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Impact of Infection Status and Cyclosporine on Voriconazole Pharmacokinetics in an Experimental Model of Cerebral Scedosporiosis[S]

Bénédicte Lelièvre, Marie Briet, Charlotte Godon, Pierre Legras, Jérémie Riou, Patrick Vandeputte, Bertrand Diquet, and Jean-Philippe Bouchara

Service de Pharmacologie-Toxicologie-Centre Régional de Pharmacovigilance, Institut de Biologie en Santé (B.L., M.B., B.D.), MITOVASC, UMR CNRS 6214, Inserm 1083, Université d'Angers (M.B.), Micro- et Nanomédecines Biomimétiques, UMR INSERM 1066–CNRS 6021, Université d'Angers (J.R.), and Laboratoire de Parasitologie-Mycologie, Institut de Biologie en Santé (J.-P.B.), Centre Hospitalier Universitaire, Angers, France; Groupe d'Etude des Interactions Hôte-Pathogène (EA 3142), Université d'Angers, Université de Bretagne Occidentale, Institut de Biologie en Santé, Angers, France (B.L., C.G., P.L., P.V., J.-P.B., B.D.); and Service Commun de l'Animalerie Hospitalo-Universitaire, Université d'Angers, Angers, France (P.L.)

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ABSTRACT

Cerebral *Scedosporium* infections usually occur in lung transplant recipients as well as in immunocompetent patients in the context of near drowning. Voriconazole is the first-line treatment. The diffusion of voriconazole through the blood-brain barrier in the context of cerebral infection and cyclosporine administration is crucial and remains a matter of debate. To address this issue, the pharmacokinetics of voriconazole was assessed in the plasma, cerebrospinal fluid (CSF), and brain in an experimental model of cerebral scedosporiosis in rats receiving or not receiving cyclosporine. A single dose of voriconazole (30 mg/kg, i.v.) was administered to six groups of rats randomized according to the infection status and the cyclosporine dosing regimen (no cyclosporine, a single dose, or three doses; 15 mg/kg each). Voriconazole concentrations in plasma, CSF, and brain samples were quantified using ultra-performance liquid chromatography–tandem mass spectrometry and high-performance liquid chromatography UV methods and were documented up to 48 hours after administration. Pharmacokinetic parameters were estimated using a noncompartmental approach. Voriconazole pharmacokinetic profiles were similar for plasma, CSF, and brain in all groups studied. The voriconazole C_{max} and area under the curve (AUC) ($AUC_{0 \geq 48\ hours}$) values were significantly higher in plasma than in CSF [CSF/plasma ratio, median (range) = 0.5 (0.39–0.55) for $AUC_{0 \geq 48\ hours}$ and 0.47 (0.35 and 0.75) for C_{max}]. Cyclosporine administration was significantly associated with an increase in voriconazole exposure in the plasma, CSF, and brain. In the plasma, but not in the brain, an interaction between the infection and cyclosporine administration reduced the positive impact of cyclosporine on voriconazole exposure. Together, these results emphasize the impact of cyclosporine on brain voriconazole exposure.

Introduction

Usually a saprophyte, *Scedosporium apiospermum* is a filamentous soil fungus that may cause a wide variety of diseases in human, ranging from localized infections such as subcutaneous mycetoma, keratitis, or bone and joint mycoses to disseminated infections (Steinbach and Perfect, 2003; Cortez et al., 2008). This old pathogen has gained increasing attention during the past two decades due to its worldwide recognition as the second most common cause of fungal respiratory infections in patients with cystic fibrosis (Cimon et al., 2000; Rainer et al., 2008; Harun et al., 2010). In this context, following lung transplantation in cystic fibrosis patients previously colonized by *S. apiospermum*, severe and often fatal disseminated infections with cerebral involvement may occur (Morin et al., 1999; Symoens et al., 2006; Morio et al., 2010; Luijk et al., 2011; Miraldi et al., 2012; Hirschi et al., 2012). In addition, cerebral scedosporiosis is also reported in immunocompetent patients in the context of near drowning (Cortez et al., 2008). Until the 1990s, the mortality rate in systemic *S. apiospermum* infections was higher than 75%; however, recent triazole antifungals have improved their prognosis (Steinbach and Perfect, 2003; Cortez et al., 2008). Current recommendations for the management of patients with a systemic *S. apiospermum* infection rely on the use of voriconazole (Tortorano et al., 2014).

