Efficacy of Ravuconazole in Treatment of Systemic Murine Histoplasmosis

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Ravuconazole (RCZ) was evaluated for efficacy in comparison to fluconazole (FCZ) and itraconazole (ITZ) in murine models of disseminated histoplasmosis. All regimens tested prolonged survival ($P < 0.05$ to 0.0001). At equivalent doses of 50 mg/kg of body weight, RCZ and ITZ were equally effective and RCZ was more effective than FCZ ($P = 0.02$). Clearance of fungal burden from the livers and spleens of mice showed RCZ and ITZ at doses of 50 mg/kg to be efficacious but not curative. These data indicate that RCZ should be studied further.

Disseminated histoplasmosis occurs in both healthy and immunocompromised patients. Therapy is necessary for these patients, with amphotericin B and itraconazole (ITZ) treatments being used most often. However, these are often non-curative therapies, and relapse and recrudescence can occur while patients are on therapy (2, 9, 15, 18, 24, 25). Improved therapeutic options are still necessary. As a result, over the last several years a number of experimental antifungal compounds have been tested in experimental models for their activities against *Histoplasma capsulatum* (4, 5, 10–12).

Recently, a new thiazole-containing triazole, ravuconazole (RCZ) (BMS 207147; Bristol-Myers Squibb, Princeton, N.J.), has been shown to be active both in vitro and in vivo against a variety of fungal pathogens (1, 6–8, 13, 14, 19, 20, 23, 26). This activity has been demonstrated in vivo against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* (13, 14). To further examine the spectrum of activity of RCZ against endemic mycoses, we compared the efficacies of RCZ, fluconazole (FCZ), and ITZ for the treatment of experimental systemic histoplasmosis in mice. Our results indicate that the efficacy of RCZ is comparable or superior to those of FCZ and ITZ against histoplasmosis.

The in vitro activity of RCZ against *H. capsulatum* G217B was tested by NCCLS methods as described previously (22). An experimental model of systemic histoplasmosis was established as described previously (4, 5). Two experiments were done using 6-week-old female CD-1 mice (average weight, 22.8 or 24.4 g; Charles River Laboratories, Portage, Mich.), which were provided food and water ad libitum. In brief, the mice were infected intravenously with 2.18×10^6 (for the survival study) or 1×10^6 (for the CFU study) viable yeasts of *H. capsulatum* G217B. Therapy began 4 days after infection. In the survival study, groups of 10 mice were given diluent (10% dimethyl sulfoxide–5% Tween 80–saline), 1, 10, or 50 mg of RCZ in this diluent per kg of body weight, 10 or 50 mg of FCZ (Pfizer, Groton, Conn.) per kg, or 50 mg of ITZ (Janssen

Pharmaceuticals, Beerse, Belgium) per kg. FCZ was suspended in 0.3% Noble agar, and ITZ was prepared in hydroxypropyl- β -cyclodextrin as described previously (16, 17); these diluents have been demonstrated in previous studies to have efficacies equivalent to that of no treatment (data not shown). All treatments were given in 0.1-ml doses by gavage once daily on days 4 through 19 of infection. In the CFU study, mice were given no treatment or 50 mg of RCZ or ITZ per kg beginning 4 days after infection for 12 consecutive days.

Numbers of deaths were tallied through 42 days (for the survival study) or 20 days (for the CFU study) of infection. All surviving mice were euthanized by CO₂ asphyxiation, and their spleens and livers were removed aseptically. The number of CFU in each organ was determined by quantitative plating of organ homogenates as described previously and expressed as the log₁₀ number of CFU per entire organ (4, 5). The lower limit of detection for determining the number of CFU in an entire organ is approximately 5 colonies.

Statistical analyses comparing the survival rates among the various treatment groups were done using a log rank test contained in Prism (version 3.02 for Windows; GraphPad Software, San Diego, Calif.). Comparison of residual burdens of *H. capsulatum* in the livers and spleens of surviving animals was done using the Mann-Whitney U test as previously described (4, 5, 21).

RCZ proved to have good in vitro activity against *H. capsulatum*, with an MIC of 0.024 μ g/ml and a minimum fungicidal concentration (MFC) of 0.097 μ g/ml. In comparison, FCZ had an MIC of 3.1 μ g/ml and an MFC of 6.3 μ g/ml (5) and ITZ had an MIC of 0.10 μ g/ml and an MFC of 1.56 μ g/ml for this strain of *H. capsulatum*. Thus, in terms of MICs, RCZ was about 4 times more active than ITZ and 129 times more active than FCZ; a comparison of MFCs showed RCZ to be 16 times and 65 times more active than ITZ and FCZ, respectively. However, *H. capsulatum* is considered susceptible to all three drugs and all three MFCs are below concentrations in serum achievable with the corresponding drug (3, 14, 16, 17).

