Therapy of systemic histoplasmosis in immunosuppressed mice with the triazole D0870

K. V. CLEMONS, M. MARTINEZ, M. E. HOMOLA & D. A. STEVENS
Department of Medicine, Division of Infectious Diseases, Santa Clara Valley Medical Center, and California Institute for Medical Research, San Jose, CA 95128; and Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA 94305, USA

Because histoplasmosis is a life-threatening disease in AIDS and other compromised patients, we examined the efficacy of D0870 (Zeneca) in immunosuppressed mice against systemic histoplasmosis. Oral therapy with fluconazole given once daily (QD) was ineffective in prolonging survival, whereas itraconazole given once or twice daily (BID), fluconazole given BID or D0870 given QD or given every other day (QOD) were efficacious (P < 0.001). Burdens of *Histoplasma capsulatum* in the liver and spleen of survivors showed that D0870 given QD or QOD and itraconazole given BID caused dose-responsive reduction of infectious burden. Infection was cleared more readily from the liver than from the spleen. Overall, D0870 was ≈20-fold more efficacious than fluconazole or itraconazole and itraconazole was > ten-fold better than fluconazole for the treatment of systemic histoplasmosis in the immunosuppressed model.

Keywords antifungal, D0870, histoplasmosis, immunosuppression

Introduction

D0870 is a novel triazole from Zeneca Pharmaceuticals (Macclesfield, UK) and is an enantiomer of the previously studied *bis*-triazole ICI 195,739 [1]. Like the parent compound, D0870 has been found to be active both *in vitro* and *in vivo* against a variety of pathogenic fungi [2-7]. In several preclinical studies, D0870 has been demonstrated to have an efficacy greater than that of fluconazole for the treatment of several systemic mycoses [3-5,7]. We have previously demonstrated that D0870 also is highly efficacious in the treatment of systemic histoplasmosis with 10- to 100-fold improved efficacy over fluconazole [5]. The continually increasing number of cases of histoplasmosis in immunocompromised patients, and particularly in AIDS patients, along with treatment failures using amphotericin B or other azoles in these patients clearly indicates the need for an improved therapeutic agent [8-10]. To better evaluate the potential of D0870 as an effective drug in immunocompromised individuals, we studied its efficacy in comparison with fluconazole and itraconazole for the treatment of histoplasmosis in immunosuppressed mice.

Materials and methods

Organism

The yeast form of *Histoplasma capsulatum* strain G217B was maintained on BHI agar (Difco Laboratories, Detroit, MI) slopes at 37 °C grown for 4–7 days [5]. The method used for inoculum preparation was that described previously [5]. The number of cells in the suspension was estimated by haemacytometer counting and further diluted in saline to the desired number. Viable CFU were determined by quantitative plating onto modified McVeigh–Morten agar containing horse serum and culture filtrate and incubation at 35 °C [5,11].

In vivo models

A model of systemic histoplasmosis was established in cortisone acetate-immunosuppressed mice similar to those described previously [5,12–14]. Six-week-old female CD-1 mice were immunosuppressed prior to infection...
by two subcutaneous injections of 5 mg of cortisone acetate (Cortone, Merck Sharp and Dohme, West Point, PA). The first injection was given 4 days prior to infection and the second injection was given on the day of infection. In experiment 1, mice were infected intravenously with $6.25 \times 10^6$ viable yeast cells, and in experiment 2 the mice were infected with $3.48 \times 10^6$ viable yeasts of *Histoplasma capsulatum*. In both experiments, therapy was given by gavage in 0.1 ml volumes on days 4-24 after infection to groups of ten mice. All mice were provided sterilized food and acidified water *ad libitum*.

The regimens and dosing schedules for experiment 1 were: no treatment, 0.5% Tween 80-saline diluent [3-5], 1 or 100 mg kg$^{-1}$ of fluconazole given in 0.3% agar once daily (QD), 1 or 10 mg kg$^{-1}$ of D0870 given QD or 1, 10 or 100 mg kg$^{-1}$ of D0870 in Tween 80-saline given every other day (QOD). The regimens for experiment 2 were: no treatment; hydroxypropyl-$\beta$-cyclodextrin (HP/$\beta$C) (Encapsin HPB, American Maize-Products, Hammond, IN), the diluent for itraconazole [15, 16]; 100 mg kg$^{-1}$ a day of fluconazole in 0.3% agar given in divided doses (BID), 10 or 100 mg kg$^{-1}$ a day of itraconazole in HP/$\beta$C [16] given BID, 100 mg kg$^{-1}$ a day of itraconazole in HP/$\beta$C given QD or 10 or 100 mg kg$^{-1}$ of D0870 in 0.5% Tween 80-saline given QOD.