Voriconazole is a triazole drug inhibiting cytochrome P450–dependent 14α-lanosterol demethylase, an enzyme that plays a key role in the synthesis of ergosterol, which is a major constituent of fungal membranes (Manavathu et al., 1998). Although voriconazole is a broad-spectrum antifungal that acts on both yeasts and filamentous fungi, differences occur between fungal species in their susceptibility to this drug. For instance, voriconazole usually exhibits high minimum inhibitory

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ABBREVIATIONS: AUC, area under the curve; CSF, cerebrospinal fluid.

concentrations against *Scedosporium* isolates (Sheehan et al., 1999; Lewis et al., 2005).

In humans, the pharmacokinetics of voriconazole follow a nonlinear profile, with 58% of the drug bound to plasma proteins (Levêque et al., 2006). The drug is metabolized by cytochrome P450s, mainly CYP3A4, CYP2C19, and to a lesser extent CYP2C9 (Purkins et al., 2002). Studies using human liver microsomes have shown that the metabolism of voriconazole presents biphasic kinetics (Hyland et al., 2003). The entire dose of voriconazole is eliminated by the renal route in 48 hours, mainly (98%) as metabolites (Levêque et al., 2006). Due to the nonlinear pharmacokinetics of voriconazole and intra- and interindividual variability, therapeutic drug monitoring of voriconazole in plasma is recommended. Nevertheless, very limited pharmacokinetics data are available regarding infections localized to sanctuary organs such as the brain or lungs. In the case of a cerebral infection, the rate of diffusion of the antifungal drugs through the blood-brain barrier remains questionable.

In the present study, we determined the pharmacokinetics of voriconazole in the plasma, cerebrospinal fluid (CSF), and brain in a rat model of disseminated scedosporiosis with cerebral involvement to evaluate its diffusion through the blood-brain barrier. In addition, the study was designed to investigate the impact of infection and/or cyclosporine administration on the voriconazole pharmacokinetics in the plasma, CSF, and brain.

Materials and Methods

Animal Model of Scedosporiosis and Study Design. Male Sprague-Dawley rats, 10 weeks old, weighing 250–274 g, were purchased from Janvier SAS (Le Genest Saint Isle, France). The animals were housed in ventilated boxes, six per cage, in a protected and a temperature- and humidity-controlled room, with 12-hour on/off light cycles. The animals were given free access to food and water. Animal care was carried out in strict accordance with French Ministry of Agriculture regulations and the animals were euthanized using CO_2 at the end of the experiments (Agreement No. 00784.02). The study design is summarized in Supplemental Fig. 1. Briefly, six randomized groups of 24 rats were studied ($n = 144$). Half of them were infected with *S. apiospermum*.

S. apiospermum (Institute of Hygiene and Epidemiology-Mycology section culture collection IHEM 3817; Scientific Institute of Public Health, Brussels, Belgium) was inoculated intravenously (Lelièvre et al., 2013). As previously described (Lelièvre et al., 2013), the inoculum size was 10^5 conidia in rats receiving cyclosporine and 10^6 conidia in rats nontreated with cyclosporine. The cerebral infection was controlled for each infected animal at sacrifice, with evaluation of the cerebral fungal load by a quantitative polymerase chain reaction method and culture of brain samples on Sabouraud dextrose agar plates (data not shown).

Voriconazole (Vfend; Pfizer (VFend; Pfizer, New York, NY)) was administered in all groups intravenously 24 hours after inoculation at 30 mg/kg, as previously described (Lelièvre et al., 2013). Cyclosporine was administered at low dosage (15 mg/kg 1 hour before administration of voriconazole, referred to as Cyclo 15) in two groups of rats (infected/noninfected) and at high dosage (15 mg/kg at D-3, D-2, Day - 3, Day - 2 and 1 hour before administration of voriconazole, referred to as Cyclo 45) in two other groups (infected/noninfected), as previously described (Lelièvre et al., 2013). Cyclosporine (Neoral; Novartis) Novartis, Basel, Switzerland) was diluted in olive oil and administered by oral gavage (Supplemental Fig. 1). Control rats received olive oil alone (referred to as Cyclo 0). For the inoculation and voriconazole administration procedures, the rats were anesthetized with 5% isoflurane for induction (5–10 minutes) and then 2% isoflurane in 20:1 air/oxygen for maintenance. As such, exposure to isoflurane never exceeded 15 minutes for each rat.

