Experimental Models of Disseminated Scedosporiosis with Cerebral Involvement

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


Received November 11, 2012; accepted February 14, 2013

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. 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 (0–9) was established, based on weight and clinical and neurologic signs evaluated by the tail suspension test. Cerebral involvement was confirmed by magnetic resonance imaging and histologic examination of brain sections after hematoxylin-eosin-safran or silver staining. Voriconazole or posaconazole was given to the rats at doses ranging from 10 to 75 mg/kg/day via 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 neurologic 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 and pharmacodynamic studies for pharmacokinetic-pharmacodynamic modeling.

Introduction

Therapeutic drug monitoring consists of 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 BBB and the activity of efflux pumps. 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 (0–9) was established, based on weight and clinical and neurologic signs evaluated by the tail suspension test. Cerebral involvement was confirmed by magnetic resonance imaging and histologic examination of brain sections after hematoxylin-eosin-safran or silver staining. Voriconazole or posaconazole was given to the rats at doses ranging from 10 to 75 mg/kg/day via 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 neurologic 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 and pharmacodynamic studies for pharmacokinetic-pharmacodynamic modeling.
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 (Steinbach and Perfect, 2003; Katragkou et al., 2007; Cortez et al., 2008). 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., 2009; 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.

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

**Materials and Methods**

**Organism and Culture Conditions.** This study was performed using *S. 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 regions 1 and 2 of ribosomal DNA genes as *S. apiospermum* sensu stricto (Gligado et al., 2009). The strain was routinely maintained by regular passages on yeast extract-peptone-dextrose agar containing chloramphenicol 0.5 g/l. After 11 days of incubation 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 hemocytometer 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 six rats to a cage in ventilated boxes kept in a protected and temperature- and humidity-controlled room, with a 12-hour on/off light cycle. 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, Basel, Switzerland), diluted in olive oil, at a dose of 15 mg/kg/day. The treatment was started 2 days before inoculation of the fungus, continued on day 1, and then administered every 2 days.

Inoculation via the penile vein was performed 2 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 and 10^6 conidia for immunosuppressed and immunocompetent animals, respectively. The animals were then followed for 8–12 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 neurologic signs, which were noted twice each day until death. Neurologic signs were estimated according to the tail suspension test described by Steru et al. (1985).

**MRI Experiments and Histologic Study.** To confirm cerebral involvement, MRI experiments were performed before and after the intravenous injection of gadolinium salt (Dotarem; Guerbet, Roissy, France), together with a histologic 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, an 84-mm-inner-diameter shielded gradient set capable of 144 mT/m maximum gradient strength, and a 64-mm-diameter birdcage resonator. Three perpendicular two-dimensional spoiled gradient echo images were acquired with a repetition time (TR) of 200 milliseconds, an echo time of 20 milliseconds, 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 (1 day after inoculation of the fungus) 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, brains were collected, fixed in formalin, and included in paraffin. Histologic sections were examined microscopically after hematoxylin-eosin-safran or Gomori’s methenamine silver staining.

**Antifungal Treatment.** Two triazole antifungal drugs, voriconazole (Pfizer, Groton, CT) and posaconazole (Schering Plough, Kenilworth, NJ), were tested in both experimental models 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/day 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/day. The dose of 10 mg/kg was not tested in immunosuppressed rats. Groups of 3 rats were studied for each dose during a first series of experiments, and the results were then confirmed with the most effective doses using 6 rats per group. Controls consisted of untreated animals. All rats were then followed for up to 3 weeks.

**Statistical Analysis.** A Cox model provided an estimate of the effect of the different inocula on rat survival. A nonparametric 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. 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 × 10⁶ and 10⁷ spores) resulted in the death of all the animals on day 3 (Fig. 1). With a lower inoculum of 10⁵, 5 × 10⁵, or 10⁵ spores, the first animals died on day 3, day 4, or day 6, respectively, and the mean survival times were 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 10⁶ or 5 × 10⁵ spores; an inoculum of 10⁶ spores was therefore selected for subsequent experiments. Statistical analysis using the Cox model showed a significant difference (P = 0.0138) between the different groups.

All immunosuppressed rats survived the follow-up period after the injection of a low-dose inoculum ranging from 10² to 10⁴ spores (Fig. 2). With a higher-dose inoculum (10⁵ 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 10⁵ spores (P < 0.0001).

