptomycin (100 μ g/ml) and were then homogenized in a tissue grinder for 15 to 30 s. Two or three 10-fold serial dilutions were made, and 0.5 ml of each dilution was plated onto Sabouraud's agar. The plates were incubated at 35°C, and colonies were counted daily for up to 5 days.

Serum D0870 and itraconazole concentrations. Itraconazole was assayed by bioassay as described previously (6). A modification of this assay with the same assay organism was used for D0870.

Statistical analysis. Mortality and quantitative culture were compared by the Mann-Whitney rank sum test. Qualitative culture results were examined by Fisher's exact test. Mice which died before 14 or 21 days (depending on the model) were assumed to have quantitative counts in their organs at least as high as the highest counts in the organs of any surviving mice. All analyses were done with the computer package Minitab (Minitab Data Analysis Software, Philadelphia, Pa.).

RESULTS

Nonimmunocompromised mouse model. Mortality is shown in Fig. 1 for mice in the first experiment with the nonimmunocompromised mouse model and in Table 1 for all experiments. An 80% lethal dose (LD_{80}) for control mice was achieved in experiments 1, 2, and 3, indicating that the model is reproducible. The efficacy of amphotericin B in this model was well demonstrated by all three experiments in which no treated animal died. Amphotericin B was statistically superior to controls and both lower doses of itraconazole. Itraconazole was effective at 25 mg/kg BID but not at any of the lower doses.

There was no statistical difference between itraconazole at 25 mg/kg BID and amphotericin B in experiment 1 ($P = 0.15$). Unfortunately, serum itraconazole concentrations were found to be extremely low in experiments 2 and 3. Retrospectively, it was found that the concentration of stock drug was approximately 10-fold less than intended because of a lack of stability of the cyclodextrin-itraconazole mixture at 4°C. There was no statistical difference between itraconazole at ≤ 10 mg/kg BID and control groups in any of the three experiments.

All three doses of D0870 were inferior to amphotericin B in experiment 1 ($P = 0.003$ to 0.031). The median survival of mice given D0870 at 25 mg/kg BID was 20 days, which was the same as those of mice given amphotericin B and itraconazole at 25 mg/kg BID. In experiments 2 and 3, the activity of D0870 at 10 mg/kg/day was statistically inferior to that of amphotericin B.

The concentrations of D0870 in serum varied with the dose and appeared to accurately reflect the range of doses (Table 2).

TABLE 1. Mortality in five experiments performed

Treatment ^a	Mortality (no. of deaths/total no. of mice)				
	Nonimmuno-compromised mouse model			Respiratory immuno-compromised mouse model	
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5
Gavage control	8/10	7/9	9/12	5/11	8/10
Intraperitoneal control				4/11	7/10
AmB	0/10	0/10	0/10	4/11	7/10
ITZ, 5 mg/kg/day	6/10				
ITZ, 5 mg/kg BID	6/10				
ITZ, 10 mg/kg BID		10/10	7/10		
ITZ, 25 mg/kg BID	2/10				
ITZ, 50 mg/kg BID				5/11	8/10
D0870, 5 mg/kg BID	5/10				
D0870, 5 mg/kg/day	6/9				
D0870, 10 mg/kg/day		7/12	9/10	3/10	8/10
D0870, 25 mg/kg/day				3/10	9/10
D0870, 25 mg/kg BID	5/10				
D0870, 50 mg/kg/day				0/11	9/10
D0870, 100 mg/kg/day				7/10	8/10

^a AmB, amphotericin B; ITZ, itraconazole.

TABLE 2. Concentrations of study drugs in serum

Model and treatment	Concn ($\mu\text{g/ml}$) in serum 2 h after dosing	
	Day 1	Day 10
Nonimmunocompromised mouse model		
D0870, 5 mg/kg/day	2.0	3.8
D0870, 5 mg/kg/dose BID	ND ^a	8.9
D0870, 25 mg/kg/dose BID	9.6	31.0
ITZ, ^b 5 mg/kg/dose	0.31	ND
ITZ, 25 mg/kg/dose	14.5	ND
No drug control, D0870	0	ND
No drug control, ITZ	0	ND
Respiratory model		
D0870, 10 mg/kg/day	1.8, 0 ^c	ND
D0870, 25 mg/kg/day	5.2, 1.6 ^c	ND
D0870, 50 mg/kg/day	4.6, 2.3 ^c	ND
D0870, 100 mg/kg/day	9, 11.5 ^c	ND
ITZ, 50 mg/kg BID	1, 1.2 ^c	ND

^a ND, not done.^b ITZ, itraconazole.^c Values obtained during a second experiment.