Sampling for Pharmacokinetic Studies. Regarding the intravenous route of administration and published data, the pharmacokinetics of voriconazole were studied for 48 hours, using eight sampling times: 5 and 20 minutes and 1, 3, 6, 10, 24, and 48 hours after administration. Blood samples were collected from three rats for each sampling time in $EDTAK_2$-containing tubes (Becton-Dickinson, Plymouth, UK) and immediately centrifuged at 3240*g* for 10 minutes at 15°C. CSF samples were collected at the same time points and immediately centrifuged for 10 minutes at 4°C. Plasma and CSF were immediately frozen and stored at −20°C until analysis. After euthanizing the animals, the brains were collected, washed with sodium chloride 0.9%, weighed, and ground in 4 ml of water. The volume of each homogenate was precisely measured, and three aliquots per homogenate were immediately frozen and stored at −20°C.

Voriconazole Assay. Voriconazole was quantified in plasma or CSF samples after protein precipitation by ultra-performance liquid chromatography–tandem mass spectrometry (Quattro Premier; Waters, Guyancourt, France). Protein precipitation was carried out using 200 μl of methanol containing d3-voriconazole as the internal standard (LGC Standards, Molsheim, France). After homogenization and centrifugation, the resulting supernatant was filtered and transferred to microvials to be analyzed using an Acquity UPLC BEH C_{18} Column (1.7 μm, 2.1 × 50 mm; Waters), maintained at 40°C. The mobile phase was nebulized using an electrospray source in positive mode. The multiple reactions monitoring transitions were m/z 350 > 127 and 350 > 280.9 for voriconazole and m/z 353 > 130 and 353 > 283.9 for d3-voriconazole. The calibration curves were quadratic over the range 0.002–20 mg/l with a coefficient of determination >0.99. Based on quality control samples, the overall relative S.D. was below 10% (intraday and interday precisions were 7.5% and 5.0%, respectively). The overall relative error was within ±10%. The lower limit of quantification was validated at 0.5 μg/l (CV = 13.5%).

For brain samples, a liquid/liquid extraction using a dichloromethane/methanol mixture (60/40, v/v) was performed after grinding the sample and adding internal standard (prazepam) to the homogenate. After centrifugation, the supernatant was evaporated to dryness using nitrogen flow. The residue was then solubilized with 80 μl water and 20 μl HCl 1 N. After homogenization and centrifugation, 20 μl of the supernatant was injected in a Kinetex C_{18} column (5 μm, 100 Å, 100 × 4.6 mm; Phenomenex, Le Pecq, France), maintained at 40°C and analyzed using a high-performance liquid chromatography-UV diode array detector (HP1100; Agilent, Massy, France). Acetonitrile/water/phosphate buffer 1 M (49/50/1, v/v) was used as the mobile phase and the running time was 10 minutes, with a flow rate of 0.8 ml/min and final detection at 254 nm. The calibration, ranging from 0.05 to 5 mg/l, was prepared by spiking different voriconazole concentrations in 500 μl of brain homogenate from a healthy rat. Three levels of internal quality control (0.05, 1.5, and 4 mg/l) were prepared similarly. The efficiency of the extraction was evaluated at more than 80% and the method was validated. The intraday and interday precisions were 7.2% and 6.4%, respectively). The limit of quantification (0.05 mg/l) was validated by measuring a control 10 times (CV = 14.8%).

Pharmacokinetic Analyses. The noncompartmental approach was performed using Phoenix 64 software (Change for Certara, Princeton, NJ) to estimate the main voriconazole pharmacokinetic parameters. Since only one sample of blood/CSF/brain was collected from each animal, data from the animals of the same group were pooled using a naive average data approach. Data were analyzed separately according to infection status or cyclosporine dosing regimen. For each group, mean maximum concentration (C_{max}) and time to C_{max} (T_{max}) were determined from experimental curves using the average concentration at each point. The voriconazole terminal half-life was derived from the elimination rate constant λ_z, which corresponds to the slope of the log-linear terminal portion of the plasma concentration versus time curve, determined using

unweighted linear least-squares regression analysis. The area under the curve (AUC) was computed from 0 to 48 hours using the log-linear trapezoidal method.

