Experimental models of disseminated scedosporiosis with cerebral involvement

Bénédicte Lelièvre, Pierre Legras, Charlotte Godon, Florence Franconi, Jean-Paul Saint-André, Jean-Philippe Bouchara, Bertrand Diquet.

L’UNAM Université, Université d’Angers, Groupe d’Etude des Interactions Hôte-Pathogène, EA 3142 (BL, PL, CG, JPSA, JPB, BD), Service Commun de l’Animalerie Hospitalo-Universitaire (PL), Plateforme d’Ingénierie et d’Analyses Moléculaires, Angers, France (FF) and L’UNAM Université, Centre Hospitalo-Universitaire, Angers, France (BL, PL, JPSA, JPB, BD)
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Corresponding author: Bénédicte Lelièvre, Groupe d’Etude des Interactions Hôte-Pathogène, Institut de Biologie en Santé-PBH, CHU, 4 rue Larrey, 49933 ANGERS cedex 9, France. E-mail: belelievre@chu-angers.fr

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List of abbreviations:
BBB: Blood-Brain Barrier; CF: Cystic Fibrosis; HES: Hematoxylin-Eosin-Safran; MIC: Minimal Inhibitory Concentration; MRI: Magnetic Resonance Imaging; YPDA: Yeast extract-Peptone-Dextrose Agar

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Abstract

*Scedosporium apiospermum* is a soil fungus which can cause severe and often fatal cerebral infections in both immunocompetent patients in the event of near drowning and immunosuppressed patients such as lung transplant recipients. Because of the low susceptibility of this fungus to antifungal drugs, and the low permeability of the blood-brain barrier (BBB), therapeutic drug monitoring is necessary to reach an effective tissue concentration with limited side effects. Indeed, diffusion of the drug in the brain is dependent on several parameters, such as the integrity of the BBB and the activity of efflux pumps. In order to evaluate drug diffusion, two experimental models were developed in immunocompetent and immunosuppressed rats. Inocula were administered via the penile vein and a clinical scale (0 to 9) was established, based on weight and clinical and neurological signs evaluated by the tail suspension test. Cerebral involvement was confirmed by magnetic resonance imaging and histological examination of brain sections after hematoxylin-eosin-safran or silver staining. Voriconazole or posaconazole were given to the rats at doses ranging from 10 to 75 mg/kg/d via the i.v. or oral routes, respectively. Whatever the immune status, the effective doses (defined by a doubling of the survival time and the absence of neurological sequelae) were 30 mg/kg/day for voriconazole and 50 mg/kg/day for posaconazole. Overall, the results demonstrated that these models may constitute valuable tools for the performance of pharmacokinetic (PK) and pharmacodynamic (PD) studies for PK-PD modeling.
INTRODUCTION

Therapeutic drug monitoring consists in evaluating the plasma concentration of a drug, on the basis that this will predict its level in target tissues. However, this extrapolation is frequently not applicable when it concerns sanctuary organs such as the brain. Indeed, the diffusion of molecules into the brain is controlled by several parameters such as the integrity of the blood-brain barrier (BBB) and the presence of efflux proteins (PgP) (Lösch et al., 2005a; Potschka et al., 2003).

To date, most studies have concerned drug diffusion in a context of epilepsy or brain cancer. Two strategies can be used to assess intracerebral diffusion: either mathematical models that have been developed and then tested in vivo, or statistical models built using the data obtained in animal experiments. In an attempt to predict diffusion via the BBB, different models have been developed, taking account of the various physicochemical properties of the drugs, including their molecular weight, lipophilicity (log P) and polarizability (Bendels et al., 2008). For example, in such models, a small molecule with molecular weight lower than about 600 Da is expected to cross the BBB. But in vivo experiments have shown that with some small anti-cancer or anti-epileptic agents (teniposide, lamotrigine, carbamazepine,...), the concentration observed in the brain differed from that which had been predicted (Potschka et al., 2003; Lösch et al., 2005a,b). In most cases, several other parameters also need to be considered. At present, PK-PD modeling is increasingly being used because unlike mathematical models it has the advantage of being based on experimental models and evidence for a dose-response relationship.

