A novel murine model of cerebral scedosporiosis: lack of efficacy of amphotericin B

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Objectives: Cerebral scedosporiosis is a life-threatening infection that is difficult to treat. The aim of this work was to develop a murine model of cerebral infection by *Scedosporium apiospermum* using intracranial inoculation and to use this model to evaluate the efficacy of amphotericin B deoxycholate and liposomal amphotericin B.

Methods: Mice were rendered neutropenic by intraperitoneal cyclophosphamide and intravenous (iv) 5-fluorouracil administration. Animals were infected with iv or intracranial inoculation of 1 $\times$ 10^4, 5 $\times$ 10^4 or 5 $\times$ 10^5 cfu of a clinical strain of *S. apiospermum*. Tissue burden reduction was determined in kidneys and brain 4 days after the infection. Efficacy of amphotericin B and liposomal amphotericin B (0.8 mg/kg/day intraperitoneally and 40 mg/kg/day iv, respectively) was evaluated in neutropenic mice infected iv or intracranially with 5 $\times$ 10^5 cfu. Survival was analysed with the log-rank test. Fungal burden values of different groups were compared using the Mann–Whitney U-test.

Results: In our model, intracranial infection produced a higher fungal load in the brain and a lower fungal load in the kidney than iv inoculation. Survival of animals infected intracranially and treated with amphotericin B or liposomal amphotericin B (mean survival time 5.8.3 and 9.2 days, respectively) was not different from the control group (P = 0.58 and 0.85, respectively).

Conclusions: We have developed a murine model of cerebral scedosporiosis, which may be useful for studying various pathological aspects of this infection and evaluating new therapeutic approaches. Amphotericin B and liposomal amphotericin B were unable to resolve the infection.

Keywords: *Scedosporium apiospermum*, brain infections, animal models

Introduction

*Scedosporium apiospermum* is a ubiquitous fungus that can cause severe infection in immunocompromised patients and commonly affects the CNS. Pneumonia or meningitis may also result in normal hosts.1 Brain involvement is usually fatal and only a few cases have been resolved with surgical drainage combined with antifungal therapy.2,3 Although recent experimental4 and clinical reports5,6 have demonstrated the efficacy of voriconazole, amphotericin B deoxycholate is still the most used antifungal drug for treating infections by this fungus. However, this drug has shown high MICs for *S. apiospermum* and clinical treatment usually fails.7,8,9 Various authors have developed animal models of disseminated infection for the study of experimental scedosporiosis,4,10,11 but up to now no cerebral model has been described. We have developed a murine model of cerebral scedosporiosis that may be useful for studying various pathological aspects of this infection and evaluating new therapeutic approaches. We have also used the model to test the efficacy of amphotericin B and liposomal amphotericin B.

Materials and methods

We used the clinical strain FMR 6694 of *S. apiospermum* stored at $-80^\circ C$ as a conidial suspension in potato dextrose broth (PDB) with 30% glycerol. The MIC of amphotericin B for this strain, determined following the NCCLS guidelines, was 4 mg/L. Inocula were prepared by defrosting the conidial suspension at room temperature and adding 100 mL of this suspension to 150 mL of PDB. Cultures were incubated in an orbital shaker (150 rpm) at 30°C for 5 days, then filtered once through sterile gauze and centrifuged at 2500 rpm for 12 min. The resulting pellet was suspended in saline solution, and the conidia concentration adjusted by counting with a haemocytometer. Five-week-old male CD-1 mice (Charles River Laboratories, Barcelona, Spain) were housed in standard conditions with water and feed ad libitum. Conditions were approved by

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the Animal Welfare Committee of the Faculty of Medicine of the University Rovira i Virgili. Animals were immunodepressed by a single administration of cyclophosphamide at 200 mg/kg intraperitoneally plus 5-fluorouracil at 150 mg/kg intravenously (iv), both of which were administered on the day of infection. Three groups of 10 animals per group were infected iv via the lateral tail vein with conidial suspensions of $1 \times 10^4$, $5 \times 10^4$ or $5 \times 10^5$ cfu per animal, respectively. Three other groups were infected intracranially by puncture at a point midline on the cranium, as previously described, with the same inocula. Groups infected intracranially were assayed in duplicate, and had been anaesthetized by inhalation of methoxyflurane. For tissue burden studies, six groups of five animals per group were infected iv or intracranially with the three inocula described above. All animals were sacrificed 4 days after infection. The brain and kidneys were removed aseptically and portions of 1 g of each organ were homogenized in 2 mL of 0.9% sterile saline. Homogenates were serially diluted and plated in duplicate on potato dextrose agar for cfu determination. For the histopathological study, a portion of each specimen was placed in 10% buffered formalin. The organs were sectioned in paraffin blocks, and sections of 3 μm were stained with periodic acid-Schiff, haematoxylin-eosin and Grocott methenamine silver stains for microscopic observation.

A complementary survival study was performed to determine the efficacy of amphotericin B and liposomal amphotericin B on both iv and intracranial animal models. Amphotericin B deoxycholate (Fungizona; Squibb Industria Farmaceutica, Barcelona, Spain) and liposomal amphotericin B (AmBisome; Gilead Sciences S.A., Barcelona, Spain) were reconstituted with sterile distilled water and further diluted in 5% sterile dextrose solution to reach the desired concentrations for administration. Six groups of 10 immunodepressed mice per group were used to assay the efficacy of amphotericin B and liposomal amphotericin B. Infection was established iv or intracranially with $5 \times 10^4$ cfu/animal. Treatments were begun 1 day after infection and were administered daily for 10 days. Amphotericin B and liposomal amphotericin B were administered iv at 0.8 and 40 mg/kg/day, respectively. Two control groups, infected iv and intracranially, received glucose 5% iv as placebo for 10 days. Survival was analysed with the log-rank test and fungus burden values were converted into log 10 cfu. Data were compared using the Mann–Whitney U test. GraphPad Prism software for windows was used for statistical analysis.

