Voriconazole Concentrations in the Cerebrospinal Fluid and Brain Tissue of Guinea Pigs and Immunocompromised Patients

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We characterized voriconazole concentrations in the cerebrospinal fluid (CSF) of immunocompetent guinea pigs and patients with invasive fungal infections. In animals, after receipt of oral doses of 4 or 10 mg/kg every 8 h, the mean ratios of CSF to plasma total and free drug concentration were 0.68 and 1.3, respectively. In humans, 1–10 h after receipt of voriconazole, the CSF concentrations ranged from 0.08 to 3.93 μg/mL, and the ratio of CSF to plasma concentration ranged from 0.22 to 1.0 (median, 0.46).

Fungal infections of the CNS are associated with a poor prognosis [1–3]. Although amphotericin B and itraconazole are active in vitro against most of the causative fungi, both agents penetrate poorly into the CSF, which may partly explain their limited efficacy [1, 4, 5]. Voriconazole is a new triazole with a spectrum of activity against organisms such as Aspergillus species (MIC90, 0.25–8 μg/mL) and Scedosporium apiospermum (MIC90, 0.5 μg/mL) [4]. Its pharmacokinetic properties are described elsewhere [6, 7].

We studied voriconazole concentrations in the CSF of immunocompetent guinea pigs and immunocompromised patients. Informed consent was obtained from patients or their parents or guardians, and animal experimentation guidelines were followed.

Methods. Adult, female, nonpigmented Dunkin Hartley guinea pigs (mean weight, 350 g) were housed in stock boxes with access to food and water ad libitum. Voriconazole doses of 2, 4, or 10 mg/kg were administered every 8 h for 5 days by oral gavage after dissolution in polyethylene glycol 200 to a final dose volume of 0.5 mL. On day 5, 1 animal at each dose level was killed by exsanguination under anaesthesia every hour after dose administration. Blood samples were collected from the inferior vena cava into heparinized tubes. Plasma was recovered from plasma via centrifugation (1500 g for 10 min). CSF samples were obtained via direct needle puncture of the cisterna magna, and the brain was removed and retained for analysis. All samples were frozen at approximately −20°C until analysis.

Validated high-performance liquid chromatography (HPLC) with UV detection at a wavelength of 254 nm was used for measuring voriconazole concentrations in specimens obtained from the animals. Brain tissue was homogenized in 4 volumes of water, and 0.1 mL of brain homogenate or 0.1 mL of plasma was diluted by addition of 1 mL of a 0.2 mol/L borate buffer solution at a pH of 9.0 before extraction into 4 mL of diethyl ether. The extracted solvents were evaporated to dryness under a stream of nitrogen, reconstituted in mobile phase, and injected into the chromatograph for HPLC analysis. CSF samples were analyzed with HPLC by direct injection into the chromatograph. CSF samples that contained visible blood were excluded from analysis. Standard curves were constructed for control plasma, brain homogenate, and 0.9% (wt/vol) saline by the addition of 0.1–10 μg/mL or 0.1–5 μg/mL voriconazole to both plasma and CSF samples or to brain homogenate, respectively. Samples in which concentrations exceeded the limits of the standard curve were diluted with CSF or brain homogenate, respectively. Internal standard UK-101,608 (2.5 μg/mL) was added to all samples and standards.

The extent of plasma protein binding was determined in guinea pig and human control plasma. Voriconazole containing the 14C-labelled isotope was added to 4 mL of plasma at a concentration of 1 μg/mL (n = 3 samples per species), and 1 mL aliquots were dialyzed (Spectrapor 1 dialysis membrane [molecular weight cutoff, 6000–8000 kDa]; Spectrum Medical Industries) against 1 mL of a 0.1 mol/L PBS solution (pH 7.4). Dialysis was performed for 2 h at 37°C with a rotating dialyzer (Dianorm MSE). Drug concentrations in PBS and plasma were determined by liquid scintillation counting.

Data for all patients in the voriconazole clinical program, which consisted of phase 2 and 3 studies of and a compassionate-use program for voriconazole, from January 1994 through December 2001 from whom CSF samples were obtained for measurement of voriconazole concentrations were included. When clinically indicated, random CSF samples for measurement of
voriconazole concentrations were obtained via lumbar or ventricular puncture after steady-state plasma levels of voriconazole had been achieved. Blood samples were obtained on the same day (but not always at the same time) as the CSF samples (table 1). After centrifugation, all supernatants were stored at −20°C. When feasible, brain tissue specimens were collected during autopsy and stored frozen at −20°C until analysis.

All samples were assayed in a central laboratory with validated reversed-phase HPLC with UV detection 2 weeks after they were obtained from patients [8]. CSF samples were measured against calibration standards, and quality-control samples were prepared in isotonic saline. Brain-tissue specimens were analyzed as described previously. The lower limits of quantification for the CSF and brain-tissue specimens were 0.01 and 0.05 μg/mL, respectively, and the upper limit of the calibration range was 3.0 μg/mL for both.

Results. Twelve animals were studied at each dose level. After administration of voriconazole doses of 2, 4, and 10 mg/kg q8h for 5 days, the mean voriconazole concentration in CSF samples was undetectable, 0.26 μg/mL, and 3.2 μg/mL, respectively. After the dose of 10 mg/kg, the ratios of the mean voriconazole concentrations in CSF samples to total and free voriconazole concentrations in plasma samples were 0.68 and 1.23, respectively (figure 1). Voriconazole concentrations in brain tissue specimens obtained 15 min and 1 h after administration of the 10-mg/kg dose were 6.8 and 2.7 μg/g, respectively. Voriconazole protein binding in guinea pigs was 45%.