Deaths were tallied to day 40 (experiment 1) or day 42 (experiment 2) after infection, at which time all surviving mice were killed by CO$_2$ asphyxiation. The spleen and liver of each surviving mouse were removed and the number of viable CFU in each organ determined by quantitative plating of serially diluted samples of organ homogenate as described previously [5]. Viable CFU were expressed as the log$_{10}$ number of CFU, and a value of 0 indicates that organisms, if any, were present at levels below the detectable level of 5 CFU per entire organ. Data analyses were performed by nonparametrical statistical methods. Comparative analyses of survival curves by day of death were performed using a Wilcoxon rank sums test [3-5, 17]. Mice that died during the course of the study were presumed, based on prior experimental observations, to have died as a result of infection with *H. capsulatum*; no culture confirmation was carried out.

Results

**Experiment 1**

The results presented in Fig. 1 illustrate the therapeutic efficacies of the various doses of D0870 and fluconazole on the prolongation of survival of mice with systemic histoplasmosis. Immunosuppression with cortisone proved to be an effective method of causing a more aggressive course of systemic histoplasmosis. All untreated and diluent-treated controls died by day 8 or 6 after infection, respectively. Fluconazole treatment at 1 or 100 mg kg$^{-1}$ on a QD schedule or 1 mg kg$^{-1}$ of D0870 given QOD were not effective, with survival indistinguishable from controls (Fig. 1). Eighty per cent of the mice that had received D0870 given QD at 1 or 10 mg kg$^{-1}$ and 100% that had received D0870 QOD at 10 or 100 mg kg$^{-1}$ survived through the 40 days of the study. These regimens of D0870 were equivalent to each other ($P > 0.05$) and superior to any control or fluconazole regimen as well as to 1 mg kg$^{-1}$ of D0870 given QOD ($P < 0.001$).

The number of viable *H. capsulatum* yeasts recovered from the spleen and liver of the surviving mice is presented in Table 1. D0870 was superior to controls or fluconazole in clearance of both organs ($P < 0.001$). D0870 was most effective in clearing the liver of infectious burden. For the QD regimens, 50% or more of surviving mice had no detectable burden and no animals given 10 or 100 mg kg$^{-1}$ of D0870 QOD had residual infection (Table 1).

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Table 1 Recovery of H. capsulatum from surviving mice in experiment 1

<table>
<thead>
<tr>
<th>Therapy group (mg kg⁻¹)</th>
<th>Number of mice</th>
<th>log₁₀ Geometric mean CFU/* (no. organs free of infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surviving</td>
<td>Free of infection</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>Liver</td>
</tr>
<tr>
<td>Untreated</td>
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<td>0</td>
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<tr>
<td>Diluent</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Fluconazole</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D0870, QD</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>D0870, QOD</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>1</td>
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</table>

*Value of 0 indicates that residual infection, if any, was below the detectable levels for the assay system used. The calculated lower limit of the assay is approximately 5 CFU per entire organ.

Both regimens of D0870 given QD were equivalent in efficacy. D0870 given QD at 10 or 100 mg kg⁻¹ were superior to 1 mg kg⁻¹ given QD (P < 0.05 or 0.001, respectively), but were equivalent to 10 mg kg⁻¹ given QD. D0870 was less efficacious in the clearance of residual burdens of H. capsulatum from the spleens of surviving mice than from livers (Table 1). All animals given D0870 QD or 10 mg kg⁻¹ of D0870 QOD carried detectable residual infection in the spleen (Table 1). At the 100 mg kg⁻¹ dosage of D0870 given QOD a single animal was cleared of spleen infection. D0870 displayed dose-responsive efficacy in the clearance of spleen infection with 10 mg kg⁻¹ of D0870 given QD superior to 1 mg kg⁻¹ given QD (P < 0.05). Likewise, 100 mg kg⁻¹ of D0870 given QD was superior to 10 mg kg⁻¹ of D0870 given QOD (P < 0.01), as well as to either QD regimen of D0870 (P < 0.001). Overall, D0870 effected complete cure of only a single animal, which had been treated with the 100 mg kg⁻¹ of D0870 given QOD whereas all other mice carried detectable infection in one or both organs.