A clinical scale was developed to assess the course of the disease. Clinical signs including animal behavior, bleeding, weight loss, and neurologic signs were graded (Table 1). Grade 0 corresponded to healthy rats. Both immunocompetent and immunosuppressed rats exhibited the same clinical signs, but the amount of time that elapsed before they occurred was longer in immunosuppressed rats, probably in relation to the use of a lower-dose inoculum. The first signs appeared 2 days after the inoculation: the rats presented with brisk hair and bleeding localized around the eyes and from the nose (grade 1). The rats then began to lose weight. Grade 2 corresponded to 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 around and could progress to paralysis (grade >7). The motor disorders were graded from + to +++ (which corresponded to the maximum). Neurologic signs were evaluated using the tail suspension test (Steru et al., 1985). 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 backward (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 neurologic disorders, with difficulty moving their hind legs (grade 4). Gadolinium-enhanced MRI revealed small hypersignals on T1-weighted imaging corresponding to small lesions, 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 neurologic signs (grade 7). Fungal invasion of the brain tissue was confirmed by histologic examination. Hematoxylin-eosin-safran 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 a median of 4.8 days (range 4.3–5.3 days) (Table 2). In view of our objective to double the survival...
time, it appeared that low doses of voriconazole (10 and 20 mg/kg/day) were unsuccessful, as they only caused a 56% increase in survival time. There was no significant difference between the animals receiving voriconazole 10 or 20 mg/kg/day and the control animals. On the other hand, with voriconazole 30 or 40 mg/kg/day, no animals died during the follow-up period.

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

Table 1. Scale used to monitor disease course

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<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 between 1% and 5%</td>
</tr>
<tr>
<td>3</td>
<td>Motor disorders + (TST: crossing one of the paws and unsuccessful attempts to straighten)</td>
</tr>
<tr>
<td>4</td>
<td>Motor disorders ++ (TST: crossing of paws, then curling up)</td>
</tr>
<tr>
<td>5</td>
<td>Weight loss greater than 10%</td>
</tr>
<tr>
<td>6</td>
<td>Motor disorders +++: paralysis (TST: paralysis of the hind legs and no reaction)</td>
</tr>
<tr>
<td>7</td>
<td>Half-closed eyes and bent back</td>
</tr>
<tr>
<td>8</td>
<td>Abdominal respiration</td>
</tr>
</tbody>
</table>

TST, tail suspension test.

*The motor disorders were graded from + to +++ (which corresponded to the maximum).*

Significant differences were observed in the groups receiving 10 or 20 mg/kg/day of voriconazole. With 30 mg/kg/day 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/day and the controls (P = 0.014). In animals receiving 40 mg/kg/day, no clinical signs were observed at any time during the experiment. When compared with the controls, rats receiving posaconazole 10 mg/kg/day also reached grade 4 on day 2, but progression of the disease to grade 7 occurred 3 days later, and these rats died on day 8. A quite similar evolution was seen in rats receiving posaconazole 25 mg/kg/day. Finally, animals receiving posaconazole 50 mg/kg/day reached grade 2 on day 4.

Fig. 3. MRI of an infected immunocompetent rat at day 1 (A) or day 3 (B), before injection of the gadolinium salt (1), immediately after injection (2), or 15 minutes later (3). No abnormalities were observed on day 1. By contrast, the MRI on day 3 revealed peripheral hypersignals, indicated by the arrows.
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 a median of 8.3 days (range 7.5–8.5 days). The four test doses of voriconazole led to a survival time longer than 16 days. Animals treated with posaconazole 25 mg/kg/day survived for a median of 14.5 days (range 14–20 days; no significant difference from the control group). No animals died when they were treated with higher doses of posaconazole.

Among control animals, the clinical score 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/day presented the first clinical signs on day 2. Three 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/day, the first signs appeared on day 3 (grade 1). The animals then improved, as attested by the decrease in the clinical score to grade 0 on day 12. None of the rats receiving 40 mg/kg/day 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/day. These rats 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/day presented few signs throughout the experimental period (little weight loss: grade 2 from days 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 neurologic sequelae), the effective doses were 30 and 50 mg/kg/day of voriconazole and posaconazole, respectively.

