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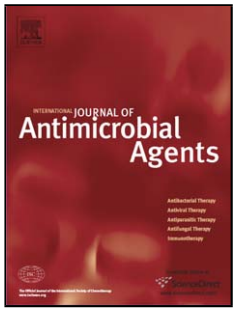

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Efficacy of voriconazole in a murine model of invasive paecilomycosis

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ABSTRACT

We studied the efficacy of voriconazole (VRC) and amphotericin B (AMB) in an immunosuppressed murine model of disseminated infection by two strains of *Paecilomyces lilacinus*. Mice were treated with VRC 60 mg/kg/day orally or AMB 3 mg/kg/day intraperitoneally, beginning 1 day after infection and continuing for 9 days. To avoid rapid clearance of VRC, animals receiving VRC and the control group were given grapefruit juice instead of water. VRC significantly prolonged survival with respect to the group treated with AMB and the control group for both strains ($P = 0.005$ and $P < 0.0001$, respectively, for strain FMR 5522; and $P = 0.0002$ and $P < 0.0001$, respectively, for strain FMR 8252). VRC reduced the fungal load in the spleen, kidneys and liver of infected mice for both strains tested. Survival of mice challenged with strain FMR 8252 treated with AMB did not differ from that of the control group ($P = 0.223$), being worse than that of the mice treated with VRC ($P = 0.0002$). AMB was not able to reduce the tissue burden in any organ with respect to the control group for both strains studied.

1. Introduction

Paecilomyces lilacinus is an opportunistic filamentous fungus causing severe infections both in immunocompetent and immunocompromised patients [1–4]. The most frequent clinical manifestations are oculomycosis and cutaneous and subcutaneous infections [5–19]. Less frequently, however, this fungus can also disseminate, probably due to this ability to sporulate in tissue, producing numerous conidia that spread haematogenously [1,20].

In ophthalmic and skin infections caused by this fungus, several drugs have been used, generally topically, but the optimal treatment for *P. lilacinus* infection has not yet been established. Amphotericin B (AMB) is probably the most commonly used drug for treating this infection, with most of the results being negative or unknown [6,8,10,15,21–26], in agreement with the poor in vitro response of *P. lilacinus* to this antifungal drug [1,27,28]. There is little data on the activity of the newer triazoles either in vitro [28,29] or in animal models [1,30], but the available data are promising. Although there is limited clinical experience in the use of voriconazole (VRC), this drug appears to be effective in the treatment of this fungal infection [1,31]. The aim of this study was to evaluate the efficacy of VRC in a disseminated *P. lilacinus* infection in neutropenic mice.

2. Materials and methods

Isolates FMR 5522 and FMR 8252, from knee joint fluid and of environmental origin, respectively, were used in this study. Isolates were identified following

morphological criteria [32] and by sequencing several genes. Their in vitro susceptibility to AMB and VRC was tested using the broth microdilution method following Clinical and Laboratory Standards Institute guidelines for filamentous fungi [33]. Isolates were stored at –80 °C and prior to testing they were subcultured on potato dextrose agar (PDA) at 30 °C. On the day of infection, cultures on PDA were suspended in sterile saline and filtered through sterile gauze to remove clumps of spores or hyphae. The resulting suspensions were adjusted to the desired inoculum based on haemocytometer counts and by serial plating on PDA to confirm viability.

Male OF1 mice (Charles River, Criffa 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. Two markers of efficacy were used, namely prolongation of survival and reduction in tissue burden. The former was evaluated through a lethal infection attained by using severe immunosuppression, and the latter through a sublethal infection attained by using moderate immunosuppression. Both regimens had been selected in a previous study [30]. For the survival study, mice were challenged with 1.2×10^8 colony-forming units (CFU)/mouse for strain FMR 5522 or 0.6×10^8 CFU/mouse for strain FMR 8252 [30]. Severe immunosuppression was reached with a dose of cyclophosphamide 200 mg/kg body weight administered intraperitoneally (i.p.) on the day of infection and two doses of 5-fluorouracil, one of 150 mg/kg body weight intravenously (i.v) on the day of infection and another one of 75 mg/kg on Day 5 post infection. For the tissue burden study, mice received cyclophosphamide 200 mg/kg body weight administered i.p. plus 5-fluorouracil 150 mg/kg body weight administered i.v. 1 day

prior to the infection to achieve moderate immunosuppression. For this study, animals were challenged with 1.2×10^7 CFU/mouse of strain FMR 5522 or 0.6×10^7 CFU/mouse of strain FMR 8252 [30]. Ten mice were used for survival studies and ten for tissue burden studies, the latter group being identified before the study started.

AMB (Fungizona®; Squibb Industria Farmacéutica S.A., Barcelona, Spain) was administered i.p. at a dose of 3 mg/kg body weight/dose once daily [8]. VRC (Vfend®; Pfizer Inc., Madrid, Spain) was administered orally at a dose of 60 mg/kg body weight/dose once daily. From 3 days prior to infection, mice receiving VRC and the control group were given grapefruit juice (Hero España, Murcia, Spain) instead of water to block VRC metabolism and to increase its serum concentration in mice to suitable levels for performing treatment studies [34,35]. The selected dose of VRC has been shown previously to deliver adequate plasma levels in mice when co-administered with grapefruit juice [36]. Preliminary experiments with strain FMR 5522 (data not shown) demonstrated that VRC 60 mg/kg/day significantly prolonged the survival of mice in comparison with VRC 40 mg/kg and the control group; survival of mice treated with VRC 40 mg/kg was not different from that of the untreated control group. Therefore, only the higher dose was tested in this study. All mice received ceftazidime (5 mg/day subcutaneously) from Days 1 to 10 after infection to prevent bacterial infection. All treatments began 24 h after challenge and the therapies lasted for 10 days. Control animals received no treatment. Survival of mice was evaluated daily for 20 days. For tissue burden studies, mice were sacrificed on Day 11 post infection. Livers, spleens and kidneys were removed