Statistical Analyses. The effects of the infection status and the cyclosporine dosing regimen on the voriconazole pharmacokinetic parameters (AUC, C_{max}, T_{max}, and $T_{1/2}$), estimated by the noncompartmental approach, were determined using multivariate linear regression analysis. The explanatory covariates were chosen based on clinical and biologic considerations, without any selection procedure to avoid any overfitting issues, as recommended (Henderson and Velleman, 1981; Hurvich and Tsai, 1990; Derksen and Keselman, 1992). In this model, interactions between cyclosporine and infection, cyclosporine and medium, or infection and medium were taken into account to evaluate their potential impact on voriconazole AUCs. In addition, comparisons of AUC between subpopulations and groups were carried out, with control of the family wise error rate (Simes, 1986). For the C_{max}, T_{max}, and global AUC values (considered for all media), the linear regression assumptions were not satisfied; therefore, the data were analyzed using nonparametric analysis (Kruskal-Wallis test and post-hoc analysis).

The heterogeneity of AUC endpoints between sample types led us to stratify this analysis on this variable. For stratified models, linear model assumptions were checked. Fixed effects included in the models were infection, cyclosporine dose, sample type, and interactions between each variable. Backward manual variables selection was performed for each model. All statistical analyses were performed using R software (https://www.R-project.org/) and a type I error rate defined at 0.05.

Results

The mean pharmacokinetic parameters for voriconazole in plasma, CSF, and brain are summarized in Table 1. After a single administration of 30 mg/kg of voriconazole, the C_{max} and $AUC_{0 \geq 48\ hours}$ values of voriconazole were significantly higher in the plasma compartment compared with the CSF compartment ($P < 0.001$ for C_{max} and $AUC_{0 \geq 48\ hours}$) (Table 1). The C_{max} CSF/C_{max} plasma and $AUC_{0 \geq 48\ hours}$ CSF/$AUC_{0 \geq 48\ hours}$ plasma ratios ranged between 0.39 and 0.55 (median 0.50) and 0.35 and 0.75 (median 0.47), respectively. The shapes of the concentration-time profiles in plasma and CSF or plasma and brain were similar (Supplemental Fig. 2).

In rats receiving no cyclosporine, plasma voriconazole $AUC_{0 \geq 48\ hours}$ was significantly increased in infected rats compared with noninfected rats ($P = 0.008$). Treatment of noninfected rats with one dose ($P = 0.047$) or three doses ($P < 0.001$) of cyclosporine was associated with a significant increase in plasma voriconazole $AUC_{0 \geq 48\ hours}$ (Table 1). In noninfected rats, a significant increase in CSF voriconazole $AUC_{0 \geq 48\ hours}$ was only observed in rats receiving three doses of cyclosporine compared with rats receiving no cyclosporine ($P < 0.001$). Conversely, treatment of infected rats with one or three doses of cyclosporine did not impact voriconazole plasma or CSF $AUC_{0 \geq 48\ hours}$ (Table 1). In multivariate analyses, administration of three doses of cyclosporine was a major determinant of CSF and plasma AUCs ($P < 0.001$). A significant interaction was shown between infection and administration of three doses of cyclosporine ($P < 0.001$). The increase in CSF and plasma $AUC_{0 \geq 48\ hours}$ after administration of three doses of cyclosporine was more important in noninfected rats compared with infected rats ($P = 0.026$). In rats receiving no cyclosporine, infection was significantly associated with an increase in plasma AUC of voriconazole ($P = 0.008$) (Table 2). Infection and cyclosporine dosing regimens were not identified as influencing the C_{max} and $AUC_{0 \geq 48\ hours}$ CSF/plasma ratio (Table 1).

In the brain, the effect of the infection was not observed in voriconazole $AUC_{0 \geq 48\ hours}$, whereas the administration of three doses of cyclosporine led to a significant increase in

TABLE 1
Main pharmacokinetic parameters of voriconazole determined by noncompartmental analysis
$N = 24$ rats per group.