Moreover, recent techniques in medical imaging, and particularly magnetic resonance imaging (MRI) and more recently positron emission tomography (PET) scan, may be helpful as they can visualize the structure of the brain and the cerebral diffusion of labeled molecules. Apart from cancer and neurological diseases, the diffusion of the drugs through the BBB is
also crucial in a context of infectious diseases with cerebral involvement (Schwartz et al., 2009). Very few studies have been performed in this field, and have mainly focused on aspergillosis (Lutsar et al., 2003). Nevertheless, the experimental model used by Lutsar et al. (2003) was developed in guinea-pigs, and it is well known that rats are more relevant when extrapolating to the human host. In addition, besides the drug and the animal species, diffusion may also depend on the pathogen involved and particularly the damage it may cause to the BBB.

*Scedosporium apiospermum* is a soil filamentous fungus which usually lives as a saprophyte but can cause localized infections resulting from the traumatic inoculation of fungal elements, such as mycetoma and bone or joint infections (Cortez et al., 2008; Katragkou et al., 2007; Steinbach et al., 2003). In recent decades, this fungus has been the subject of increasing attention because of several reports of its relatively high frequency as a causal agent in the airway colonization of patients with cystic fibrosis (CF). With a frequency ranging from 6.5% to 10%, *S. apiospermum* ranks second among the filamentous fungi recovered from airway secretions from CF patients (Cimon et al., 2000; Kaltseis et al., 2009; Harun et al., 2010). Because of its propensity to disseminate in an immunocompromised host and its low susceptibility to current systemic antifungal agents, the early detection of chronic airway colonization by this fungus is essential. Fatal disseminated infections with cerebral involvement have been described recently following lung transplantation in CF patients previously colonized by *S. apiospermum* (Symoens et al., 2006; Morio et al., 2010). Cerebral scedosporiosis has also been reported in immunocompetent patients, occurring in the context of near-drowning (Cortez et al., 2008). Until the 1990s, the mortality rate seen with *S. apiospermum* meningitis was higher than 75%, but recent triazole antifungals have enabled an improvement in the prognosis of these patients. Nevertheless, in lung transplant recipients, the balance between the efficacy and toxicity of the antifungal agent needs to be determined with
respect to drug interactions with immunosuppressive treatments, and the modeling of drug diffusion through the BBB is essential for dose adjustment.

The aim of this study was therefore to develop experimental models of disseminated scedosporiosis with cerebral involvement in both immunocompetent and immunosuppressed rats, in order to reproduce the two clinical contexts of cerebral scedosporiosis.

MATERIAL AND METHODS

Organism and culture conditions. This study was performed using *Scedosporium apiospermum* strain IHEM 3817 (Institute of Hygiene and Epidemiology-Mycology section culture collection) obtained from the Scientific Institute of Public Health in Brussels, Belgium. This strain, initially isolated in the Netherlands from a case of cerebral scedosporiosis, was identified by sequencing the internal transcribed spacer (ITS) regions 1 and 2 of ribosomal DNA genes as *Scedosporium apiospermum sensu stricto* (Gilgado et al., 2009). The strain was routinely maintained by regular passages on yeast extract-peptone-dextrose agar (YPDA) containing chloramphenicol 0.5g/L. After 11 days of inoculation at 37°C, the conidia were harvested by scraping the colonies in sterile distilled water, filtering them through a 25-µm pore size nylon filter and enumerating them by hematocytometer counts. The final density of the conidial suspension was adjusted in sterile distilled water.

Animals. Male Sprague Dawley rats (10 weeks old) weighing 250-274 g were purchased from Janvier (Le Genest Saint Isle, France). They were housed 6 rats to a cage in ventilated boxes kept in a protected and temperature- and humidity-controlled room, with a 12-h on-off light cycles. The animals were given free access to food and water. Animal care was ensured in strict compliance with the regulatory requirements of the French Ministry of Agriculture, and the animals were euthanized using CO₂ at the end of the experiments.
Experimental models of disseminated scedosporiosis. Two models were developed in immunocompetent or immunosuppressed rats. Immunosuppression was induced by the oral administration of cyclosporine (Novartis) diluted in olive oil at a dose of 15 mg/kg/d. The treatment was started two days before inoculation of the fungus, continued on day -1 and then every two days.

Inoculation via the penile vein was performed two weeks after the animals arrived at the facility. This vein was chosen because it enables the simple administration of inocula and preservation of the coccygeal vein for drug administration and collection of the numerous blood samples required. For the inoculation procedure, the rats were anesthetized with 5% isoflurane for induction and then 2% isoflurane for maintenance in 20:1 air/oxygen. Unless otherwise stated, the animals were inoculated with $10^5$ or $10^6$ conidia for immunosuppressed and immunocompetent animals, respectively. The animals were then followed for 8 to 12 days after the inoculation, depending on their immune status.

