Efficacy of Posaconazole in Murine Experimental Sporotrichosis

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We developed a murine model of systemic sporotrichosis by using three strains of each of the two commonest species causing sporotrichosis, i.e., Sporothrix schenckii sensu stricto and Sporothrix brasiliensis, in order to evaluate the efficacy of posaconazole (PSC). The drug was administered at a dose of 2.5 or 5 mg/kg of body weight twice a day by gavage, and one group was treated with amphotericin B (AMB) as a control treatment. Posaconazole, especially at 5 mg/kg, showed good efficacy against all the strains tested, regardless of their MICs, as measured by prolonged survival, tissue burden reduction, and histopathology.

Sporotrichosis is the most common and cosmopolitan subcutaneous mycosis (13). The most usual clinical manifestation of the disease is the subacute or chronic lymphocutaneous form, followed by fixed cutaneous infections (5); however, disseminated infections have also been described for those with underlying alcoholism and for immunosuppressed patients, especially those with AIDS or those receiving chemotherapy or corticoids (11, 13, 17). Sporotrichosis is caused by a group of species belonging to the Sporothrix schenckii complex which have various individual in vitro responses to antifungal agents. The differential antifungal activity among Sporothrix species could explain the variability in antifungal susceptibility reported for S. schenckii in studies prior to the recent recognition of the S. schenckii complex (5, 20, 21).

The recommended procedures for the management of sporotrichosis include local measures such as hyperthermia and systemic measures such as administration of a saturated solution of potassium iodide or administration of azoles (itraconazole and fluconazole), amphotericin B, or terbinafine (15). Itraconazole has become the drug of choice for treatment of the lymphocutaneous and cutaneous forms. In those patients with intolerance to itraconazole, fluconazole is the recommended alternative. Amphotericin B is the first choice for the treatment of disseminated sporotrichosis, and itraconazole is recommended as the step-down therapy after patients respond to the initial treatment with amphotericin B (18).

Posaconazole has shown activity in vitro against the species of the S. schenckii complex (21). Since treatment options for disseminated sporotrichosis are limited and there is only scarce information on the effectiveness of posaconazole in vivo, we evaluated the response to this drug in a murine model of disseminated sporotrichosis.

MATERIALS AND METHODS

Fungi were stored in slant cultures covered with sterile paraffin oil and subcultured on potato dextrose agar (PDA) plates at 30°C for 7 days.

In vitro antifungal susceptibility to posaconazole of 5 isolates of S. brasiliensis and 10 of S. schenckii sensu stricto was determined using a broth microdilution method according to the CLSI guidelines for filamentous fungi (8).

For the in vivo studies, strains of S. brasiliensis (n = 3) and S. schenckii sensu stricto (n = 5) showing different MIC values were chosen. The inocula were prepared by flooding the surface of an agar plate with saline solution and scraping the sporulating mycelium. The resulting solutions were transferred to 100 ml of potato dextrose broth (PDB) and incubated in an orbital shaker at 150 rpm at 30°C for 5 days. Cultures were then filtered twice through sterile gauze and centrifuged at 325 × g. The pellets were washed once with saline solution, and the conidium concentrations were adjusted to the desired concentrations by hemocytometer counting. To verify the viability and size of inocula, 10-fold dilutions were placed in PDA to determine the CFU.

Four-week-old OF-1 male mice (Charles River, Cripta S.A., Barcelona, Spain) with a mean weight of 30 g were used. Animals were housed in standard boxes with corncob bedding and free access to food and water. All animal care procedures were supervised and approved by the University Rovira i Virgili Animal Welfare and Ethics Committee. Mice were infected intravenously (i.v.) in the lateral tail vein with 2 × 107 CFU of fungi in 0.2 ml of sterile saline. This inoculum level was chosen based upon previous studies with strain FMR 8314 that indicated that this concentration was the minimum dose that killed all the infected animals within 18 days (data not shown).