In addition, there appeared to be marked accumulation over the time course of the experiment when dosing was twice daily, indicating a long half-life of D0870 in mice. Toxicity was possibly observed at the highest D0870 dose (25 mg/kg BID), in that the animals showed a staring coat, became razor-backed, and lost weight. We did not observe any diarrhea or other toxicity in experiments 2 and 3 (10 mg/kg/day), despite the longer duration of therapy (14 days) in experiment 3.

Of the 41 survivors in experiment 1, only 9 had negative renal cultures; 5 of them were in the amphotericin B group, 3 were in the group receiving D0870 at 5 mg/kg BID, and 1 was in the group receiving itraconazole at 5 mg/kg BID. By Fisher's exact test, culture negativity was statistically more frequent in the amphotericin B group than in the control groups and in the groups receiving itraconazole at 5 and 25 mg/kg BID and D0870 at 5 mg/kg/day and 25 mg/kg BID ($P = 0.02$). The

results for the group receiving D0870 at 5 mg/kg BID were statistically superior to those for the same groups mentioned above ($P = 0.01$).

Renal colony counts from experiment 1 are given in Fig. 2. The results for the amphotericin B group were statistically superior to those for all other groups ($P < 0.001$ to 0.03), with the least significant difference being with the group receiving D0870 at 5 mg/kg BID ($P = 0.03$). Itraconazole at 25 mg/kg BID was statistically better than control treatment ($P = 0.02$) and treatment with itraconazole at 5 mg/kg BID and approached significance compared with treatment with D0870 at 5 mg/kg/day ($P = 0.09$). D0870 was not statistically superior to any other treatment group.

Similar results were seen for brain cultures. Amphotericin B was superior to all treatments except D0870 at 5 mg/kg BID by Fisher's exact test ($P < 0.00006$ to 0.001). D0870 at 5 mg/kg BID was also superior to all other treatments except amphotericin B and D0870 at 5 mg/kg/day ($P = 0.04$). With respect to the quantitation of culture results, only amphotericin B was superior to the other treatments ($P = 0.0001$ to 0.01). There were no statistically significant differences between any groups in the brain counts in experiments 2 and 3.

Respiratory model. Figure 3 shows the neutrophil counts after administration of cyclophosphamide. The neutrophil count was falling by day 2, reached a nadir of zero on days 4 to 6, and rose rapidly on days 7 and 8. Mice were infected on day 3.

Mortality in the first respiratory model experiment (experiment 4) is shown in Fig. 4. Figure 4 excludes data for two mice from the itraconazole group which died during gavage on day 2. In this experiment the two control groups had mortalities of 50 and 60%, respectively. However, in experiment 5 mortalities of 80 and 90% were produced with a 70% larger inoculum in the control group (Fig. 5). The model is therefore sensitive to changes in inoculum size but is again reproducible, given the parity between the two control groups in each experiment.

Amphotericin B was less effective in this respiratory model than in the nonimmunocompromised mouse model, with mortality being indistinguishable from that of controls. Itracon-

