Activity of Posaconazole against *Pseudallescheria boydii*: In Vitro and In Vivo Assays

Gloria M. González, Rolando Tijerina, Laura K. Najvar, Rosie Bocanegra, Michael G. Rinaldi, David Loebenberg, and John R. Graybill

Division of Infectious Diseases (7881), Department of Medicine, and Department of Pathology, The University of Texas Health Science Center at San Antonio, and Audie L. Murphy Division, South Texas Veterans Health Care System, San Antonio, Texas 78229-3900; Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico; and Schering-Plough Research Institute, Kenilworth, New Jersey 07033-0530

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Thirty isolates of *Pseudallescheria boydii* were tested to compare the in vitro activity of posaconazole with those of fluconazole and itraconazole, using NCCLS methods. Posaconazole was evaluated in an immunosuppressed mouse model of disseminated pseudallescheriasis. Posaconazole was more effective than itraconazole and as effective as fluconazole in preventing death and significantly reducing the CFU of *P. boydii* from tissues.

In the last few decades, *Pseudallescheria boydii* has emerged as an important human pathogen, particularly in the immunocompromised host (1, 13, 14). The diagnosis of invasive pseudallescheriasis is difficult to make histologically, since the tissue specimens show only sepsate, branching hyphae that resemble *Aspergillus*. This is a major problem frequently leading to unsuitable therapy, since amphotericin B (AMB) is the treatment of choice for aspergillosis but *P. boydii* is resistant to AMB.

It has been reported that *P. boydii* exhibits resistance to AMB in vitro and resistance to fluconazole and that many isolates are only moderately susceptible to miconazole, ketoconazole, and itraconazole (ITRA) (3, 7, 10). There have been some reports of successful treatment of fungal infections with miconazole, ketoconazole, and the newer triazole voriconazole (2, 4, 8). Posaconazole (POS) (SCH 56592) is a new antifungal agent currently undergoing clinical development by Schering-Plough Research Institute (Kenilworth, N.J.). POS is a broad-spectrum triazole and has been shown to have potent in vitro and in vivo activity against *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., and numerous other opportunistic fungi that are likely to be encountered clinically (6, 11, 12).

The purpose of this study was to evaluate the in vitro and in vivo activities of POS against *P. boydii*. We compared the activity of POS with the activities of the triazoles ITRA and fluconazole (FLU) against a series of *P. boydii* strains using National Committee for Clinical Laboratory Standards (NCCLS) methods. An animal model of disseminated pseudallescheriasis was used to determine the in vivo efficacies of POS and other drugs.

Thirty clinical isolates of *P. boydii* were used in this study. All isolates were obtained from the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio. Each isolate of *P. boydii* was grown for 10 days at 35°C on potato flake agar slants. Isolates were evaluated using the NCCLS broth macrodilution proposed standard reference method M38-P for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi (9). The conidial suspensions were vortexed and adjusted for a transmittance of 68 to 70% at 530 nm in a spectrophotometer (Spectronic 21; Milton Roy Company); this was verified by plating 10 μL of each inoculum onto potato dextrose agar plates, incubating the plates at 35°C, and measuring the resulting growth. POS (Schering-Plough Research Institute) and ITRA (Janssen Pharmaceutica, Beerse, Belgium) were dissolved in polyethylene glycol 400 (Sigma, St. Louis, Mo.). FLU (Pfizer Central Research, Groton, Conn.) was dissolved in sterile distilled water. The final drug concentrations were as follows: POS and ITRA, 0.015 to 8 μg/ml; and FLU, 0.125 to 64 μg/ml. *Pseudallescheria boydii* was subcultured in sterile potato flake agar slants. Isolates were evaluated using the NCCLS broth macrodilution proposed standard reference method M38-P for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi (9). The conidial suspensions were vortexed and adjusted for a transmittance of 68 to 70% at 530 nm in a spectrophotometer (Spectronic 21; Milton Roy Company); this was verified by plating 10 μL of each inoculum onto potato dextrose agar plates, incubating the plates at 35°C, and measuring the resulting growth. POS (Schering-Plough Research Institute) and ITRA (Janssen Pharmaceutica, Beerse, Belgium) were dissolved in polyethylene glycol 400 (Sigma, St. Louis, Mo.). FLU (Pfizer Central Research, Groton, Conn.) was dissolved in sterile distilled water. The final drug concentrations were as follows: POS and ITRA, 0.015 to 8 μg/ml; and FLU, 0.125 to 64 μg/ml. A *Paecilomyces variotii* control strain, UTHSC 90-459, was included for all testing. All testing was performed in duplicate.

