Activities of the Triazole D0870 In Vitro and against Murine Blastomycosis

KARL V. CLEMONS,1,2,* LINDA H. HANSON,2 AND DAVID A. STEVENS1,2,3

Department of Medicine, Division of Infectious Diseases, Santa Clara Valley Medical Center, 1 and California Institute for Medical Research, 2 San Jose, California 95128, and Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, California 94305

Received 19 November 1992/Accepted 17 February 1993

The novel triazole D0870 was tested for in vitro activity, as well as in vivo in a murine model of pulmonary blastomycosis. In vitro, D0870 had inhibitory and fungicidal activity against Blastomyces dermatitidis (MIC = 0.048 μg/ml; minimal fungicidal concentration = 0.097 μg/ml). In vivo, D0870 was approximately 100-fold more active than fluconazole on the basis of milligrams per kilogram of body weight given once daily (QD) against blastomycosis. D0870 doses of both 1 or 10 mg/kg given QD and 10 or 100 mg/kg given every other day prolonged survival (P < 0.001) over fluconazole (100 mg/kg given QD). A D0870 dosage of 1 mg/kg QD was equivalent to fluconazole given at 100 mg/kg in reduction of lung burdens of B. dermatitidis, and D0870 administered at 10 mg/kg QD and 10 or 100 mg/kg every other day caused greater reduction (P < 0.001). However, D0870 at 100 mg/kg given QD was lethally toxic, whereas fluconazole at 100 mg/kg was not. These results indicate that D0870 is an effective therapy for murine blastomycosis and should be further tested.

As the number of serious fungal infections continues to increase, safer and more effective therapeutic agents are sought. The imidazoles and triazoles currently in use are readily administered in the oral form and have been shown to have efficacy against a variety of fungal infections. However, relapses and drug-related toxicities have occurred (4, 9, 10). Because of these newer agents are under investigation. D0870 is an enantiomer of the previously studied bis-triazole, ICI 195,739, which has been demonstrated to be efficacious in murine models of blastomycosis (8) and candidosis (5). We examined the activity of the purified compound in vitro and in vivo.

D0870 (ICI Pharmaceuticals, Macclesfield, Cheshire, England) and fluconazole (Pfizer, Groton, Conn.) were obtained as powders. D0870 was dissolved in 100% dimethyl sulfoxide at 900 μg/ml to serve as a stock. In vitro assays for MICs of D0870 and fluconazole were performed by broth dilution in defined medium (8). The minimum fungicidal concentrations were determined by plating 0.05 ml from MIC tubes with no growth onto sheep blood agar plates and scoring for colonies after incubation at 35°C (2, 8).

The in vivo activities of D0870 and fluconazole were compared in the murine model of pulmonary blastomycosis as described previously (1–3, 7). In brief, 4-week-old male CD-1 mice (mean weight, 23.6 g) under methoxyflurane anesthesia were infected by intranasal instillation of 27,900 viable CFU of Blastomyces dermatitidis isolate V (ATCC 26199) to establish disease resulting in >50% mortality. Four days postinfection, various therapy regimens were initiated. Therapy was given by gavage in volumes of 0.1 ml once daily (QD) or once every other day (QOD) from days 4 through 25 postinfection. Fluconazole was given in 0.3% agar, and D0870 was given in 0.5% Tween 80 in normal saline. D0870 doses were made fresh daily, starting from powder. Groups of 10 mice received either no treatment, fluconazole at 1 or 100 mg/kg of body weight QD, D0870 at 1, 10, or 100 mg/kg QD, or D0870 at 1, 10 or 100 mg/kg QOD. Previous studies (7, 8) had demonstrated that the diluents used for D0870 and fluconazole gave the same results as untreated controls, and these were not included in this study.

Additional groups of uninfected mice were given either a single 100-mg/kg dose of D0870 or multiple doses (QD or QOD) on days 4 through 25. At various times after the first and last doses, two mice were exsanguinated and the serum was pooled. A sample of the serum was tested in a bioassay for the purpose of estimating the concentration of bioactive drug present in the serum (8).

Deaths were tallied through 49 days postinfection, at which time all surviving mice were killed and the lungs were homogenized in 5 ml of sterile saline (2). The log_{10} number of CFU in the lungs was determined by quantitative plating of homogenates onto sheep blood agar plates (2). This assay has a lower limit of approximately 5 CFU per entire organ (8).

