Posaconazole efficacy in a murine disseminated infection caused by *Paecilomyces lilacinus*

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Received 18 August 2008; returned 6 October 2008; revised 11 November 2008; accepted 12 November 2008

**Objectives:** We have compared the efficacy of posaconazole and amphotericin B in an experimental murine model of paecilomycosis.

**Methods:** Immunosuppressed mice were treated with posaconazole at 25, 50, 75 or 100 mg/kg/day orally, amphotericin B at 1.5 or 3 mg/kg/day intraperitoneally or liposomal amphotericin B at 5 mg/kg/day intravenously. Treatment began 1 day after infection and continued for 10 days post-infection. Two strains of *Paecilomyces lilacinus* were tested.

**Results:** Posaconazole at 50 mg/kg/day was the only treatment able to significantly reduce fungal loads in the spleens, kidneys and livers of the mice infected by each of the two strains.

**Conclusions:** The results suggest that posaconazole may have a clinical role in the treatment of disseminated paecilomycosis.

Keywords: amphotericin B, murine model, paecilomycosis

**Introduction**

*Paecilomyces lilacinus* is a fungus distributed worldwide, which produces severe infections in immunocompromised hosts, although it can affect immunocompetent hosts too.1 A recent review of 119 infections caused by this fungus since 1964 has shown that although most of them were ocular, many other sites of the body were also affected.1 In general, these infections are very difficult to treat, recovery of neutropenia and removal of central venous catheters, if present, being crucial for resolving the infection. *P. lilacinus* shows a high *in vitro* resistance to the classical antifungal drugs, including amphotericin B, which is still the most commonly used drug despite its poor *in vitro* activity1 and its controversial *in vivo* results. While it usually fails in ocular, cutaneous and subcutaneous infections, it has shown efficacy in other types of infections.1 The novel triazoles have shown *in vitro* activity,1 but there has been little experience of its clinical use. Since posaconazole has showed good *in vitro* activity against *P. lilacinus*1 and favourable outcomes in several *in vivo* studies,2,3 we have evaluated the effectiveness of this drug against two strains of this fungus in murine models of *Paecilomyces* infection.

**Materials and methods**

The isolates tested were: FMR 5522 of clinical origin and FMR 8252 of environmental origin. Their *in vitro* antifungal susceptibility was assayed using a broth microdilution method following the CLSI (formerly the NCCLS) guidelines for filamentous fungi.4 The MICs were 32 mg/L for amphotericin B and 1 mg/L for posaconazole for both strains. The isolates were stored at −80°C in potato dextrose broth with glycerol, and prior to testing they were subcultured on potato dextrose agar (PDA) at 30°C for 7 days. On the day of infection, they were suspended in sterile saline and filtered through sterile gauze to remove clumps of cells or hyphae. The resulting suspensions, containing ≥95% conidia, were adjusted to the desired inoculum based on the haemocytometer counts. Dilutions of the original suspension were cultured on PDA plates to confirm the haemocytometer count.

OF1 male mice (Charles River, Criffa S.A., Barcelona, Spain) with a mean weight of 30 g were used. All animal care procedures were supervised and approved by the Universitat Rovira i Virgili Animal Welfare Committee. The efficacy of the drugs was evaluated by prolonging survival and reducing tissue burden. Survival studies were performed by lethal infection attained by using severe immunosuppression. Tissue burden studies were performed by a sublethal infection attained by using moderate immunosuppression. Severe immunosuppression was reached after a dose of cyclophosphamide at 200 mg/kg of body weight administered intraperitoneally on the day of infection and two doses of 5-fluorouracil, one of 150 mg/kg of body weight intravenously on the day of infection and another of 75 mg/kg on day 5. In prior studies, we found that this immunosuppressive regimen provokes severe neutropenia with polymorphonuclear leukocyte (PMN) counts of around 50/µL from days 3 to 14.