Parameter	Cyclo 0		Cyclo 15		Cyclo 45	
	Noninfected	Infected	Noninfected	Infected	Noninfected	Infected
T_{max} (h)						
Plasma	0.333	1	1	0.333	0.083	1
CSF	1	0.33	0.33	0.33	3	0.33
Brain	1	0.33	0.083	0.33	0.083	0.33
C_{max} ± S.D. (mg/l)						
Plasma	16.9 ± 1.5	18.1 ± 2.3	20.2 ± 7.2	17.1 ± 0.4	23.1 ± 2.7	17.9 ± 0.6
CSF	8.7 ± 1.2	8.1 ± 0.6	7.3 ± 1.8	9.4 ± 0.6	9.3 ± 0.1	8.6 ± 0.8
C_{max} ± S.D. (μg/g)						
Brain	28.8 ± 2.3	33.5 ± 2.1	22.8 ± 4.3	29.0 ± 5.2	35.4 ± 2.0	32.5 ± 1.5
C_{max} CSF/C_{max} plasma ratio	0.52 ± 0.11	0.51 ± 0.03	0.39 ± 0.03	0.55 ± 0.02	0.43 ± 0.13	0.50 ± 0.07
$AUC_{0 \geq 48\ hours}$ ± S.D. (mg·h/l)						
Plasma	145.0 ± 13.2	189.8 ± 10.5[a],**	193.2 ± 13.3[b],*	174.8 ± 5.7	272.3 ± 15[c],***	214.6 ± 5.8
CSF	104.3 ± 6.5	96.0 ± 13.6	66.5 ± 4.7	95.3 ± 4.1	120.2 ± 37.3[c],***	92.5 ± 1.4
$AUC_{0 \geq 48\ hours}$ ± S.D. (μg·h/g)						
Brain	399.1 ± 70.1	420.5 ± 38.0	276.3 ± 18.7	388.7 ± 32.4	510.3 ± 23.4[c],***	374.2 ± 16.5
$AUC_{0 \geq 48\ hours}$ CSF/plasma ratio ± S.D.	0.75 ± 0.13	0.50 ± 0.04	0.35 ± 0.02	0.56 ± 0.01	0.44 ± 0.04	0.43 ± 0.01
$T_{1/2}$ (h)						
Plasma	6.1	10.4	7.3	9.4	12.2	11.6
CSF	15.0	10.7	8.0	9.8	11.8	9.3
Brain	14.4	13.5	9.2	9.4	13.1	9.5

$AUC_{0 \geq 48\ h}$, area under the curve from 0 to 48 h; C_{max}, maximum concentration; Cyclo 0, rats receiving no cyclosporine; Cyclo 15, rats receiving a single dose of cyclosporine, 15 mg/kg; Cyclo 45, rats receiving three doses of cyclosporine, 15 mg/kg each; SD, standard deviation; $T_{1/2}$, half-life; T_{max}, maximum time corresponding to C_{max}.
[a]Cyclo 0 infected vs. Cyclo 0 noninfected.
[b]Cyclo 15 noninfected vs. Cyclo 0 noninfected.
[c]Cyclo 45 noninfected vs. Cyclo 0 noninfected.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

TABLE 2
Multivariate analysis of voriconazole AUCs in plasma and CSF

Fixed Effects	Beta	CI 95%	*P* Value
Intercept	150,957	130,094.3; 17,1819.7	<0.001
Infection (yes vs. no)	33,576	9485.5; 57,665.8	0.008
Cyclosporine (15 vs. 0)	29,873	368.3; 59,376.9	0.047
Cyclosporine (3 × 15 vs. 0)	112,801	83,296.3; 142,304.9	<0.001
Medium (CSF vs. plasma)	−83,793	−107,882.8; −59,702.5	<0.001
Infection (yes vs. no): cyclosporine (15 vs. 0)	−27,395	−61,463.4; 6673.8	0.111
Infection (yes vs. no): cyclosporine (3 × 15 vs. 0)	−75,741	−109,809.7; −41,672.5	<0.001
Cyclosporine (15 vs. 0): medium (CSF vs. plasma)	−18,242	−52,310.8; 15,826.4	0.028
Cyclosporine (3 × 15 vs. 0): medium (CSF vs. plasma)	−52,569	−86,637.8; −18,500.6	0.004

CI, confidence interval.

voriconazole $AUC_{0 \geq 48\ hours}$ (Tables 1 and 3). The C_{max} value in the brain was similar for the six groups of rats ($P = 0.106$) (Table 1). The relations between brain and plasma voriconazole concentrations were weak (Supplemental Fig. 3). Taking into account CSF instead of plasma, the correlation coefficients were higher under all conditions (Supplemental Fig. 3).

Discussion

In the present study, we demonstrated that voriconazole pharmacokinetic profiles were similar in the plasma, CSF, and brain compartments. Both infection and cyclosporine administration impacted voriconazole exposure in the plasma, whereas only repeated cyclosporine administration influenced voriconazole exposure in the brain and CSF.

To the best of our knowledge, this is the first experimental study evaluating cerebral diffusion of voriconazole in a rat model of scedosporiosis with cerebral involvement. In our model, voriconazole exposure in CSF was about half of the exposure in plasma (Table 1). This observation is in line with other experimental studies involving voriconazole in immunocompetent noninfected guinea pigs (Lutsar et al., 2003), and in a cryptococcal meningitis rat model (Alves et al., 2017).

