Murine model of a disseminated infection by the novel fungus *Fonsecaea monophora* and successful treatment with posaconazole

Running title: Posaconazole against *Fonsecaea monophora*.

Enrique Calvo, F. Javier Pastor, M. Mar Rodríguez, Emilio Mayayo, Valentina Salas, and Josep Guarro*

Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, IISPV, Universitat Rovira i Virgili, Reus, Spain

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* Corresponding author. Mailing address: Unitat de Microbiologia, Facultat de Medicina, Universitat Rovira i Virgili. Carrer Sant Llorenç, 21432 Reus, Spain. Phone 977-759359. Fax: 977-759322. E-mail: josep.guarro@urv.cat
ABSTRACT

We have evaluated the efficacy of posaconazole, amphotericin B and itraconazole in a murine model of disseminated infection by *Fonsecaea monophora*. Of those three antifungals drugs tested, Posaconazole prolonged survival significantly and reduced the fungal load in most of the organs tested. Bioassay studies demonstrated the relationship between posaconazole levels and dose escalation in serum and brain tissue. Posaconazole may have a clinical role in the treatment of disseminated infections by *F. monophora*.
The dematiaceous fungus *Fonsecaea monophora* is a causal agent of cerebral phaeohyphomycosis (10) and chromoblastomycosis (11-13). On the basis of molecular studies (3, 7), this fungus has recently been segregated from *Fonsecaea pedrosoi*, a traditionally well-known pathogen. Since very little is known about the pathogenicity and antifungal susceptibility of this novel fungus, the aim of this study was to develop a murine model of disseminated infection by *F. monophora* to evaluate its virulence and compare the therapeutic efficacy of amphotericin B (AMB), itraconazole (ITZ) and posaconazole (PSC).

Two clinical strains of *Fonsecaea monophora* were used: CBS 269.37 and CBS 117236. Their *in vitro* antifungal susceptibility tests (8) showed MICs of 1 µg/ml for AMB, 0.5 and 0.25 µg/ml for ITZ respectively, and 0.25 and 0.12 µg/ml for PSC respectively.

Male OF1 mice were immunosuppressed by a single intraperitoneal (i.p.) injection of 200 mg/kg of cyclophosphamide plus 5-fluorouracil at 150 mg/kg intravenously one day prior to the infection.

**Development and characterization of an infection model.** For each strain, groups of 20 mice (10 for survival and 10 for tissue burden studies) were challenged with $2 \times 10^5$ CFU into the lateral tail vein. This was the lowest dose tested to produce an acute infection from which all the animals infected with the strain CBS 117236 died within 15 days post-infection (data not shown). Animals were checked daily for 30 days. For the tissue burden study, mice were sacrificed on day 6 post-infection. Lungs, brain, spleen, liver and kidneys were aseptically removed and approximately half of each organ was weighed and homogenized in 1 ml of sterile normal saline. Dilutions of the homogenates were plated on PDA, incubated at 30°C and examined...
daily for 7 days. For the histopathology study, half of each organ was fixed, dehydrated, paraffin embedded, and sliced into 2 µm sections, which were stained with Haematoxylin-Eosin, Periodic acid Schiff or Grocott methamine silver. The Kaplan-Meier method and the log rank test were used for survival studies. When necessary, tissue burden studies were analysed using the Kruskal-Wallis test, the Mann-Whitney U-test and the Bonferroni correction. P<0.05, was considered statistically significant.

Virulence studies (Figure 1) did not reveal significant differences in mortality rates between the isolates (P=0.054). Fungi were present in all organs tested, and important differences were shown in the fungal loads of the different organs within and between both strains.

**Treatment studies.** Mice were challenged with 2x10⁵ CFU of the strain CBS 117236, or 1x10⁶ CFU of the strain CBS 269.37. Both inocula were chosen from previous studies and were able to produce acute infections, with all the animals dying within 15 days post-infection. The drugs assayed were: AMB (Fungizone), PSC (Noxafil), and ITZ (Canadiol). Their efficacy was evaluated by prolongation of survival and reduction of fungal load in kidneys, lungs, spleens and brains. The procedures were as indicated above. The different groups (20 mice) were treated as follows: AMB at 1.5mg/kg i.p. once daily; PSC at 10, 20 or 40 mg/kg orally once daily; and ITZ at 25 mg/kg orally twice daily. Control animals received no treatment. All treatments began 1 day after challenge and the therapy lasted for 7 days. An additional group of 5 mice were similarly infected with the strain CBS 269.37 and treated with the same doses used in the treatment study. These mice were used to determine, by bioassay, the level of each drug in serum and brain (1, 2, 4), 4 hours after last dosing on day 6 of therapy.
PSC 20 mg/kg and 40 mg/kg significantly prolonged survival with respect to the control group and to the other therapies, although the lower dose only in mice infected by the strain CBS 117236 (Figure 2).