Statistical analyses of the survival study by day of death were done by a Wilcoxon rank sums test (6). Analyses of the residual lung burdens of B. dermatitidis were done by a Mann-Whitney U test (6) with datum points missing because of the death of an animal assigned a log_{10} CFU value of 8. This is the approximate number of CFU in the lungs per animal just prior to death and has been established from prior studies (3). This assignment also ensures that death as a result of infection is scored as a worse outcome than is survival with any amount of residual CFU.

The yeast form of B. dermatitidis was exceptionally sensitive to D0870 (MIC and minimal fungicidal concentration, 0.048 and 0.097 μg/ml, respectively). The MIC and minimal fungicidal concentration for fluconazole against B. dermatitidis were both 3.1 μg/ml. Thus, the MIC for D0870 was 64-fold lower and the minimal fungicidal concentration was 32-fold lower than for fluconazole.

The results of the in vivo survival studies are presented in Fig. 1 and show that treatment with D0870 affects survival in a dose-responsive manner. Of the untreated controls and the mice given 1-mg/kg doses of fluconazole, 90% died between days 12 and 26 postinfection. All mice treated with 100-mg/kg doses of fluconazole or with D0870 at 1 mg/kg QOD...
died by day 41. Of the regimens tested, 100-mg/kg doses of fluconazole and D0870 at 1 or 10 mg/kg QD and 10 or 100 mg/kg QOD were efficacious in prolonging survival beyond that of untreated controls ($P < 0.001$). Although only 20% of the mice given D0870 at 1 mg/kg QD survived, this regimen was superior to fluconazole at 100 mg/kg ($P = 0.05$). All mice treated with D0870 at 10 mg/kg QD and 10 or 100 mg/kg QOD survived, making these regimens superior to fluconazole at 100 mg/kg ($P < 0.001$) in both absolute survival and time to death. In addition, a 1-mg/kg dose of D0870 QOD was better than a 1-mg/kg dose of fluconazole ($P = 0.001$). On the basis of these results, D0870 given QD is at least 100-fold more active than fluconazole and, when given QOD, D0870 is at least 10-fold better in prolonging survival.

At equivalent 100-mg/kg QD dosages, D0870 was lethally toxic, whereas fluconazole was not. At the 100-mg/kg QD dosage of D0870, all infected and uninfected mice died. Deaths began after the 7th daily dose and continued through the 16th dose. Upon necropsy, no gross pathology was observed for any organ in mice that died of toxicity. No evidence of toxicity was noted in surviving infected or uninfected mice that were given D0870 at 100 mg/kg QOD or fluconazole at 100 mg/kg. The observation of no toxicity with larger total doses of D0870 in the QOD regimens suggests that the toxicities observed in mice given 100 mg/kg D0870 are somehow related to the pharmacokinetics of D0870 and that the 100-mg/kg QD regimen allows sufficient time for clearance of D0870 or any postulated metabolites to nontoxic levels prior to the next dose. However, it is interesting that a total of 700 mg of D0870 per kg was given before the first deaths occurred. Further studies would be required to define the toxic dose of D0870 given QD for this species. The present study indicates that fluconazole may be approximately 10-fold less toxic than D0870 in a milligram-per-kilogram comparison.

Further evaluation of in vivo efficacy was derived from the residual organ burdens, as presented in Table 1. Although B. dermatitidis was exquisitely sensitive to D0870 in vitro, no regimen of D0870 cleared all mice of residual infection. However, clearance by D0870 was dose responsive. D0870 at 10 mg/kg QD and 100 mg/kg QOD cleared five and seven mice of infection, respectively. These regimens were equivalent to each other but significantly better than all other regimens of fluconazole or D0870 ($P < 0.05$ to 0.001, depending on the regimen). A D0870 dosage of 10 mg/kg QOD was also better in reduction of burden than 100-mg/kg doses of fluconazole or lower doses of D0870 ($P < 0.001$). Other regimens of D0870 and fluconazole were not effective in clearing survivors of infectious burden (Table 1). With QD administration, D0870 was estimated to be >100-fold more active than fluconazole, and with QOD administration, it was >10-fold more effective than the QD fluconazole regimen in reduction of infectious burden. Our results with QD dosing with fluconazole are in agreement with previous studies showing prolongation of survival but progression to death after cessation of therapy (1, 7).