Interestingly, repeated cyclosporine administration (3 × 15 mg/kg) increased voriconazole exposure in the CSF and brain (Table 3). The impact of cyclosporine on voriconazole concentration in the brain could be explained by the increase in plasma voriconazole exposure. As expected, cyclosporine administration increased plasma voriconazole exposure. This finding is consistent with the inhibition effect of cyclosporine on the metabolism of voriconazole (Roffey et al., 2003; Groll et al., 2004). In vitro, voriconazole is metabolized mainly by CYP2C19 and CYP3A4 following a biphasic kinetic pattern (Hyland et al., 2003). The respective involvement of these two cytochromes is dependent on voriconazole concentration. In the case of high concentrations of voriconazole, the low-affinity and high-capacity enzyme CYP3A4 is mainly responsible for the metabolism (about 73%), whereas about 93% of voriconazole is metabolized by the high-affinity and low-capacity enzyme CYP2C19 in the case of low concentrations (Hyland et al., 2003). Since cyclosporine is a potent inhibitor of CYP3A4 (Hope et al., 2013), it should modify voriconazole disposition at high concentrations of voriconazole.

Cyclosporine, through its local effect on brain vascular permeability, may also positively and directly impact voriconazole brain diffusion. Indeed, cyclosporine was shown to inhibit adrenomedullin-mediated autocrine regulation of the blood-brain barrier by lowering adenyl cyclase/cAMP/protein kinase A signaling, which increases the brain endothelial permeability (Takata et al., 2009; Dohgu et al., 2010).

In this experimental model involving *S. aspiospermum*, infection did not influence voriconazole brain diffusion. This result may appear conflicting with those reported by Alves et al. (2017), who used another infectious rat model involving a yeast, *Cryptococcus neoformans* and not a filamentous fungus. In addition, only free voriconazole was quantified in the brain by Alves et al. (2017), using microdialysis technologies.

It is worth noting that in the present study an interaction was observed between cyclosporine administration and infection in plasma. In rats receiving three doses of cyclosporine, the increase in CSF and plasma $AUC_{0 \geq 48\ hours}$ was more important in noninfected rats. Sepsis is known to impact pharmacokinetic parameters, particularly the volume of distribution. Sepsis and inflammation increase vascular permeability, decrease heart flow rate, and modify peripheral resistances. The modification of the volume of distribution by the infection could partly explain this observation (De Paepe et al., 2002; Kulmatycki and Jamali, 2005).

In our study, C_{max} was not reached at the first sampling time as is usually observed following intravenous bolus administration. The mean concentration in the whole rat population increased slowly to reach a maximum between 20 minutes and 1 hour after administration. The administration of voriconazole was performed after anesthetizing the animals using isoflurane, which exhibits cardiac effects, including negative inotropic and chronotropic effects, and reduction of ventricular ectopic automaticity (Eger, 1981; Miralles et al., 1989; Hanouz et al., 1998). These pharmacological properties may explain the observed delay of voriconazole diffusion in systemic circulation after administration in the caudal vein.

In conclusion, our data highlight the impact of cyclosporine on plasma, CSF, and brain pharmacokinetics of voriconazole. These results offer promising developments in terms of drug

TABLE 3
Multivariate analysis of voriconazole AUCs in the brain

Fixed Effects	Estimate	S.E.	CI 95%	*P* Value
Intercept	12.118	0.063	11.990; 12.245	<0.001
Infection (yes vs. no)	0.005	0.056	−0.109; 0.120	0.925
Cyclosporine (15 vs. 0)	−0.055	0.069	−0.195; 0.085	0.431
Cyclosporine (3 × 15 vs. 0)	0.225	0.069	0.084; 0.365	0.003

CI, confidence interval.

monitoring and strategies to improve cerebral diffusion of voriconazole.

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Authorship Contributions

Participated in research design: Lelièvre, Diquet, Bouchara.

Conducted experiments: Lelièvre, Godon, Legras, Vandeputte.

Performed data analysis: Lelièvre, Briet, Riou, Bouchara.

Wrote or contributed to the writing of the manuscript: Lelièvre, Briet, Riou, Bouchara.

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Address correspondence to: Dr. Bénédicte Lelièvre, Department of Pharmacology, Angers University Hospital, 4 Rue Larrey, 49100 Angers, France. E-mail: belelievre@chu-angers.fr