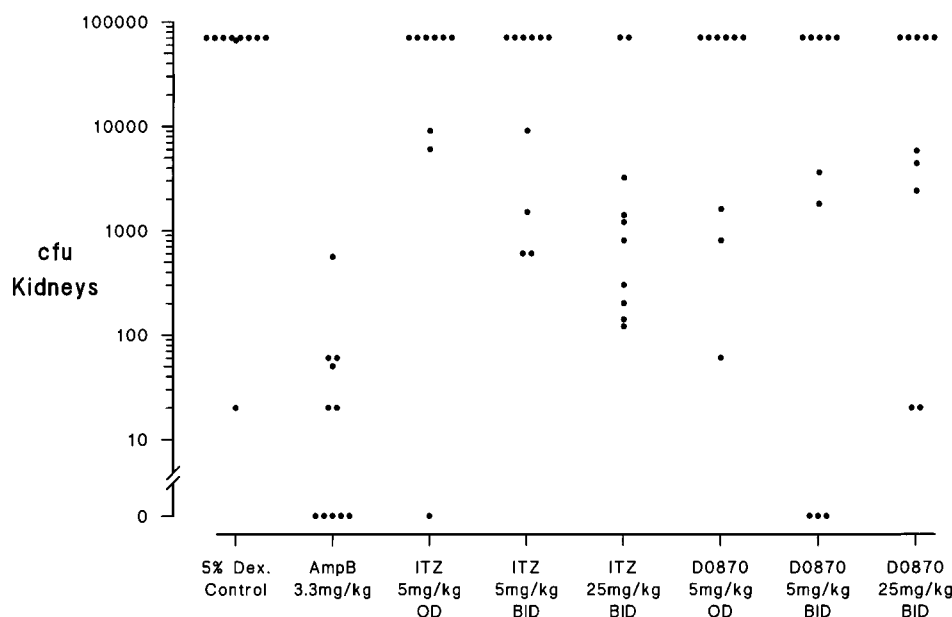


FIG. 2. Scatter plot of renal culture results from experiment 1. Dex, dextrose (glucose); AmpB, amphotericin B; ITZ, itraconazole; OD, once daily.

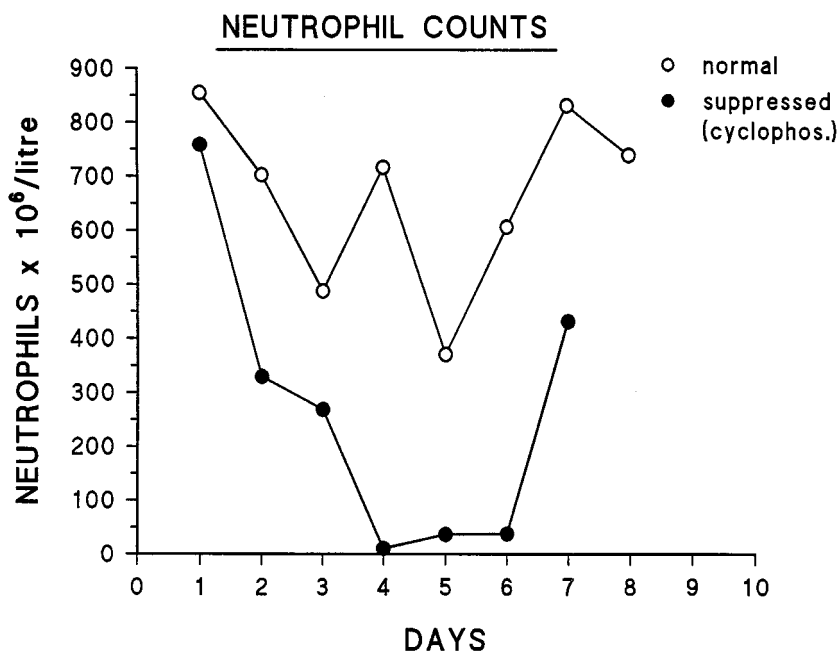


FIG. 3. Neutrophil count against time following administration of cyclophosphamide in respiratory model. ○, normal mice; ●, suppressed mice. The other symbols are defined in the Fig. 1 legend.

azole at 50 mg/kg BID was also ineffective, perhaps attributable to very low concentrations (1 to 1.2 μ g/ml) (Table 2). The most efficacious regimen was D0870 at 50 mg/kg, which was statistically superior to all other regimens ($P < 0.001$ to 0.03) except the two lower-dose D0870 regimens ($P = 0.06$). D0870 at 100 mg/kg, however, yielded a higher mortality, which could reflect toxicity because this dose yielded concentrations similar to those obtained with D0870 at 25 mg/kg BID in the nonimmunocompromised mouse model, in which toxicity was observed. This result implies a narrow therapeutic window of D0870 against *A. fumigatus*.

Culture results of the lungs from the respiratory model are shown in Fig. 6. There was less consistency in culture results

from one dilution to the next in lung cultures compared with the results for brain or renal cultures, and so all results were rounded to the nearest 10-fold dilution result. D0870 at 25 and 50 mg/kg were statistically better than all other treatments ($P = 0.005$ to 0.04) except amphotericin B, to which they were superior but not statistically so. There was some discordance between the dichotomized comparison and the quantitative results.

Although higher drug doses were administered in the respiratory model, the concentrations of both itraconazole and D0870 were lower. This could reflect the use of younger mice or, more likely, the administration of cyclophosphamide and the general ill health of the mice.