PSC showed a dose response efficacy in fungal load reduction (Figures 3 and 4). PSC at 40 mg/kg was the most effective in reducing the fungal recovery in kidney for both strains and in lung for the strain CBS 269.37 with respect to the other therapies. For the strain CBS 117236, PSC at 10 or 20 mg/kg also improved fungal load in kidney and lung with respect to ITZ and AMB. All doses of PSC reduced significantly the fungal load in brain in both strains.

AMB only prolonged the survival of mice infected with the strain CBS 269.37 and ITZ was not able to prolong the survival of mice in any case. The ability of AMB and ITZ to reduce tissue burden was considerably lower than PSC.

At day 6 of treatment, for all treatments administered, antifungal levels in serum and brain were above the corresponding MICs. PSC levels increased with dose escalation (Table 1).

Histological studies only showed evidence of kidney and lung invasion by the strain CBS 117236 (Figure 5). Kidney sections showed glomerular and tubular invasion by hyphae. In lung, this isolate caused focal interstitial infiltration. None of the organs showed any inflammatory response.

In this study, PSC was the most effective drug in prolonging mice survival and showed a dose-dependent efficacy in reducing tissue burden. These results correlated with drug levels in serum and brain obtained by bioassay. Although there is little clinical data on the use of these drugs in the treatment of phaeohyphomycosis (9), PSC has also shown efficacy in murine studies of disseminated infections by other dematiaceous fungi (5, 6). ITZ has also been recommended in the treatment of such
infections (10). However, in our model the results obtained with this drug were very modest and clearly inferior to those of PSC.

*F. monophora* has been considered a neurotropic fungus (10). In our study, both strains, including an isolate from a brain abscess, were able to affect brain, although to a lesser degree than the other organs tested. Our data demonstrate a correlation between the *in vitro* activity of PSC, the brain concentration levels and efficacy of this drug, even at low concentrations, in brain tissue burden.

In conclusion, this work confirms PSC as an alternative to ITZ in the treatment of phaeohyphomycoses.

REFERENCES


Figure 1. Cumulative mortality of mice infected with $2 \times 10^5$ CFU of *F. monophora* CBS 269.37 and CBS 117236 (A). Colony counts in spleen, kidney, liver, lung and brain of mice infected with $2 \times 10^5$ CFU of *F. monophora* strain CBS 269.37 (B) and the strain CBS 117236 (C). Horizontal lines indicate mean values.
Figure 2. Cumulative mortality of mice infected with $2 \times 10^5$ CFU of *F. monophora* of the strain CBS 269.37 (A), and $1 \times 10^6$ CFU of the strain CBS 117236 (B); a, $P < 0.05$ versus control; b, $P < 0.05$ versus PSC 10 mg/kg; c, $P < 0.05$ versus PSC 20 mg/kg; d, $P < 0.05$ versus ITZ 50 mg/kg; e, $P < 0.05$ versus AMB 1.5 mg/kg.
Figure 3. Effects of the antifungal treatments on CFU counts in mice infected by CBS 269.37 in spleen, kidney, brain and lung. \(^a\), P < 0.003 versus control; \(^b\), P < 0.003 versus PSC 10 mg/kg; \(^c\), P < 0.003 versus PSC 20 mg/kg; \(^d\), P < 0.003 versus ITZ 50 mg/kg; \(^e\), P < 0.003 versus AMB 1.5 mg/kg. Horizontal lines indicate mean values.
Figure 4. Effects of the antifungal treatments on CFU counts in mice infected by *CBS 117236* in spleen, kidney, brain and lung. a, P < 0.003 versus control; b, P < 0.003 versus PSC 10 mg/kg; c, P < 0.003 versus PSC 20 mg/kg; d, P < 0.003 versus ITZ 50 mg/kg; e, P < 0.003 versus AMB 1.5 mg/kg. Horizontal lines indicate mean values.
Figure 5. (A) Kidney section showing hyphae and conidia of *F. monophora* CBS 117236 (P.A.S. x400). (B) Lung section with hyphae invasion of *F. monophora* CBS 117236 with focal interstitial infiltration (H-E. x400).
Table 1. Drug levels in serum and brain tissue measured by bioassay on day 6 of the therapy and 4 hours after last dosing. Results are expressed as the mean ± standard deviation.

<table>
<thead>
<tr>
<th>Drug and dose (mg/kg)</th>
<th>Serum (µg/ml)</th>
<th>Brain (µg/g)</th>
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<tbody>
<tr>
<td>PSC 10</td>
<td>5.17 ± 0.99</td>
<td>2.02 ± 0.19</td>
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<tr>
<td>PSC 20</td>
<td>6.35 ± 0.97</td>
<td>2.84 ± 0.74</td>
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<td>PSC 40</td>
<td>9.72 ± 0.57</td>
<td>6.73 ± 0.88</td>
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<td>ITZ 50</td>
<td>7.32 ± 3.44</td>
<td>6.88 ± 1.52</td>
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<tr>
<td>AMB 1.5</td>
<td>6.45 ± 0.54</td>
<td>5.71 ± 0.43</td>
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