A clinical tool was developed to determine the clinical stage of the disease in each animal and the timing of their sacrifice (limit point) according to ethical guidelines. Different clinical signs were taken into account, i.e. animal behavior, bleeding, weight and neurological signs, which were noted twice a day until death. Neurological signs were estimated according to the tail suspension test described by Steru et al. (1975).

Magnetic resonance imaging (MRI) experiments and histological study. To confirm cerebral involvement, MRI experiments were performed before and after the intravenous injection of gadolinium salt (Dotarem®), together with an histological study of the brain after sacrifice of the animals. MRI experiments were performed on a Bruker Avance DRX system (Bruker Biospin SA, Wissembourg, France) operating on a Paravision (version 4.0) software platform (Bruker Biospin SA). The system was equipped with a 150 mm vertical super-wide bore magnet operating at 7 Tesla, a 84-mm-inner diameter shielded gradient set capable of
144 mT/m maximum gradient strength and a 64-mm-diameter birdcage resonator. Three perpendicular 2D spoiled gradient echo images were acquired with a TR of 200 ms, an echo time of 20 ms, a flip angle of 20°, a slice thickness of 2 mm, a field-of-view of 40 mm and a matrix size of 128 µm. Immunocompetent (n = 6), immunosuppressed (n = 6), and healthy control (n = 2) rats were studied, and cerebral MRI was performed at D1 and D3 for immunocompetent rats and at D1, D3 and D5 for immunosuppressed rats.

After sacrifice of the animals at the end of the experiment, the brain was collected, fixed in formalin and included in paraffin. Histological sections were examined microscopically after hematoxylin-eosin-safran or Gomori’s methenamine silver staining.

**Antifungal treatment.** Two triazole antifungal drugs, voriconazole purchased from Pfizer (Groton, CN, USA) and posaconazole from Schering Plough (Kenilworth, New Jersey, USA), were tested in both experimental models in order to determine the effective dose, i.e., that which resulted in a doubling of the survival time. Commercially available drugs were used; for voriconazole, the i.v. form was chosen as it enabled better bioavailability (F=100%), and doses ranging from 10 to 40 mg/kg/d were administered via the caudal vein. By contrast, posaconazole which has a low aqueous solubility and is only available as an oral suspension, was given orally, by gavage, and four doses were tested: 10, 25, 50 and 75 mg/kg/d. Groups of three rats were studied for each dose during a first series of experiments, and the results were then confirmed with the most effective doses using six rats per group. Controls consisted of untreated animals. All the rats were then followed for up to three weeks.

**Statistical analysis.** A Cox model provided an estimate of the effect of the different inocula on rat survival. A non parametric bilateral Mann Whitney test (α 5%) was used to compare the survival times in treated and control rats, as well as between groups receiving different doses of the antifungal drugs.
RESULTS

Development of experimental models of scedosporiosis. In order to reproduce the two clinical contexts of cerebral scedosporiosis, two experimental models of scedosporiosis with cerebral involvement were developed in both immunocompetent and immunosuppressed rats. In the case of immunocompetent rats, the two highest doses (5 x 10^6 and 10^7 spores) resulted in the death of all the animals at day 3 (Fig. 1). With a lower inoculum of 10^6, 5 x 10^5 or 10^5 spores, the first animals died on day 3, day 4 and day 6, respectively, and the mean survival times were found to be 4.7 ± 1.1, 5.1 ± 1.0 and 7.8 ± 1.8 days, respectively. These experiments were then repeated and produced similar results with mean survival times of 4.7 ± 0.7 or 5.1 ± 0.5 days for animals inoculated with 10^6 or 5 x 10^5 spores; an inoculum of 10^6 spores was therefore selected for subsequent experiments. Statistical analysis using the Cox model showed a significant difference (p = 0.0138) between the different groups.

For immunosuppressed rats, all the animals survived throughout the follow-up period after the injection of a low-dose inoculum ranging from 10^2 to 10^4 spores (Fig. 2). With a higher dose inoculum (10^5 spores), almost all the animals died between days 7 and 10 after the injection (mean survival time: 8.9 ± 1.2 days). Statistical analysis revealed a significant difference for rats receiving 10^5 spores (p < 0.0001).