**Results**

**Intravenous infection**

The fungus proved to be highly lethal when it was inoculated iv. Figure 1(a) shows the survival percentages of infected mice. The inoculum of $1 \times 10^4$ cfu caused a mortality of 100% by day 11, with a mean survival time (MST) of 8 days. MST was shorter in animals infected with higher inocula, i.e. 5.8 days with an inoculum of $5 \times 10^4$ cfu and 3.6 days with an inoculum of $5 \times 10^5$ cfu. Tissue burden studies demonstrated a similar dose–response, the kidneys being more affected than the brain (Figure 2). The mean cfu/g in the kidneys of the infected animals, from the lowest to the highest inoculum, were $2.15 \pm 0.51$, $2.57 \pm 0.39$ and $3.96 \pm 0.43 \log_{10}$cfu/g, and $0.90 \pm 0.54$, $1.90 \pm 0.32$ and $2.17 \pm 0.39 \log_{10}$cfu/g in the brain. A histopathological study showed low tissue infection in brain and kidneys with few microabscesses and/or inflammatory cells.

**Intracranial infection**

The groups of mice infected intracranially were tested in duplicate. Since there were no statistical differences between the two tests for each inoculum, the results were grouped in a single curve (Figure 1b). Animals infected with $1 \times 10^4$ cfu showed symptoms of neurological infection, such as disorientation, convulsion and anorexia. However, they all survived to the end of the experiment (day 14 post-infection). By contrast, higher inocula, such as $5 \times 10^4$ and $5 \times 10^5$ cfu/animal, caused 100% mortality (MST of...
Tissue burden correlated with the results obtained in the survival study. Fungal load was significantly higher in the brain with the lowest inoculum than in the kidneys with the highest inoculum (Figure 2). Animals infected with the lowest inoculum showed 2.57 and 1.03 log_{10} cfu/g in the brain and kidneys, respectively. cfu counts were higher with higher inocula, i.e. 3.38 and 1.10 log_{10} cfu/g in the brain and kidneys, respectively, when the inoculum was 5 \times 10^4 cfu/g, and 3.97 and 1.56 log_{10} cfu/g, respectively, when it was 5 \times 10^5 cfu/animal. The histopathological study revealed the presence in the brain of macro- and microabscesses with necrosis and hyphae (conidia were not observed) (Figure 3). The number and severity of the lesions increased with the size of the inoculum.

**Treatment study**

The MST of control animals infected iv or intracranially were 5.8 and 8.5 days, respectively. In the iv model, treatments with amphotericin B or liposomal amphotericin B (Figure 4) increased the MST (6.4 and 7.2 days, respectively) but not significantly in comparison with the control group (P = 0.43 and P = 0.072, respectively). Animals infected intracranially and treated with amphotericin B or liposomal amphotericin B did not show increased survival (MST = 8.3 and 9.2 days, respectively) in comparison with the control group (P = 0.58 and 0.85, respectively).

**Discussion**

Cerebral scedosporiosis is one of the commonest clinical manifestations of *S. apiospermum*, which is difficult to resolve and usually fatal. In a recent revision of solid organ transplant patients, 48% of those infected by this fungus had CNS involvement and almost all died. In another revision of 38 cases of scedosporiosis with cerebral involvement, 76% resulted in death after the failure of antifungal therapy. The severity of this infection means that it is necessary to test new therapeutic strategies and suitable animal models are critical if efficacy and tolerance are to be determined. As far as we know, in this study we have developed the first animal model of cerebral scedosporiosis using an intracranial infection. Using this route of infection, the strain of *S. apiospermum* assayed seems to be less virulent, causing less mortality and lower tissue burden in the kidneys than the systemic infection achieved by iv inoculation. Interestingly, the brain was affected more by the intracranial inoculation and the cerebral fungal load was also higher, which makes this model more suitable for studying cerebral scedosporiosis.
We have used mice to develop our model because they are relatively inexpensive and easy to handle. This model, which is easy to perform and reproducible, can be used to evaluate the efficacy of various antifungal drugs. Our study demonstrated the lack of efficacy of amphotericin B and liposomal amphotericin B to resolve the infection, which confirms the poor results with these drugs in clinical settings.2,4,8,14 Nowadays, the recommended drug for treating scedosporiosis is voriconazole. However, its use in cerebral infection is poorly documented. It has been demonstrated that voriconazole can reach concentrations of up to 3.93 mg/L in the brain of immunocompromised patients,16 which is clearly higher than the usual MIC shown by this drug for S. apiospermum.17,18 The rapid clearance of voriconazole in mice may mean that guinea pigs are more useful for evaluating the activity of this drug,15 but these animals are expensive and hard to handle. Grapefruit juice has been shown to increase the serum level of voriconazole in mice due to the inhibition of cytochrome P450 enzymes, which are responsible for the induction of the metabolism of this drug.19 Mice given grapefruit to drink have also proven to be an appropriate model for evaluating the efficacy of voriconazole2 and are a good alternative. Other antifungal agents such as posaconazole or tetracycline CMT-3 have demonstrated activity against S. apiospermum,7,17,20 and should also be tested in this new infection model.

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References