Posaconazole, provided as Noxfil (Schering-Plough Ltd., Hertfordshire, United Kingdom), was administered at 2.5 or 5 mg/kg of body weight twice a day (BID) by gavage. These doses were chosen based upon preliminary studies using higher (10 and 20 mg/kg BID) and lower (1 and 2 mg/kg BID) doses of posaconazole (Fig. 1). Controls received no treatment. The efficacy of posaconazole was evaluated as prolonged survival, reduced tissue burden, and differences in histopathology. Treatments began 1 day after infection and lasted for 18 days. For survival studies, groups of 10 mice were randomly established for each strain and each treatment and checked daily for 30 days after challenge. For tissue burden studies, groups of 10 mice were also established, and the animals were sacrificed on day 13 postinfection in order to compare the results with controls. The liver and spleen, which are the most affected organs in experimental systemic sporotrichosis (2), were removed aseptically, and one half of each was homogenized in 1 ml sterile saline. Serial 10-fold dilutions of the homogenates were plated on PDA and incubated for 72 h at 30°C. The CFU/g of tissue were calculated. For the histopathology study, half of each organ was fixed with 10% buffered formalin. Samples were dehydrated, paraffin embedded, and sliced into 2-μm sections, which were then stained with hematoxylin-eosin (H-E), periodic acid-Schiff (PAS) stain, or Grocott methenamine silver and examined in blinded fashion by light microscopy. Additionally, one group of 10 animals was infected with S. brasiliensis FMR 8326 and treated i.v. with amphotericin B (Fungizone; Squibb Industria Farmacéutica S.A., Barcelona, Spain) at 0.8 mg/kg for 18 days.

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Statistical analysis was performed using Graph Pad Prism 5 for Windows. The mean survival time was estimated by the Kaplan-Meier method and compared among groups by using the log rank test. The colony counts from tissue burden studies were analyzed using the Mann-Whitney U test. The observed differences were considered statistically significant for P values of <0.05.

RESULTS
Two of 5 S. brasiliensis strains tested and 4 of 10 S. schenckii sensu stricto strains tested showed posaconazole MICs of 0.5 to 1 µg/ml, which are close to the suggested breakpoint indicative of the susceptibility of filamentous fungi, i.e., ≤1 µg/ml for posaconazole (8). The other strains of S. brasiliensis (3) showed a MIC of 2 µg/ml (intermediate susceptibility). Three strains of S. schenckii sensu stricto showed intermediate susceptibility, and another 3 showed MICs of >2 µg/ml.

The posaconazole MICs for those strains included in the in vivo study were as follows: for S. brasiliensis, 0.5 µg/ml (FMR 8319), 1 µg/ml (FMR 8314), and 2 µg/ml (FMR 8326); and for S. schenckii sensu stricto, 1 µg/ml (FMR 8606), 2 µg/ml (FMR 8609 and FMR 9018), 4 µg/ml (FMR 9010), and 8 µg/ml (FMR 8604).

Systemic infection caused 100% mortality in untreated control groups within 11 to 18 days after challenge, regardless of the species or strain tested, with the exception of strains of S. schenckii sensu stricto with high MICs (FMR 9010 and FMR 8604), which did not cause death; these strains were discarded from the treatment study.

Posaconazole given at 2.5 or 5 mg/kg BID significantly prolonged survival with respect to the controls (Fig. 2), and all treated animals survived to the end of the experiment, regardless of the species and strain tested.

Posaconazole given at 2.5 or 5 mg/kg BID was able to significantly reduce the fungal loads in the liver and spleen in comparison to the control group for animals infected with S. schenckii sensu stricto or S. brasiliensis (Fig. 3). Moreover, the high dose of posaconazole significantly reduced the fungal loads in both organs in comparison to the low dose (P < 0.05). Amphotericin B was effective at reducing the fungal loads in both organs in comparison to controls (P < 0.0001), despite the fact that the amphotericin B MIC for the tested strain was 4 µg/ml.

Histopathological studies of control mice showed an abundance of typical cigar-shaped cells in the liver and spleen. In mice infected with S. schenckii sensu stricto, these fungal cells were usually surrounded by a granuloma, while in those infected with S. brasiliensis a massive infiltration of tissue with fungal cells was observed, with an absence of granulomas (Fig. 4A and B). In general, there was a scarcity of fungal cells in mice treated with posaconazole at 2.5 mg/kg BID (Fig. 4C), with a recovery of tissue structure, and in both those treated with posaconazole at 5 mg/kg BID (Fig. 4D) and those treated with amphotericin B, fungal elements were not observed and tissue structure was normal.