A clinical scale was developed in order to assess the course of the disease. Clinical signs including animal behavior, bleeding, weight loss and neurological signs were graded (Table 1). Grade 0 corresponded to healthy rats. Both immunocompetent and immunosuppressed rats exhibited the same clinical signs, but the time elapsing before they occurred was longer in immunosuppressed rats, probably in relation with the use of a lower dose inoculum. The first signs appeared two days after the inoculation: the rats presented with bristling hair and bleeding localized around the eyes and from the nose (grade 1). The rats then began to lose
weight. Grade 2 corresponded to a weight loss ranging from 1% to 5% of their initial weight. However according to our observations, weight loss was significant when it exceeded 5% of the initial weight (grade 3, between 5% and 10%; or grade 6, more than 10%)

Motor disorders were also observed, mainly affecting the hind legs, which drove the rats to turn round and could progress to paralysis (grade >7). Neurological signs were evaluated using the tail suspension test (Steru et al., 1975). A healthy rat would immediately try to regain its balance and straighten up. As the disease progressed, it became increasingly difficult for the rat to return to a normal position, turning around initially without managing to straighten up (grade 4) and then also crossing its paws (grade 5) until, at an advanced stage of the disease, it remained unresponsive (grade 7).

The later stage was characterized by paralysis, the rats having half-closed eyes and being bent backwards (grade 8); a few hours before death, the animals presented with “abdominal respiration” (grade 9).

In line with ethical guidelines, the limit point for further experimentation was defined as corresponding to grade 7.

**Confirmation of cerebral involvement and loss of BBB integrity.** At day 1, MRI findings were normal in all rats. At day 3, immunocompetent rats displayed neurological disorders, with difficulty in moving their hind legs (grade 4). Gadolinium-enhanced MRI revealed small areas, almost invisible before injection of the gadolinium salt, opacification of which confirmed BBB dysfunction (Fig. 3).

In immunosuppressed rats, the disease course was more rapid, with hypersignals localized in the frontal areas of the brain on day 3, and diffusion of the gadolinium salt throughout the brain which revealed the damage to the BBB (Fig. 4). On day 5, MRI showed that the lesions had spread to the ventricles; this disease progression was confirmed by marked weight loss.
and neurological signs (grade 7). Fungal invasion of the brain tissue was confirmed by histological examination. HES staining showed inflammatory areas with eosinophilic zones at their center (Fig. 5A), particularly in a section of the brain ventricle (Fig. 5B). In these areas, fungal hyphae were clearly visible after Gomori’s methenamine silver staining (Fig. 5C).

**Dose-ranging study to evaluate the efficacy of antifungal agents.** In the immunocompetent group, the control animals died after 4.8 days (median, [4.3 d; 5.3 d]) (Table 2). In view of our objective to double the survival time, it appeared that low doses of voriconazole (10 and 20 mg/kg/d) were unsuccessful, as they only caused a 56% increase in survival time. There was no significant difference between the animals receiving voriconazole 10 mg/kg/d or 20 mg/kg/d and the control animals. On the other hand, with voriconazole 30 or 40 mg/kg/d, no animals died during the follow-up period.

With posaconazole 10 or 25 mg/kg/d, the animals died after 7 days (median, [6.5 d; 7 d]) or 7.5 days (median, [6 d-10 d]), respectively (no significant difference from the control group). All animals receiving a dose of 50 mg/kg/d or 75 mg/kg/d survived for 21 days.

Treatment efficacy was also investigated using the clinical scale. In control animals, the disease progressed rapidly, starting from grade 4 at day 2 and reaching grade 8 on day 5 just before death (Fig. 6). Compared to the controls, no significant differences were observed in the groups receiving 10 mg/kg/d or 20 mg/kg/d voriconazole. With 30 mg/kg/d voriconazole, the clinical score reached grade 2 much later (between day 8 and day 11), but the score then returned to 0 on day 11, and remained thus until the end of the experiment. Contrary to the low dose groups, there was a significant difference between rats receiving 30 mg/kg/d and the controls ($p = 0.014$). In animals receiving 40 mg/kg/d, no clinical signs were observed at any time during the experiment. When compared to the controls, rats receiving posaconazole 10 mg/kg/d also reached grade 4 on day 2, but progression of the disease to grade 7 occurred
three days later and they died on day 8. A quite similar evolution was seen in rats receiving posaconazole 25 mg/kg/d. Finally, animals receiving posaconazole 50 mg/kg/d reached grade 2 on day 4 or 5, the clinical score returning to 0 on day 6 and remaining thus until the end of the experiment.