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or more (data not shown). In this case, the mice were challenged with $1.2 \times 10^8$ cfu/mouse for the strain FMR 5522 and $0.6 \times 10^8$ cfu/mouse for the strain FMR 8252, both inocula being chosen in previous studies (data not shown). Moderate immunosuppression was reached by a single administration of cyclophosphamide at 200 mg/kg of body weight intraperitoneally plus 5-fluorouracil at 150 mg/kg of body weight intravenously, given 1 day prior to the infection. Previously, we had demonstrated that with this immunosuppressive regimen, the peripheral blood PMN counts were <100/μL from days 3 to 9 or more. Mice were challenged with $1.2 \times 10^7$ cfu/mouse; and $0.6 \times 10^7$ cfu/mouse for the strains FMR 5522 and FMR 8252, respectively; both inocula were chosen in previous studies (data not shown).

Amphotericin B, purchased as Fungizone (Squibb Industria Farmacéutica S.A., Barcelona, Spain), was administered at doses of 1.5 or 3 mg/kg of body weight once daily intraperitoneally; liposomal amphotericin B, kindly provided by Gilead Sciences S.A. (Madrid, Spain), was administered at a dose of 5 mg/kg of body weight/dose once daily intravenously; and posaconazole, purchased as Noxafil (Schering Plough Ltd, Hertfordshire, UK), was administered at doses of 25, 50, 75 or 100 mg/kg of body weight/dose once daily orally by gavage. To prevent bacterial infection, all mice received ceftazidime (5 mg/day subcutaneously) from days 1 to 10 post infection.

Treatments were started 24 h after challenge and lasted for 10 days. Control animals received no treatment. Groups of 10 mice were randomly established for each strain and each treatment. Mice were checked daily for 30 days. For tissue burden studies, groups of 10 mice were randomly established too. The animals were sacrificed on day 11 post-infection. The livers, spleens and kidneys were aseptically removed, and portions of each organ were homogenized with sterile glass rods with the cutting edge in 1 mL of sterile saline. Serial 10-fold dilutions of the homogenates were plated on PDA, incubated at 30°C and examined daily for 3 days. The numbers of cfu/g of tissue were calculated. Mean survival time was estimated by the Kaplan–Meier method and compared among groups by using the log rank test. Colony counts in tissue burden studies were analysed using the Mann–Whitney U-test. Calculations were made using SPSS 15.0 and Graph pad 4.0 for Windows.

Results

The results of the survival studies are shown in Figure 1. For the strain FMR 5522, the control group and the treatment with posaconazole at 50 mg/kg/day were repeated and the results were pooled. In this case, there were 20 control mice and 20 mice in that particular group. Posaconazole at 50 mg/kg/day significantly prolonged survival at a rate of over 65%, the results being significantly better than for the other treatments at the end of the study. Posaconazole at 75 mg/kg significantly prolonged survival with respect to posaconazole at 25 and 100 mg/kg and to the control group. Amphotericin B at 3 mg/kg/day significantly prolonged survival with respect to posaconazole at 25 and 100 mg/kg and to the control group. Amphotericin B at 3 mg/kg/day significantly prolonged survival with respect to the control group and the other therapies with the exception of posaconazole at 50 and 75 mg/kg. Survival of groups treated with posaconazole at 100 mg/kg, liposomal amphotericin B at 5 mg/kg and amphotericin B at 1.5 mg/kg

Figure 1. Cumulative mortality of severe immunosuppressed mice infected with $1.2 \times 10^8$ cfu/mouse of P. lilacinus FMR 5522 (a) or $0.6 \times 10^8$ cfu/mouse of P. lilacinus FMR 8252 (b). Amphotericin B (AMB) at 1.5 or 3 mg/kg/day; liposomal amphotericin B (LAMB) at 5 mg/kg/day; posaconazole (PSC) at 25, 50, 75 or 100 mg/kg/day. a $P$ value, 0.05 versus the control; b $P$ value, 0.05 versus AMB 3; c $P$ value, 0.05 versus AMB 1.5; d $P$ value, 0.05 versus PSC 100; e $P$ value, 0.05 versus PSC 75; f $P$ value, 0.05 versus PSC 25; g $P$ value, 0.05 versus LAMB 5. All treatment groups contained 10 mice with the exception of the group treated with posaconazole at 50 mg/kg/day and the control group for the strain FMR 5522, which contained 20 mice each.
was not significantly different from that of the control group. For the strain FMR 8252, posaconazole at 50 mg/kg was the only drug able to significantly prolong survival with respect to the control group, although less impressively than for the FMR 5522 strain.