The results of the long-term survival study indicate that the model of systemic histoplasmosis established with CD-1 mice was extremely acute and lethal (Fig. 1). All untreated and diluent-treated mice succumbed to infection within 7 days. All drug regimens proved efficacious in the prolongation of sur-

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FIG. 1. Cumulative mortality rates of mice infected intravenously with *H. capsulatum* and left untreated or treated with RCZ, FCZ, ITZ, or RCZ-diluent (survival study).

vival in comparison with either control regimen ($P = 0.02$ to 0.0001 , depending on the comparison). Groups treated with 50 mg of RCZ or ITZ per kg had a 70 or 60% survival rate, respectively, versus a 20% survival rate for those treated with 50 mg of FCZ per kg (Fig. 1). Other regimens resulted in a survival rate of 30% or less. Comparisons showed that the efficacies of RCZ and ITZ at 50 mg/kg were equal. Both were superior to RCZ or FCZ at 10 mg/kg ($P = 0.03$ to 0.007 , depending on the comparison); RCZ at 50 mg/kg was superior to FCZ at 50 mg/kg ($P = 0.03$), and RCZ at 1 or 10 mg/kg was equivalent to FCZ at 50 mg/kg. However, neither RCZ nor ITZ given at 50 mg/kg was significantly more effective than RCZ at 1 mg/kg.

Recovery of *H. capsulatum* from the spleens and livers of surviving mice in the survival study indicated that none of the mice given 50 mg of RCZ per kg were free of infection, whereas two of the six surviving mice given 50 mg of ITZ per kg were free of infection in both organs; all other mice had infections in both organs (data not shown). However, the results of these treatments were equivalent statistically.

In the second experiment, the CFU study, a smaller infecting inoculum was used to reduce the overall mortality rate of the

mice in the model in order to compare the efficacies of ITZ and RCZ in the clearance of infectious burden. No RCZ- or ITZ-treated mice died, whereas 7 of 11 control mice died between days 7 and 12 . Statistical comparison showed that both RCZ and ITZ significantly prolonged survival ($P = 0.003$). Figure 2 shows the numbers of CFU recovered from the surviving animals. The mean burdens of *H. capsulatum* in the spleens and livers of untreated mice were significantly higher than they were after treatment with either RCZ or ITZ ($P < 0.001$). Comparison of the burdens of RCZ- and ITZ-treated mice showed that both treatments were equally effective in controlling infection in the livers ($P > 0.05$). In the spleens, ITZ-treated mice had a lower mean burden than RCZ-treated mice ($P = 0.012$). Neither treatment cured any animals of infection in either organ assayed.

Both the survival study and the CFU study indicate that RCZ has efficacy in treating experimental systemic murine histoplasmosis. This efficacy was only partially dose responsive in the prolongation of survival in that RCZ at 50 mg/kg was significantly superior to RCZ at 10 mg/kg but not to RCZ at 1 mg/kg. In comparison with FCZ, RCZ was superior (a 50 -mg

FIG. 2. Scattergram plot (CFU study) of individual CFU burdens on day 20 postinfection in the spleens and livers of surviving mice that were not treated or treated with RCZ or ITZ at 50 mg/kg. Horizontal lines represent mean numbers of CFU, closed symbols indicate numbers of CFU from the spleens, and open symbols indicate numbers of CFU from the livers. Only 4 of 11 untreated mice survived.

dose of RCZ per kg was more effective than an equal dose of FCZ), perhaps as much as 50 times more potent in a milligram-per-kilogram comparison (1 mg of RCZ per kg was equivalent to 50 mg of FCZ per kg). The results of both studies indicate that RCZ and ITZ could be considered equal in efficacy at the 50-mg/kg doses. However, the CFU study showed that neither RCZ nor ITZ at 50 mg/kg was curative.

It is possible that a twice-daily dosing regimen would improve the efficacy of RCZ in this model. RCZ and FCZ have been reported to have half-lives ($t_{1/2}$) of about 4 h in mice (3, 14), whereas ITZ has been reported to have a $t_{1/2}$ of less than 2 h in mice (14). However, ITZ given in cyclodextrin appears to have a prolonged $t_{1/2}$ (16) which would be at least equivalent to those of RCZ and FCZ. Peak levels of RCZ after the administration of a single dose occurred after 6 h and remained higher than the MICs reported here for *H. capsulatum* for at least 12 h (14). Both FCZ and ITZ have improved efficacies against histoplasmosis when they are given on a twice-daily schedule (4). Thus, additional studies are necessary to answer the questions of the comparative efficacies of those drugs when dosing is more frequent. The diluent formulation of RCZ used in our studies might not result in optimal bioavailability. It has been reported that use of a diluent of dimethyl sulfoxide-carboxymethyl cellulose results in a rate of RCZ absorption similar to that of ITZ in mice (14). Formulation of ITZ in cyclodextrin has been shown to dramatically improve the drug's pharmacokinetics and bioavailability, resulting in an improved therapeutic outcome (16, 17). Thus, testing different formulations to improve the bioavailability of RCZ should be considered. Overall, our studies clearly indicate that RCZ has efficacy against this infection and should be studied further for its activity against systemic histoplasmosis.

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