Regarding the immunosuppressed rats, the control animals died within 8.3 days (median, [7.5 d; 8.5 d]). The four test doses of voriconazole led to a survival time longer than 16 days. Animals treated with posaconazole 25 mg/kg/d survived for 14.5 days (median, [14 d; 20 d]) (no significant difference from the control group). No animals died when they were treated with higher doses of posaconazole.

With respect to the clinical score, among control animals it reached grade 2 on day 2, then grade 4 on day 3 and grade 7 on day 8 (Fig. 7). Rats receiving voriconazole 20 mg/kg/d presented the first clinical signs on day 2. Three out of the 6 rats reached grade 4 on day 7. All animals remained grade 4 until the end of the experiment, because of the presence of motor disorders. In rats receiving voriconazole 30 mg/kg/d, the first signs appeared on day 3 (grade 1). The animals then improved, as attested by the fall in the clinical score to grade 0 on day 12. None of the rats receiving 40 mg/kg/d displayed any clinical signs during the entire experimental period.

The first clinical signs were seen on day 2 in rats receiving posaconazole 25 mg/kg/d. They lost weight from day 3 (grade 2). There was an improvement by day 11 as their clinical scores had returned to 0. Rats treated with posaconazole 50 mg/kg/d presented few signs throughout the experimental period (little weight loss: grade 2 from day 4-5 to day 6). In these two groups, the clinical score returned to grade 0 after a few days, and all the animals survived until the end of the experiment. They never presented with any motor disorders.

According to our criteria (a doubling of survival time and an absence of neurological sequelae), the effective doses were 30 mg/kg/d and 50 mg/kg/d of voriconazole and
DISCUSSION

In this study, our objective was to develop an experimental model for fungal infection with cerebral involvement in order to determine whether the activity of antifungal drugs was dependent on them crossing the BBB, and to clarify the existence of a sanctuary compartment, or not.

The first step consisted in the choice of appropriate animal species. Using rats, mice, rabbits, dogs and cynomolgus monkeys, Nomeir et al. (2000) tested a variety of compounds and clearly demonstrated marked variations in the parameters, such as plasma concentrations, half-life or AUC$_{0\rightarrow\infty}$, which were dependent on the animal species studied. Similar observations were made by Roffey et al. (2003). For the present model of diffusion through the BBB, rats were preferred to other species (including mice) because they allow sampling a large volume of cerebrospinal fluid and allometric extrapolations to humans.

Regarding the fungal species, *Scedosporium apiospermum* was chosen as it can cause meningitis, and strain IHEM 3817 (initially isolated from a case of cerebral scedosporiosis) was selected. The size of the inoculum was determined in both immunocompetent and immunosuppressed rats. Immunosuppression was achieved using cyclosporine, because of the magnitude of its immunosuppressive effects. In addition, an antifungal activity has been reported for cyclosporine; however, this latter property has only been demonstrated *in vitro* to date (Cruz et al., 2000; Dreyfuss et al., 1976).

To estimate the degree and kinetics of disease development, different clinical stages were defined using a scale that included clinical signs, weight loss and neurological symptoms in order to assess the disease course and establish a comparative dose-ranging profile for drugs of interest.
As far as the clinical course of the disease was concerned, and because *Scedosporium apiospermum* is known to have various localizations, including the cerebral compartment, in case of blood dissemination, it was necessary to assess the cerebral localization of the fungus, which was achieved using MRI. MRI revealed a difference between the two groups: in immunocompetent rats, hypersignals were first of all detected at the periphery and then throughout the brain, whereas in the immunosuppressed animals, the lesions spread rapidly to the ventricles. The focal hypersignals led to the localization of *S. apiospermum* brain abscesses. The inflammatory process and loss of BBB integrity are pharmacodynamic parameters which are able to modify the diffusion of antifungal drugs to the brain.