Outbred ICR mice 4 to 6 weeks old (25 to 30 g) purchased from Harlan Sprague Dawley Inc. were used in all experiments. There were 12 mice in each treatment or control group for the survival study, and groups of eight mice were used in the tissue burden study. Animal studies were repeated for verification of data. Mice were housed in cages of four mice each and were provided food and water ad libitum.

For all experiments, mice were immunosuppressed by one intraperitoneal injection of cyclophosphamide (Bristol-Myers Squibb Co., Princeton, N.J.) at a dose of 200 mg/kg of body weight given 24 h before inoculation of the fungus. This immunosuppression regimen produced absolute neutrophil counts of 2.48 × 10^5, 0.11 × 10^5, and 11.48 × 10^5/μL at days 1, 4, and 7 after administration, respectively.

Conidia of a single clinical isolate of *P. boydii* (UTHSC 00-180) were used in in vivo experiments. *P. boydii* was subcultured onto potato dextrose agar plates for 10 days at 35°C. The mycelium was overlaid with 0.85% sterile saline solution, and suspensions were made by gently scraping the colonies with a microbiological loop. Cell suspensions were filtered through glass wool, washed three times, and suspended in
saline, and the cells were counted using a hemocytometer. To corroborate hemocytometer counts, diluted cell suspensions were cultured onto potato dextrose agar plates at 35°C for 48 h. Mice received an intravenous injection of 10⁵ conidia. Prior experiments had shown that this number of conidia caused the mice to die within 6 days of infection (5).

For in vivo assays, POS was provided as a suspension, diluted with sterile distilled water, and administered orally in a 0.2-ml volume. POS was given in doses of 0.5, 1, 5, 10, 30, and 50 mg/kg once daily (OD) and 25 mg/kg twice a day (BID), FLU was administered orally at 20 mg/kg BID, and ITRA cyclodextrin solution was administered orally at 30 mg/kg three times a day (TID); all drugs were given on days 1 through 10 postinfection. A control group received oral sterile distilled water. Deaths were recorded through day 20 postinfection. Moribund mice were sacrificed, and deaths were recorded as occurring on the next day. Animals that survived to day 20 were sacrificed by inhalation of metofane, followed by cervical dislocation. The kidneys and brains were removed aseptically, organs were homogenized in 2 ml of sterile saline, and the entire organs were plated onto potato dextrose agar and incubated at 35°C for 4 days.

For tissue burden studies, mice received POS at 10 and 40 mg/kg OD, FLU at 5 and 20 mg/kg BID, or ITRA at 30 mg/kg TID on days 1 through 7. As in the survival studies, control mice were treated orally with sterile distilled water, and mice were sacrificed on day 8. The kidneys, spleens, and brains of dead mice and sacrificed survivors were assessed for fungal burdens utilizing quantitative culture. Organs were removed aseptically, weighed, and transferred to sterile glass homogenizers containing 2 ml of sterile saline. Serial 10-fold dilutions of the suspensions were plated onto potato dextrose agar and incubated at 35°C for 4 days to determine the number of viable CFU in each organ.

For survival studies, the log rank and Wilcoxon tests were used. P values for determining significance varied because of correction for multiple comparisons. For tissue burden studies, Dunnett’s two-tailed t test or the rank sum test (Wilcoxon scores) was used. Values were considered significantly different from the control values at a P value of ≤0.05.

The MIC ranges of POS, ITRA, and FLU 72 h after the drug was given were 0.125 to 1, 0.5 to 4, 32 to >64 µg/ml, respectively. The MICs (in micrograms per milliliter) necessary to inhibit 50 and 90% of isolates, respectively, were as follows: 0.5 and 1 for POS; 1 and 4 for ITRA; and 64 and >64 for FLU.