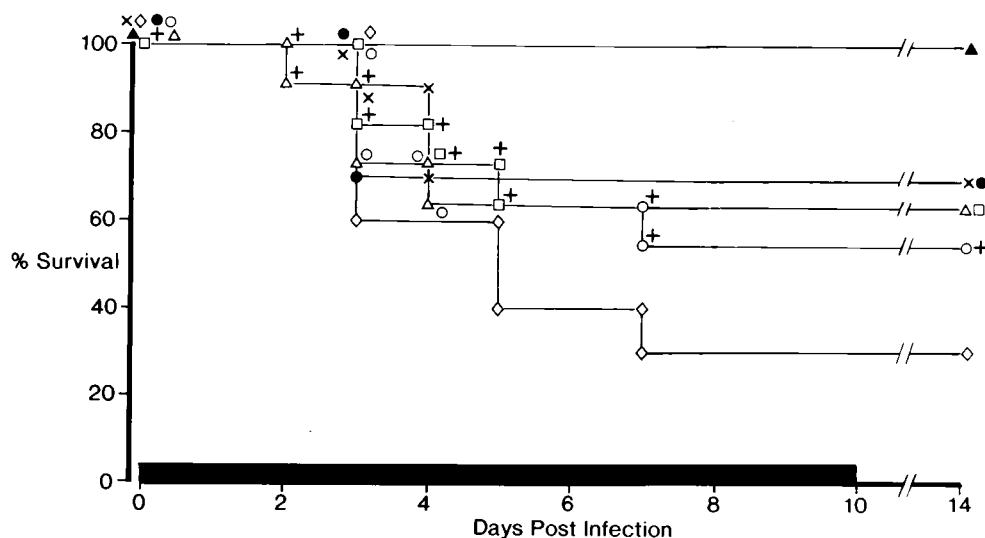


FIG. 4. Mortality against time in first respiratory model (experiment 4; isolate AF15) with an inoculum of 8.7×10^6 conidia. Δ, amphotericin B; +, itraconazole at 50 mg/kg BID; ◇, D0870 at 100 mg/kg/day; ▲, D0870 at 50 mg/kg/day; ●, D0870 at 25 mg/kg/day; ×, D0870 at 10 mg/kg/day; ○, 5% glucose control (by gavage); □, 5% glucose control (intraperitoneally).

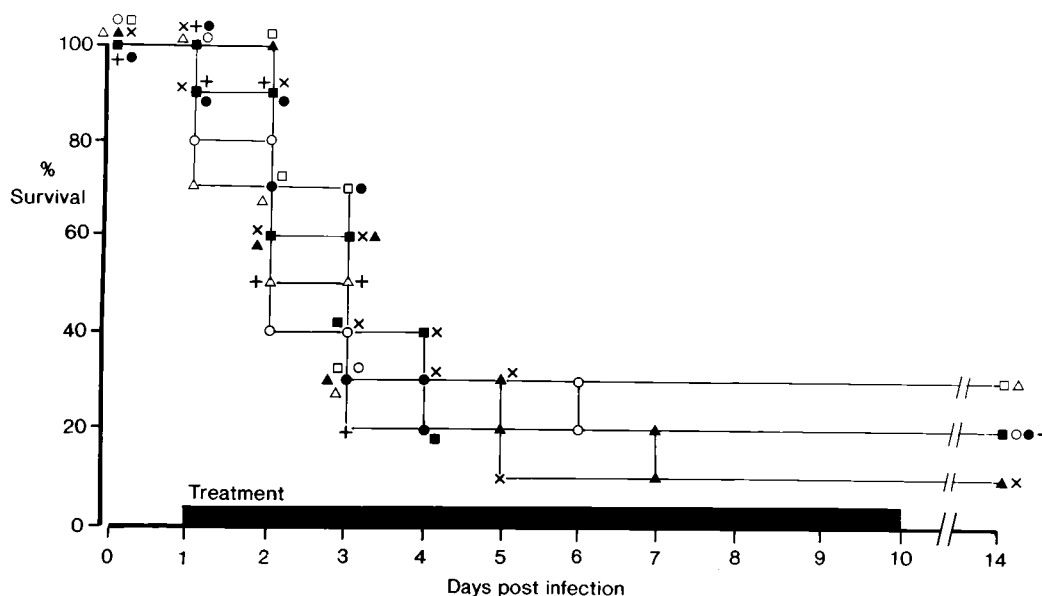


FIG. 5. Mortality against time in second respiratory model (experiment 5) (isolate AF15) with an inoculum of 15×10^6 conidia. △, amphotericin B at 3.3 mg/kg/day; ■, itraconazole at 50 mg/kg BID; ●, D0870 at 100 mg/kg/day; ▲, D0870 at 50 mg/kg/day; ×, D0870 at 25 mg/kg/day; +, D0870 at 10 mg/kg/day; ○, 5% glucose control (by gavage); □, 5% glucose control (intraperitoneally).