The choice of the drugs used for this model was based on previous observations. Several systemic antifungal agents are available, and include amphotericin B, fluconazole, voriconazole, posaconazole and echinocandins. Echinocandins and the first two drugs cannot be used to treat scedosporiosis and/or meningitis. Therefore, voriconazole and posaconazole were selected since they are active *in vitro* against *S. apiospermum* and can be used for treatment of meningitis. They both inhibit lanosterol 14-α demethylase, resulting in inhibition of the synthesis of ergosterol, an essential component of the fungal membrane. Voriconazole and posaconazole present the same mechanism of action but major differences in their pharmacokinetic profiles. The dose of each agent was determined in order to ensure an overlap of molar concentrations (as they differ in terms of molecular weight).

The first test doses of the antifungal drugs were determined on the basis of data published on mice, rabbits and guinea pigs (Nomeir et al., 2000; Meletiadis et al., 2002; Lutsar et al., 2003; Rodriguez et al., 2010; Capilla et al., 2003, 2004; Imai et al., 2004; Roffey et al., 2003) and of the doses used in humans (Pitisuttithum et al., 2005) after extrapolation to rats according to FDA criteria. The doses thus selected (30 mg/kg/d for voriconazole and 50 mg/kg/d for posaconazole) were higher than the calculated doses. According to our scale, such doses
would be able to cure rats, as assessed by a regression of clinical signs. When they were administered at a lower dose, the rats never returned to their baseline status. Indeed, their clinical score remained at around grade 4, as attested by their motor impairments.

In our model, voriconazole appeared to be more potent. However, in view of the molecular weight of posaconazole and voriconazole (700 and 349 g/mol, respectively), the effective doses obtained in our experiments were very similar, being 0.71 µmol/kg and 0.86 µmol/kg, respectively. Nevertheless, the equivalence of the two drugs, or the superiority of one of them, cannot be ruled out because of differences between them in terms of their pharmacokinetic profiles (related to their different routes of administration and their specific physicochemical properties).

The difference between the effective doses in immunocompetent and immunosuppressed rats may be due at least in part to the use of cyclosporine. Indeed, cyclosporine is known to inhibit both CYP3A4 and P-glycoprotein. As triazole molecules are metabolized by CYP3A4, cyclosporine may inhibit their metabolism. Moreover, some studies have shown that, in combination with cyclophilin A, cyclosporine may reduce fungal growth by inhibiting the calcineurin pathway, and may therefore enhance the effect of antifungal agents (Cruz et al., 2000, Dreyfuss, 1976).

**CONCLUSION**

Finally, these models of disseminated scedoporiosis with cerebral involvement, studied in immunocompetent or immunosuppressed animals, are the first to have been described and validated in rats. The results obtained indicate that an inoculum of $10^6$ spores in immunocompetent rats, and of $10^5$ spores in immunosuppressed rats, fitted our objective well. Indeed, the size of the inoculum and the virulence of the *S. apiospermum* strain warrant a broad range of clinical effects, thus enabling a finely tuned ranking of the potency of
compounds belonging to the same therapeutic class. Such models are suitable for drugs administered via both the i.v. and oral routes, as we were able to demonstrate that with both models, access to the sanctuary compartment was possible provided the permeability of the BBB was modulated.

Finally, the differences observed between the two models and between the two drugs lead to the hypothesis that the pharmacokinetic and pharmacodynamic parameters of these agents could be influenced by development of the disease (inflammation, loss of BBB integrity, ...), the concomitant use of cyclosporine, and perhaps the activity of efflux pumps. These models might therefore be implemented for in vivo preclinical screening of antifungal agents.

ACKNOWLEDGMENTS

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Lelièvre, Legras, Godon, Bouchara, Diquet.

Conducted experiments: Lelièvre, Legras, Godon.

Performed data analysis: Lelièvre, Legras, Godon, Franconi, Saint-André, Bouchara.

Wrote or contributed to the writing of the manuscript: Lelièvre, Franconi, Bouchara, Diquet.
REFERENCES


LEGENDS FOR FIGURES

Figure 1. Survival curves of male immunocompetent Sprague Dawley rats inoculated i.v. with four different inocula (ranging from $10^5$ to $10^7$ spores) from *S. apiospermum* IHEM 3817.

Figure 2. Survival curves of male immunosuppressed Sprague Dawley rats inoculated i.v. with *S. apiospermum* IHEM 3817. Four different inoculums (ranging from $10^2$ to $10^5$ spores) were tested. Only data obtained with $10^4$ and $10^5$ spores are shown since no lethality was observed with lower inoculum sizes.