### Discussion

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

The first step consisted of 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 area under the concentration-time curve from time 0 to infinity (AUC<sub>0→∞</sub>), 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 of a large volume of cerebrospinal fluid and allometric extrapolations to humans.

---

**Fig. 4.** MRI of an infected immunosuppressed rat at day 3 before injection of the gadolinium salt (A), immediately after injection (B), or 15 minutes 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 (2 and 3).

**Fig. 5.** Histologic examination of brain sections from an infected immunosuppressed rat stained with hematoxylin-eosin-safran (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 to the cerebral involvement. Magnification 100×.
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 parentheses.

<table>
<thead>
<tr>
<th>Antifungal Drugs and Daily Doses</th>
<th>Survival Time (Days)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunocompetent Rats</td>
<td>Immunosuppressed Rats</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>----------------------</td>
<td>--------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Controls</td>
<td>4.8 (4.3–5.3)</td>
<td>8.3 (7.5–8.5)</td>
<td></td>
</tr>
<tr>
<td>Voriconazole (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7 (6.5–7.5)</td>
<td>&gt;21</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>7.25 (7–8.5)</td>
<td>&gt;21</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>&gt;21</td>
<td>&gt;21</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>&gt;21</td>
<td>&gt;21</td>
<td></td>
</tr>
<tr>
<td>Posaconazole (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7 (6.5–7)</td>
<td>Not tested</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>7.5 (6–10)</td>
<td>14.5 (14–20)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>&gt;21</td>
<td>&gt;21</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>&gt;21</td>
<td>&gt;21</td>
<td></td>
</tr>
</tbody>
</table>

Regarding the fungal species, *S. apiospermum* was chosen because 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, antifungal activity has been reported for cyclosporine; however, this latter property has only been demonstrated in vitro to date (Dreyfuss et al., 1976; Cruz et al., 2000).

To estimate the degree and kinetics of disease development, different clinical stages were defined using a scale that included clinical signs, weight loss, and neurologic symptoms to assess the disease course and establish a comparative dosering profile for drugs of interest.

As far as the clinical course of the disease was concerned, and because *S. 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.

**Fig. 6.** Disease course in immunocompetent rats infected with *S. apiospermum* IHEM 3817 (10⁶ spores) and treated with voriconazole (10–40 mg/kg/day i.v.) (A) or posaconazole (10, 25, or 50 mg/kg/day orally) (B). Data are presented as the median and interquartile ranges.

**Fig. 7.** Disease course in immunosuppressed rats infected with *S. apiospermum* IHEM 3817 (10⁵ spores) and treated with voriconazole (20, 30, or 40 mg/kg/day i.v.) or posaconazole (25 or 50 mg/kg/day orally). Data are presented as the median and interquartile ranges.
The focal hypersignals led to the localization of \textit{S. apiosepernum} 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 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 \textit{S. apiosepernum} and can be used for treatment of meningitis. They both inhibit lanosterol 14-o-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 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; Capilla et al., 2003; Lutsar et al., 2003; Roffey et al., 2003; Capilla and Guarro, 2004; Imai et al., 2004; Rodriguez et al., 2010) and on the doses used in humans (Pitsisuttithum et al., 2005) after extrapolation to rats according to Food and Drug Administration criteria. The doses thus selected (30 mg/kg/day for voriconazole and 50 mg/kg/day 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 the drugs were administered at a lower dose, the rats never returned to their baseline status. Indeed, their clinical score remained 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 (0.71 and 0.86 \textmu mol/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 (Dreyfuss et al., 1976; Cruz et al., 2000).

**Conclusion**

Finally, these models of disseminated scedosporiosis with cerebral involvement, studied in immunocompetent or immunosuppressed animals, are the first to be described and validated in rats. The results obtained indicate that an inoculum of 10⁶ spores in immunocompetent rats, and 10⁵ spores in immunosuppressed rats, fit our objective well. Indeed, the size of the inoculum and the virulence of the \textit{S. apiosepernum} 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 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 led 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, etc.), 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**

The authors thank Pfizer and Schering Plough for supplying the voriconazole and posaconazole reference substances used for the experiments and publications.

**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**


Address correspondence to: 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