Experiment 2

A second study was performed giving fluconazole on a twice daily schedule, D0870 on a QOD schedule and also included was itraconazole as a comparator. All untreated or diluent-treated control mice died by day 7 or 8 after infection, respectively (Fig. 2). All regimens of all three drugs significantly prolonged survival compared with either untreated controls or HP/C-diluent controls (P < 0.01-0.001, dependent on comparison). Comparison of the drug regimens showed that both regimens of D0870 (90 and 100% survival) as well as 100 mg kg⁻¹ of itraconazole given BID or QD (90 and 100% survival, respectively) were equivalent (P > 0.05) and all were superior to 10 mg kg⁻¹ of itraconazole BID (10% survival) (P < 0.01-0.001). Both D0870 regimens and itraconazole at 100 mg kg⁻¹ QD were also superior to 100 mg kg⁻¹ BID of fluconazole (40% survival) (P < 0.05). Although the 10 mg kg⁻¹ BID regimen of itraconazole prolonged survival over controls, it was not equivalent to 10 mg kg⁻¹ of D0870 given QD (P < 0.001).

A further evaluation of comparative efficacies was again based on recovery of viable yeasts from the spleen and liver of surviving mice (Table 2). Both doses of D0870 and the 100 mg kg⁻¹ regimens of itraconazole were reasonably effective in the clearance of burden from the liver; 20% or fewer mice carried any detectable infection, whereas three of the four surviving mice that had been treated with fluconazole had detectable burdens of H. capsulatum (Table 2). Both itraconazole and D0870 showed dose-responsiveness in the clearance of liver infection. Itraconazole given BID or QD at 100 mg kg⁻¹ and D0870 at 10 or 100 mg kg⁻¹ given QOD were superior to 10 mg kg⁻¹ of itraconazole given BID (P < 0.001), and were also better than 100 mg kg⁻¹ of fluconazole given BID (P < 0.01-0.001). Both 100 mg kg⁻¹ regimens of itraconazole were equivalent to both regimens of D0870 (P > 0.05).

Although the D0870 and itraconazole regimens were reasonably effective in reduction of spleen burdens, no mice given any drug regimen were free of detectable infection in one or both organs.
mice given 100 mg kg\(^{-1}\) had slightly lower burdens \((P > 0.05)\), and carried the lowest mean burdens of any treated mice. In the spleen, D0870 at 100 mg kg\(^{-1}\) given QOD was superior to any regimen of itraconazole or fluconazole \((P < 0.05-0.001\), dependent on comparison). D0870 at 10 mg kg\(^{-1}\) was superior to all but 100 mg kg\(^{-1}\) BID of itraconazole \((P < 0.01-0.001)\). The 100 mg kg\(^{-1}\) BID regimen of itraconazole was more effective than the 100 mg kg\(^{-1}\) QD regimen \((P < 0.05)\), demonstrating that BID dosing improved the efficacy of itraconazole. However, both 100 mg kg\(^{-1}\) regimens of itraconazole were superior to fluconazole \((P < 0.01-0.001)\); 10 mg kg\(^{-1}\) of itraconazole was equivalent to 100 mg kg\(^{-1}\) of fluconazole \((P > 0.05)\). Both D0870 and itraconazole showed limited dose-responsiveness in reduction of spleen burdens.

### Discussion

In previous studies we have demonstrated that D0870 is highly efficacious as a therapy for blastomycosis, coccidioidomycosis and histoplasmosis in models utilizing immunocompetent mice [3-5]. In addition, D0870 has been demonstrated by others to be effective against a
variety of other fungal infections [2,6,7]. Against histoplasmosis D0870 was 10- to 100-fold more efficacious than fluconazole, with up to 80% of treated mice cured of infection [5]. Because histoplasmosis in AIDS patients is a serious and life-threatening infection, we have further examined the potential of D0870 as a therapy by using a more lethal model to more closely mimic the disease in AIDS patients. The current study was carried out using an immunosuppressed model of histoplasmosis to obtain comparative efficacy data among D0870, fluconazole and itraconazole; the immunosuppression resulting in dramatic differences in survival. For example, with a similar inoculum, 50% of immunocompetent mice survived for 11 days and at 44 days only 90% had died [5], whereas 100% of immunosuppressed mice died by day 7 or 8. To account for differences in pharmacokinetic profiles, different dosing schedules of D0870, itraconazole and fluconazole were used. As in our previous studies using immunocompetent mice [5], D0870 was found to be highly efficacious against systemic histoplasmosis. This efficacy was confirmed in that D0870 prolonged survival and restricted the proliferation of *H. capsulatum* in the spleen and liver of treated animals.