DISCUSSION

D0870 is a new azole compound with impressive activity against *Candida* spp. (1, 7, 10, 11, 13), *Cryptococcus neoformans* (10, 13), *Histoplasma capsulatum* (11), *Blastomyces dermatitidis* (12), and *Coccidioides immitis* (2). It has shown activity against human oropharyngeal candidosis even in a few patients with fluconazole-resistant disease (1, 7). Its activity against *Aspergillus* spp. is less remarkable in vitro, although it was clearly active (9).

The present study compared the activity of D0870 with those of amphotericin B and itraconazole in two models of invasive

aspergillosis. Both models were highly reproducible. The non-immunocompromised mouse model reflects the known activities of amphotericin B and itraconazole and thus offers a useful comparison for the activity of D0870. The neutropenic respiratory model more closely mimics the clinical setting, with relatively poor activities of amphotericin B and itraconazole.

In the nonimmunocompromised mouse model, amphotericin B was clearly superior to D0870 in all comparisons. Only three doses of amphotericin B, as in the second experiment, were superior to D0870 given at 10 mg/kg once daily. The highest dose of itraconazole used in this model was also supe-

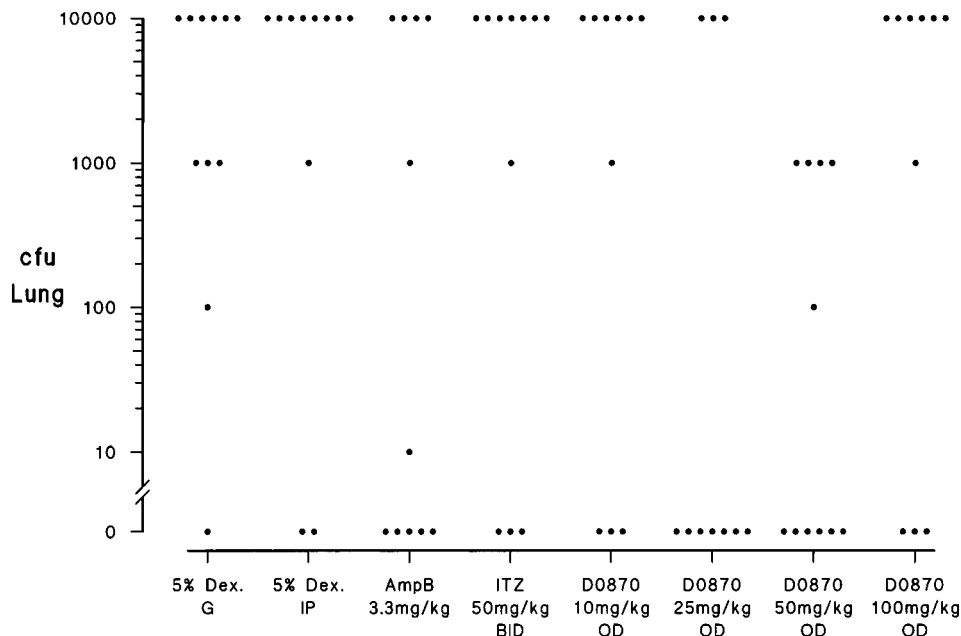


FIG. 6. Scatter plot of pulmonary culture results from experiment 4. Dex, dextrose (glucose); AmpB, amphotericin B; ITZ, itraconazole; OD, once daily.

rior to D0870 in the one experiment in which adequate concentrations of itraconazole were achieved in serum. However, D0870 was much more bioavailable and demonstrated useful activity at higher doses. This was particularly true in the neutropenic pulmonary model, in which D0870 was superior to amphotericin B and itraconazole with regard to mortality and to some extent in tissue cultures. Therefore, these results indicate that D0870 has useful activity at concentrations in serum above 4 mg/ml.