DISCUSSION
Although cases of systemic sporotrichosis are fortunately not frequent, several cases have been described (1, 3, 15, 19, 22, 26). Amphotericin B is the most common drug used in the treatment of disseminated sporotrichosis, but the dosage is limited by its toxicity and by the long-term therapy required.

In this study, we evaluated the activity in vitro and efficacy in vivo of posaconazole against several strains of S. schenckii sensu stricto and S. brasiliensis. Only a few previous studies evaluated the in vitro activity of posaconazole against Sporothrix spp., and they reported very variable results, with MIC values ranging from 0.03 to 16 µg/ml. The MICs obtained in our study are consistent with those obtained by previous authors (12, 14, 21, 25), although they did not establish MICs for each individual species of the complex as was done later by Marimón et al. (21). Although some studies have evaluated the efficacy of some antifungals (16) and/or determined the virulence of strains of various origins (10), all of them were carried out prior to the reclassification of Sporothrix organisms as a species complex. Only a comparative study on virulence in mice had been carried out for this complex of species (2). Our study shows that the spleen and liver have high fungal loads, in agreement with previous reports on animal models of sporotrichosis (2, 9, 10). To our knowledge, this is the first study to explore the efficacy of posaconazole in a murine model of disseminated sporotrichosis. Our results demonstrated good efficacy of posaconazole administered at 2.5 or 5 mg/kg BID against disseminated sporotrichosis caused by the two species tested. In addition, lower (1 or 2 mg/kg BID) and higher (10 or 20 mg/kg BID) doses showed in vivo efficacy in our model of infection. However, the best statistical significance was observed at 2.5 mg/kg and 5 mg/kg BID. These results should be considered ex-
excellent for prolonging survival, since none of the infected mice died during the experiment, even considering that the doses were relatively low in comparison with those tested in other studies of treatment of infections by other fungi under similar conditions (7, 23, 24). For instance, a dose of 20 or 30 mg/kg BID against Cryptococcus gattii (7), Rhizopus oryzae (25), or the Aspergillus terreus complex (24) achieved a survival rate of only 20%, 40%, or 60%, respectively.

Surprisingly, our results indicate that outcome does not seem to correlate with the MIC in the range observed among our strains, since in those strains with a MIC of 0.5 or 2 μg/ml, tissue burden reductions were similar (approximately 4 to 5 log with respect to the control group). Furthermore, we also observed that the efficacies of posaconazole were similar against the two species tested.

As demonstrated experimentally (16) and corroborated in clinical reports (19, 26), amphotericin B is effective for the treat-
FIG 3 Effects of antifungal treatment with posaconazole (PSC) (A to F) or amphotericin B (AMB) (G) on fungal loads of mice infected with the indicated strains. (A) S. brasiliensis FMR 8314 (MIC = 1 μg/ml); (B) S. brasiliensis FMR 8319 (MIC = 0.5 μg/ml); (C) S. brasiliensis FMR 8326 (MIC = 2 μg/ml); (D) S. schenckii sensu stricto FMR 9018 (MIC = 2 μg/ml); (E) S. schenckii sensu stricto FMR 8609 (MIC = 2 μg/ml); (F) S. schenckii sensu stricto FMR 8606 (MIC = 1 μg/ml); (G) FMR 8326 (MIC = 4 μg/ml). a, P < 0.005 versus control; b, P < 0.05 versus PSC 2.5. Posaconazole was administered BID by gavage at 2.5 mg/kg or 5 mg/kg. Amphotericin B was administered at 0.8 mg/kg i.v. once a day. Horizontal lines indicate median values.
ment of disseminated sporotrichosis. It should be noted that *Sporothrix* infections are frequently chronic and consequently require long-term therapy. Our results suggest that treatment with posaconazole could be an alternative to that with amphotericin B. Despite the reductions in fungal load in posaconazole-treated animals being lower than those achieved by using amphotericin B, the reductions were notably significant with respect to untreated animals being lower than those achieved by using amphotericin B, itraconazole, posaconazole and terbinafine in infected animals, and together with the reduced toxicity reported for posaconazole, this allows us to propose posaconazole as an alternative therapeutic for sporotrichosis. However, further studies including more strains and treatment durations are required in order to establish the usefulness of posaconazole against sporotrichosis.

REFERENCES


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