The results of the survival study are displayed in Fig. 1. Control mice started to die at day 3, and all died by day 8. Ninety percent of mice treated with POS at 0.5 and 1 mg/kg
TABLE 1. Fungal burdens in spleens, kidneys, and brains of mice infected with P. boydii strain 00-180

<table>
<thead>
<tr>
<th>Treatment group (mg/kg)</th>
<th>Range of CFU/g (10^3) ± SD</th>
<th>Mean log_{10} CFU/g of organ/P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleens</td>
<td>Kidneys</td>
</tr>
<tr>
<td>None (control)</td>
<td>3.9–10 ± 1.8</td>
<td>30–90 ± 19</td>
</tr>
<tr>
<td>POS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10, OD</td>
<td>0.2–3.8 ± 1.2</td>
<td>16–74 ± 21</td>
</tr>
<tr>
<td>40, OD</td>
<td>0.2–1.1 ± 0.32</td>
<td>0.3–1.3 ± 0.4</td>
</tr>
<tr>
<td>ITRA 30, TID</td>
<td>0.7–6.4 ± 2.3</td>
<td>24–97 ± 29</td>
</tr>
<tr>
<td>FLU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5, BID</td>
<td>3–9.3 ± 2</td>
<td>16–82 ± 29</td>
</tr>
<tr>
<td>20, BID</td>
<td>0.24–2.4 ± 0.7</td>
<td>1–13 ± 7.2</td>
</tr>
</tbody>
</table>

* Values that were significantly lower (P < 0.05) than the control values are indicated by asterisks. NA, not applicable

were dead by the end of the treatment. Eighty percent of mice treated with POS at 10 mg/kg and ITRA at 30 mg/kg TID were dead by the end of the treatment. There was no statistically significant difference in survival between treatment with doses of 0.5, 1, 5, and 10 mg/kg of POS and ITRA at 30 mg/kg TID (P > 0.05). However, there were significant improvements in survival results using higher doses of POS. Wilcoxon test showed a statistical difference among these survival curves. POS at concentrations of 30 and 50 mg/kg OD or 25 mg/kg BID significantly prolonged survival of mice (70 to 75%) compared to that of the control group (P < 0.0001). FLU at a dose of 20 mg/kg BID also prolonged survival of mice (55%) compared to survival of control mice (P < 0.002).

To assess the activity of each antifungal agent on the fungal load of P. boydii, comparative quantitative cultures from spleens, kidneys, and brain homogenates were determined. The results are shown in Table 1. Treatment with ITRA at 30 mg/kg TID and POS at 10 mg/kg OD did not reduce the fungal burden in the kidneys and brains (P > 0.05) as effectively as treatment with FLU at 20 mg/kg BID and POS at 40 mg/kg once daily. Fungal burden results showed that POS at 40 mg/kg OD and FLU at 20 mg/kg BID significantly reduced the fungal load in all three tissues from those of untreated controls (P < 0.0002).

This study demonstrated that POS was more active in vitro against P. boydii than ITRA and FLU. However, in vivo experiments POS was effective only at higher doses (>25 mg/kg), confirming the relative resistance of this organism to these drugs and suggesting that maximum dosages of POS may have to be used, particularly in the setting of neutropenia. In general, a dose-related response in mouse mortality was observed with POS. One hundred percent survival was not achieved under our experimental conditions. It is remarkable that survival of approximately 75% could be obtained with POS in this model.

FLU did not appear to be active against P. boydii in vitro, with MICs ranging from 32 to >64 μg/ml 72 h after FLU was given under carefully controlled conditions. However, treatment with FLU at 20 mg/kg BID showed 50% survival in this model of disseminated pseudallescheriasis, and the quantitative tissue burden results were similar to the results for POS treatment (40 mg/kg OD).

ITRA at 30 mg/kg TID had a minimal effect on survival and on the number of organisms in tissues. Despite the fact that both POS and FLU were effective in controlling systemic pseudallescheriasis, neither drug was able to eradicate the fungus from the tissues, at least not at the dosage schedules employed. However, the in vivo data from this study indicate that POS and FLU may be suitable alternatives in the treatment of disseminated P. boydii infection.

REFERENCES