D0870 was easily deliverable, and the means of preparing the drug for administration was simple. Within each model reproducible concentrations in serum were achieved, given the different doses and regimens used. The bioassay used was a modification used for itraconazole and appeared to accurately reflect what is anticipated from pharmacological considerations of D0870 administration. In particular, accumulation over time was shown, which was anticipated given its long half-life. High concentrations of D0870 in the serum of mice in the high-dose groups appeared to reflect toxicity. In this sense, therefore, the concentrations in serum are probably meaningful. There may be a relatively narrow therapeutic window for the treatment of invasive aspergillosis, depending on the doses that can be administered to patients. In addition higher concentrations in serum (e.g., ≥ 10 $\mu\text{g/ml}$) were not more effective.

In previous work we showed that D0870 has consistent activity against *Aspergillus* spp. in vitro, but at higher concentrations than those at which itraconazole and amphotericin B have activity. Given the difficulties of consistently achieving therapeutic concentrations of itraconazole in serum, we were not able to assess from the results of the present experiments whether the higher MICs of D0870 in vitro really reflect reduced in vivo activity at the same concentration.

There is an urgent need for new compounds with activity against *Aspergillus* species. Invasive aspergillosis represents one of the most devastating complications of neutropenia, bone marrow transplantation, and organ transplantation (4). It is emerging as a significant cause of death in patients with AIDS (3). Patients with chronic granulomatous disease are also at grave risk of fatal invasive aspergillosis (4). Current therapy is often toxic or ineffective. The present study indicates that D0870 has potentially useful activity against invasive aspergillosis at modest concentrations in serum.

REFERENCES

1. Cartledge, J. D., D. Denning, B. Dupont, N. Clumeck, S. De Wit, D. A. Hawkins, and B. G. Gazzard. 1994. Treatment of fluconazole (FCZ) resistant (res) oral candidosis (OC) with D0870 in patients with AIDS (PWA), abstr. M89, p. 248. In Program and abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
2. Clemons, K. V., and D. A. Stevens. 1994. Utility of the triazole D0870 in the treatment of experimental systemic coccidioidomycosis. *J. Med. Vet. Mycol.* **32**:323–326.
3. Denning, D. W., S. Follansbee, M. Scolaro, S. Norris, D. Edelstein, and D. A. Stevens. 1991. Pulmonary aspergillosis in AIDS. *N. Engl. J. Med.* **324**:654–662.
4. Denning, D. W., and D. A. Stevens. 1990. Antifungal and surgical treatment of invasive aspergillosis: review of 2121 published cases. *Rev. Infect. Dis.* **12**:1147–1201.
5. Denning, D. W., and D. A. Stevens. 1991. Efficacy of cilofungin in a murine model of disseminated aspergillosis. *Antimicrob. Agents Chemother.* **35**:1329–1333.
6. Denning, D. W., R. W. Tucker, L. H. Hanson, and D. A. Stevens. 1989. Treatment of invasive aspergillosis with itraconazole. *Am. J. Med.* **86**:791–800.
7. De Wit, S., B. Dupont, J. D. Cartledge, D. A. Hawkins, D. Denning, N. Clumeck, and B. G. Gazzard. 1994. Pilot study of a new triazole derivative (D0870) in HIV patients with oral candidiasis (OPC), abstr. I225, p. 213. In Program and abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
8. Meunier, F. 1994. Alternative modalities of administering amphotericin B: current issues. *J. Infect.* **28**:51–56.
9. Moore, C. B., D. Law, and D. W. Denning. 1993. In vitro activity of D0870 compared with amphotericin B and itraconazole against *Aspergillus* spp. *J. Antimicrob. Chemother.* **32**:831–836.
10. Peng, T., and J. N. Galgiani. 1993. In vitro studies of a new antifungal triazole, D0870 against *Candida albicans*, *Cryptococcus neoformans*, and other pathogenic yeasts. *Antimicrob. Agents Chemother.* **37**:2126–2131.
11. Ross, S., D. A. McGough, A. W. Fothergill, and M. G. Rinaldi. 1992. In vitro susceptibility testing of D-0870, an investigational antifungal triazole, abstr. 1045, p. 284. In Program and abstract of the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
12. Tucker, R. M., L. M. Hanson, E. Brummer, and D. A. Stevens. 1989. Activity of ICI 195,739, a new oral triazole, compared with that of ketoconazole in the therapy of experimental murine blastomycosis. *Antimicrob. Agents Chemother.* **33**:573–575.
13. Yamada, H., T. Tsuda, T. Watanabe, M. Ohashi, K. Murakami, and H. Mochizuki. 1993. In vitro and in vivo antifungal activities of D0870, a new triazole agent. *Antimicrob. Agents Chemother.* **37**:2412–2417.