Although the organism was exquisitely sensitive to D0870, we doubt that the number of CFU recovered from mice is artificially low as a result of residual drug in the tissues carrying over into the plating assay. Our reasoning is several-fold. While the tissue pharmacokinetics of D0870 in this model remain to be determined, it is expected that the time (24 days) between the last dose and plating, in conjunction with the significant dilution factors of the plating assay, serves to diminish the concentration of any residual drug. Additionally, had residual drug influenced the number of CFU, we would expect that the less dilute the homogenate plated, the fewer colonies proportionately would arise. This did not occur, and numbers of colonies increased as expected from most to least dilute. Finally, the gross pathology of the lungs of surviving mice at necropsy was indicative of the dose-responsive efficacy of D0870. The survivors on the 1-mg/kg QD regimens of D0870 and fluconazole were severely infected, with multiple lesions encompassing >90% of the tissue in all lobes. In contrast, observable lesions in mice that had been given higher dosages of D0870, QD or QOD, were well defined, individual, and usually confined to one or two lobes, leaving >75% of the lung tissue appearing normal. At the most efficacious dosage of D0870 (100 mg/kg QOD), the lung tissues appeared normal in all animals, even though three mice had low residual burdens. Thus, we feel that the number of CFU recovered is indicative of the in vivo efficacy of D0870 in this model.

The results of pharmacokinetic analysis of bioactive drug present in the serum from uninfected mice are presented in Fig. 2. After a single 100-mg/kg dose of D0870, peak serum

![FIG. 1. Survival of CD-1 mice infected with B. dermatitidis and treated by gavage with fluconazole (Flu) or D0870.](http://aac.asm.org/)

### TABLE 1. Recovery of B. dermatitidis from lungs of surviving mice

<table>
<thead>
<tr>
<th>Therapy (mg/kg)</th>
<th>No. of survivors</th>
<th>Log$_{10}$ CFU/animal$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Infection free</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>D0870, QD</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>D0870, QOD</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

$^a$ A value of 0 indicates that residual infection, if any, was below detectable levels for the assay system used. The calculated lower limit of the assay is approximately 5 CFU per entire lung.
concentrations of 31 μg/ml occurred 12 h postdose, with measurable activity persisting through 120 h postdose. In the chronic-dosing study (12 doses of 100 mg/kg given QOD), concentrations were higher and the predose concentration suggested steady-state pharmacokinetics. A peak of 58 μg/ml occurred 8 h postdose, with activity still present in the serum 192 h postdose. These data indicate a prolonged terminal phase in the serum with an estimated half-life of approximately 50 h. Whether this activity represents D0870 alone or a combination of D0870 and one or more active metabolites remains to be determined. As the half-life of fluconazole is shorter than that of D0870 in mice (7), the comparisons of efficacy between fluconazole QD and D0870 QOD may be the most pertinent. The results with fluconazole may be further improved with regimens giving the drug more than once per day.

Our present results are similar to those reported (8) with ICI 195,739, the parent compound of D0870. In that study, ICI 195,739 proved to be >50 times more potent than ketoconazole in prolonging survival, and doses of 10 mg/kg or greater cured infections in >90% of the mice. Similarly, fluconazole has been demonstrated to be >10-fold more potent than ketoconazole against murine blastomycosis (1, 7). By comparing these data, we estimate that D0870 is >500-fold more active than ketoconazole. However, D0870 cleared fewer mice of infection in our study than ICI 195,739 did previously (8). Reasons for this discrepancy might be that different strains of mice were used (i.e., CD-1 versus BALB/c) and that we used a 36-fold-greater inoculum to infect the animals. Thus, it is possible that the much greater inoculum of B. dermatitidis used in our study could not be controlled effectively by D0870. In contrast to the results reported previously, we found that a D0870 dosage of 100 mg/kg QD caused lethal toxicity, whereas no toxicities were reported with the same dosage in the study with ICI 195,739 (8).

In conclusion, D0870 was found to be highly efficacious for treatment of murine blastomycosis, a disease that is difficult to treat. D0870 should be further tested in studies with other animal models and in preliminary clinical trials.

These studies were funded in part by a grant from ICI Pharmaceuticals.

REFERENCES