The results from experiment 1 demonstrate that dosages of D0870 at 1 or 10 mg kg⁻¹ QD or 10 or 100 mg kg⁻¹ QOD effectively prolonged survival, whereas 100 mg kg⁻¹ of fluconazole given QD did not. The effectiveness of D0870 was also demonstrated by comparative residual burdens of *H. capsulatum* from the spleen and liver of survivors. D0870 showed dose-responsive efficacy in the reduction of yeast burdens in these organs. The liver was most readily cleared of infectious burden. Mice that had received 100 mg kg⁻¹ QOD of D0870 carried the lowest mean burden in the spleen. These results indicate that D0870 was ≥100-fold more efficacious than fluconazole on a milligram-per-kilogram basis given QD.

The lack of efficacy of fluconazole in experiment 1 may have reflected the once daily treatment schedule and the comparative pharmacokinetics of D0870 and fluconazole [3,4]. Thus, to account for these differences in pharmacokinetic profiles, D0870 was dosed every other day and fluconazole twice a day in experiment 2. Itraconazole solubilized in HPBC to improve its bioavailability as described previously [15] and given once or twice daily was also included. Twice daily administration of fluconazole improved its efficacy. Although this increase in efficacy could possibly be because the infecting inoculum was slightly lower, it is more likely that the BID schedule improved its pharmacokinetic profile and also its efficacy. However, all three drugs significantly prolonged survival and, overall, the comparative efficacies were estimated as follows: itraconazole was about ten-fold more active than fluconazole; D0870 was 20-fold more active than itraconazole; D0870 was >20-fold more active than fluconazole.

Both doses of D0870 and the 100 mg kg⁻¹ regimens of itraconazole were effective in clearance of burden from the liver and spleen. Mice that had been given fluconazole carried the highest mean burdens in both organs, whereas mice given D0870 carried the lowest mean burdens and D0870 was more effective than regimens of itraconazole or fluconazole. The 100 mg kg⁻¹ a day BID regimen of itraconazole was more effective than the 100 mg kg⁻¹ QD regimen, demonstrating that twice daily dosing also improved the efficacy of itraconazole. Comparisons of infectious burdens recovered from the spleen indicate that itraconazole is about ten-fold more active than fluconazole and that D0870 was >20-fold better than itraconazole or fluconazole. In the liver, D0870 was >20-fold better than fluconazole and more than two-fold better than itraconazole; itraconazole was better than fluconazole.

If the daily doses administered are considered alone, in order to regard the differences in half-life predominantly and ignore the total drug amount administered, then D0870 was superior to itraconazole and more than ten times better than fluconazole in survival; itraconazole was superior to fluconazole. In clearing the spleen, D0870 was superior to itraconazole and more than ten times better than fluconazole, and itraconazole was superior to fluconazole. In clearing the liver, D0870 was superior to itraconazole and more than ten times better than fluconazole, and itraconazole was superior to fluconazole.

Our results with itraconazole and fluconazole are consistent with reported data, which have demonstrated that both have efficacy against histoplasmosis [5,12–14,18]. As previously noted [14], we found itraconazole to be more efficacious than fluconazole. It should also be noted that the current study demonstrated that efficacy is reduced by the use of a more lethal immunosuppressed model. When efficacy was assessed in normal mice we found that D0870 cured up to 80% of treated animals, whereas <10% of treated immunosuppressed mice were free of infection [5]. This reduction in efficacy also occurred with fluconazole given on a once daily regimen; with a similar challenge, 100% of immunocompetent mice surviving 44 days [5], whereas 100% of immunosuppressed mice died by day 8. These results are also similar to that reported previously for fluconazole [12].

In conclusion, D0870 was highly efficacious against systemic histoplasmosis in immunosuppressed animals. Even with dosing schedules optimized for the pharmacokinetics of the comparator drugs and the use of HPBC diluent for itraconazole to increase its bioavailability, D0870 was >20 times as potent as itraconazole or fluconazole when the drugs were compared on a
milligram-per-kilogram basis; itraconazole was more than ten-fold more efficacious than fluconazole. These results indicate that D0870 is a promising treatment for histoplasmosis in the immunocompromised host and that further studies and clinical trials are warranted.

Acknowledgements

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References