For both strains, the fungal load was at least two log units higher in the spleen and the liver than in the kidneys (Figure 2). Posaconazole at 50 mg/kg was the only treatment able to reduce the fungal load in all the organs studied for both strains.

**Discussion**

No previous data exist on the efficacy of posaconazole in experimental paecilomycosis, and there is a very little clinical experience of its use in human infections. Although experimental infections by some species of *Paecilomyces* have been evaluated in different animal models, such as mice and rabbits using different routes of infection (intraperitoneal, intracorneal and intravenous), this is the first time they have been used to evaluate different antifungal treatments against *P. lilacinus*.

Here, we have demonstrated the efficacy of posaconazole in two experimental murine models of paecilomycosis, one for survival and another for tissue burden studies. Due to the low virulence of *P. lilacinus*, and in order to find an immunosuppressive regimen that, combined with a high fungal inocula, would be able to provoke an acute infection with 100% of the animals dying within 10 days post-infection, we used a lethal inoculum in both strains. This condition was achieved with 10^7 cfu/mouse of *P. lilacinus* FMR 5522 (a, b and c) or 0.6 × 10^7 cfu/mouse of *P. lilacinus* FMR 8252 (d, e and f) in the spleen, liver and kidneys of mice. Amphotericin B (AMB) at 1.5 or 3 mg/kg/day; liposomal amphotericin B (LAMB) at 5 mg/kg/day; posaconazole (PSC) at 25, 50, 75 or 100 mg/kg/day. *P value < 0.05 versus the control; *P value < 0.05 versus PSC 100, LAMB 5 and AMB 3; *P value < 0.05 versus PSC 25 and PSC 75. Horizontal lines indicate mean values.

Figure 2. Effects of the antifungal treatments on colony counts in mice moderately immunosuppressed and infected with 1.2 × 10^7 cfu/mouse of *P. lilacinus* FMR 5522 (a, b and c) or 0.6 × 10^7 cfu/mouse of *P. lilacinus* FMR 8252 (d, e and f) in the spleen, liver and kidneys of mice. Amphotericin B (AMB) at 1.5 or 3 mg/kg/day; liposomal amphotericin B (LAMB) at 5 mg/kg/day; posaconazole (PSC) at 25, 50, 75 or 100 mg/kg/day. *P value < 0.05 versus the control; *P value < 0.05 versus PSC 100, LAMB 5 and AMB 3; *P value < 0.05 versus PSC 25 and PSC 75. Horizontal lines indicate mean values.
studies. For the tissue burden studies, we used a less aggressive immunosuppressive regimen that allowed the animals to survive until the completion of treatment. The best results were obtained with posaconazole at 50 and 75 mg/kg, the 50 mg/kg dose being the most effective in getting a high survival rate and reducing the fungal load with respect to the control group for all the organs tested. We did not observe a dose–effect relationship for posaconazole in this model, as higher doses of this drug were ineffective in prolonging survival and in reducing the fungal load. Similarly, other authors did not obtain better results with higher doses versus lower doses.5,7 No data exist to explain these observations, but a pharmacokinetic study in mice suggests that posaconazole absorption could be lower when high doses were administered.9 No studies on posaconazole toxicity in mice have been reported, but multiple daily 40 mg/kg doses have been administered to dogs with no adverse effects observed.9 Our study agrees with that of Imai et al.,7 who observed an apparent lack of clinical signs of toxicity since respiratory, nervous system, cardiovascular or gastrointestinal effects were not seen and the state of the skin, hair and pallor of the mucosae were similar to those of the controls. Even the mice treated with high doses of posaconazole appeared healthier than those treated with the low dose of this drug.

In our study, posaconazole has shown efficacy in the treatment of murine paecilomycosis. Although further studies are needed to ascertain its clinical relevance, we think that this drug must be considered of potential clinical use in the treatment of human infections by \textit{P. lilacinus}.

**Funding**

This work was supported by a grant from Fondo de Investigaciones Sanitarias from the Ministerio de Sanidad y Consumo of Spain (PI 050031).

**Transparency declarations**

None to declare.

**References**