Figure 3. MRI of an infected immunocompetent rat at day 1 (A) or day 3 (B), before injection of the gadolinium salt (I), immediately after injection (2) or 15 min later (3). No abnormalities were observed on day 1. By contrast, the MRI on day 3 revealed peripheral hypersignals indicated by the arrows.

Figure 4. MRI of an infected immunosuppressed rat at day 3 before injection of the gadolinium salt (A), immediately after injection (B) or 15 min later (C). Hypersignals were seen before the injection of gadolinium salt, localized in the frontal areas of both cerebral hemispheres (arrows). After the injection, a rapid diffusion of the gadolinium salt was seen, thus demonstrating the loss of BBB integrity (B and C).

Figure 5. Histological examination of brain sections from an infected immunosuppressed rat stained with HES (A and B) or Gomori’s methenamine silver (C). Inflammatory granulomas were seen at the periphery of the brain section centered by eosinophilic zones (A), as well as in the ventricle, associated with an infiltration by phagocytic cells of the edge of the brain tissue which was detached in places (B). Gomori’s methenamine silver staining revealed the presence of thin septate hyphae within these inflammatory granuloma (C), attesting the cerebral involvement. Magnification x100.

Figure 6. Disease course in immunocompetent rats infected with *S. apiospermum* IHEM 3817 ($10^6$ spores) and treated with voriconazole (A; 10 to 40 mg/kg/d, i.v.) or posaconazole (B; 10,
25 or 50 mg/kg/d, per os). Median and interquartile ranges.

Figure 7. Disease course in immunosuppressed rats infected with *S. apiospermum* IHEM 3817 (10^5 spores) and treated with voriconazole (A; 20, 30 or 40 mg/kg/d, i.v.) or posaconazole (B; 25 or 50 mg/kg/d, per os). Median and interquartile ranges.
# TABLES

## TABLE 1.

Scale used to monitor disease course.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>No symptoms (healthy rat)</td>
</tr>
<tr>
<td>1</td>
<td>Bristling hair and bleeding from the nose</td>
</tr>
<tr>
<td>2</td>
<td>Weight loss of between 1% and 5%</td>
</tr>
<tr>
<td>3</td>
<td>Weight loss of between 5% and 10%</td>
</tr>
<tr>
<td>4</td>
<td>Motor disorders +</td>
</tr>
<tr>
<td></td>
<td>(TST: Crossing one of the paws and unsuccessful attempts to straighten)</td>
</tr>
<tr>
<td>5</td>
<td>Motor disorders ++</td>
</tr>
<tr>
<td></td>
<td>(TST: Crossing of paws, then curling up)</td>
</tr>
<tr>
<td>6</td>
<td>Weight loss greater than 10%</td>
</tr>
<tr>
<td>7</td>
<td>Motor disorders +++: Paralysis</td>
</tr>
<tr>
<td></td>
<td>(TST: paralysis of the hind legs and no reaction)</td>
</tr>
<tr>
<td>8</td>
<td>Half-closed eyes and bent back</td>
</tr>
<tr>
<td>9</td>
<td>Abdominal respiration</td>
</tr>
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</table>
TABLE 2.
Efficacy of antifungal drugs in rat models of disseminated scedosporiosis.

Survival times (in days) were determined in immunocompetent and immunosuppressed rats treated with intravenous voriconazole or oral posaconazole. Data correspond to median values, with minimum and maximum values shown in square brackets.

<table>
<thead>
<tr>
<th>Antifungal drugs and daily doses</th>
<th>Immunocompetent rats</th>
<th>Immunosuppressed rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4.8 [4.3-5.3]</td>
<td>8.3 [7.5-8.5]</td>
</tr>
<tr>
<td>Voriconazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>7 [6.5-7.5]</td>
<td>&gt; 21</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>7.25 [7-8.5]</td>
<td>&gt; 21</td>
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<tr>
<td>Posaconazole</td>
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<tr>
<td>10 mg/kg</td>
<td>7 [6.5-7]</td>
<td>/</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>7.5 [6-10]</td>
<td>14.5 [14-20]</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>&gt; 21</td>
<td>&gt; 21</td>
</tr>
<tr>
<td>75 mg/kg</td>
<td>&gt; 21</td>
<td>&gt; 21</td>
</tr>
</tbody>
</table>
Figure 1

[Graph showing percent survival over days for different concentrations of a substance, with symbols for different concentrations: $10^5$, $5 \times 10^5$, $10^6$, $5 \times 10^6$, and $10^7$.]
Figure 4
Figure 6