aseptically and were homogenised in 1 mL of sterile saline. Serial 10-fold dilutions of the homogenates were plated on PDA and incubated for 24–72 h at 30 °C. Mean survival time was estimated by the Kaplan–Meier method and compared among groups 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 (SPSS Inc., Chicago, IL) and GraphPad 4.0 for Windows (GraphPad Software Inc., La Jolla, CA).

3. Results

Minimal inhibitory concentrations were 32 μg/mL for AMB and 0.5 μg/mL for VRC for both strains. Fig. 1 shows the results of the survival studies. VRC 60 mg/kg/day significantly prolonged survival with respect to the group treated with AMB and the control group for both strains. Fig. 2 shows that with both strains the fungal load was at least two log units higher in the spleen and liver than in the kidneys. For strain FMR 8252, VRC 60 mg/kg/day was able to reduce significantly the fungal load with respect to the control group and the group receiving AMB in all three organs (Fig. 2d–f). For strain FMR 5522, VRC 60 mg/kg/day was able to reduce significantly the fungal load with respect to the control group and the group receiving AMB in the spleen and kidneys only (Fig. 2a–c).

4. Discussion

In this study, we investigated the effect of VRC against a *P. lilacinus* infection using the same murine model where previously posaconazole showed efficacy

[30]. The low virulence of *P. lilacinus* previously described in an experimental murine model of disseminated infection was also corroborated [37]. In the survival study, severe immunosuppression was used to provoke an acute infection, with all of the control animals dying within 10 days post infection.

Some data exist on the efficacy of triazoles in human paecilomycosis, especially VRC [26,31,38,39]. Our results agree with these favourable data, since VRC was able to reduce the fungal burden in the three organs studied for one strain and in two organs for the other strain. Our results suggest that VRC may have a clinical role in the treatment of disseminated paecilomycosis.

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Competing interests

None declared.

Ethical approval

All animal care procedures were supervised and approved by the Universitat Rovira i Virgili Animal Welfare Committee (Reus, Spain).

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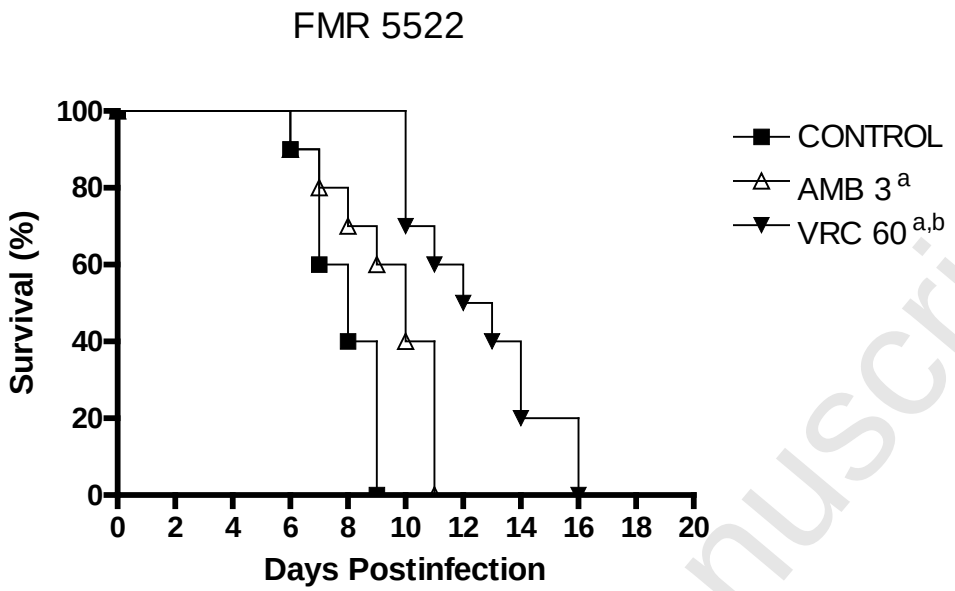
Fig. 1. Cumulative mortality of severely immunosuppressed mice infected with (a) 1.2×10^8 colony-forming units (CFU)/mouse of *Paecilomyces lilacinus* FMR 5522 or (b) 0.6×10^8 CFU/mouse of *P. lilacinus* FMR 8252 and treated with amphotericin B (AMB) 3 mg/kg/day or voriconazole (VRC) 60 mg/kg/day, or untreated (control). [a] $P < 0.05$ versus the control; [b] $P < 0.05$ versus AMB.

Fig. 2. Effect of antifungal treatment with voriconazole 60 mg/kg/day or amphotericin B (AMB) 3 mg/kg/day on colony counts of *Paecilomyces lilacinus* (a–c) strain FMR 5522 and (d–f) strain FMR 8252 in the spleen (a,d), liver (b,e) and kidneys (c,f) of mice. [a] $P < 0.05$ versus the control and AMB. Horizontal lines indicate mean values.

(a)



(b)