A total of 36 CSF samples obtained from 14 patients (age range, 5–50 years) were assayed for voriconazole concentrations. For all patients, data on demographic characteristics, voriconazole doses received, and CSF and plasma concentrations are presented in table 1. CSF samples were collected either because of CNS fungal infection (n = 9 patients) or because of a high suspicion of brain involvement in patients with disseminated fungal infection (n = 5 patients). For 3 patients, all of whom had intraventricular catheters in place, 13, 7, and 3 CSF samples were collected. Only 1 or 2 samples per patient were obtained from the remaining 11, and all were obtained via lumbar puncture. The CSF and plasma concentrations of voriconazole 1–10 h after administration ranged from 0.08 to 3.93 μg/mL (median, 0.65 μg/mL; interquartile range, 0.22–0.97 μg/mL) and from <0.01 to 7.23 μg/mL (median, 1.08 μg/mL; interquartile range, 0.37–6.14 μg/mL), respectively. The ratio of CSF to plasma concentrations of voriconazole varied from 0.22 to 1.0 (median, 0.46; table 1). There was a linear relationship between the voriconazole dose and concentration in CSF samples.

From 2 patients who died after receiving treatment for pulmonary aspergillosis, post mortem brain tissue specimens were obtained. One was a 36-year-old woman who had received intravenous voriconazole therapy at a dose of 4.5 mg/kg q12h, and the second patient was a 13-year-old boy who had received intravenous voriconazole therapy at a dose of 7.2 mg/kg q24h. Both patients died 9–10 h after therapy was stopped, and voriconazole concentrations in the brain-tissue specimens were 11.8 μg/mg (female patient) and 58.5 μg/mg (male patient).

Discussion. These data show that voriconazole penetrates the CSF and brain tissue and that penetration (at least in animals) is not dependent on meningeal inflammation. CSF samples were collected randomly, and the majority of voriconazole levels in these samples exceeded the MIC90 values for most Aspergillus species and S. apiospermum isolates.

The CSF concentrations presented here were not from a prospectively designed study but were determined in samples that were randomly collected from patients enrolled in the voriconazole clinical program. Therefore, they represent data on individual patients, rather than data from a formal pharmacokinetic profile of voriconazole in the CSF.

Evaluation of drug pharmacokinetics in the CSF of immunocompromised patients is often complicated. Studies with antibiotics have shown that penetration of a drug through the blood-brain barrier (BBB) in laboratory animals does not differ from that in humans [9]. Whether this statement is valid also for fungal infections is less clear. However, the ratio of CSF to plasma fluconazole concentrations reported in laboratory animals is similar to that reported in humans [10–12]. In this study, the mean ratio of CSF to plasma concentrations of voriconazole among guinea pigs without CNS inflammation was slightly higher than that among patients with CNS fungal infections (0.68 vs. 0.46). This difference may be caused by various factors, including differences in plasma protein binding (the percentage of free plasma was ~55% in guinea pigs and ~42% in humans) and differences in sampling times, or it may be because 23 of 36 CSF samples were obtained from ventricles, where drug concentrations are likely to be lower than in the lumbar space [13].

The exact mechanism of voriconazole penetration through the BBB is not known. However, in guinea pigs without CNS infection, voriconazole concentrations in the CSF are equivalent to free-drug concentrations in plasma, which suggests passive diffusion. The moderate lipophilicity of voriconazole (logD7.4, 1.8), the observed ratio of CSF to free plasma concentrations of voriconazole of >1 in animals without CNS inflammation, and the rapid achievement of peak voriconazole concentrations in the CSF all suggest that transcellular penetration of voriconazole through the BBB occurs (data in file, Pfizer) [14].

In cases of bacterial meningitis, achievement of an optimal therapeutic effect depends on the presence of bactericidal concentrations of antibiotics in the CSF [9, 15]. However, what constitutes sufficient efficacy of antifungal agents is less clear. Successful use of amphotericin B to treat animals with Candida meningitis has been demonstrated, although amphotericin B
Table 1. Demographic and clinical characteristics of patients who received voriconazole (Vor) therapy for CNS fungal infection.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age in years</th>
<th>Underlying infection</th>
<th>Response to therapy</th>
<th>Vor dose(s) b.i.d., mg/kg</th>
<th>Time(s) of sampling, h&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Vor concentration(s), µg/mL</th>
<th>Time(s) of sampling, h&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Vor concentration(s), µg/mL</th>
<th>Ratio of Vor in CSF to Vor in plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>Pulmonary aspergillosis</td>
<td>Partial</td>
<td>9.4 po</td>
<td>NS</td>
<td>0.85</td>
<td>NS</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>S. apiospermum brain abscess</td>
<td>Partial</td>
<td>8.4 iv</td>
<td>1</td>
<td>7.23</td>
<td>3</td>
<td>3.93</td>
<td>0.54</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>Cerebral aspergillosis</td>
<td>Stable</td>
<td>6.3 po</td>
<td>1</td>
<td>7.16</td>
<td>2</td>
<td>2.41</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>C. immitis meningitis</td>
<td>Stable</td>
<td>5.3 po</td>
<td>NS</td>
<td>9.99</td>
<td>N</td>
<td>2.87 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.29</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>Pulmonary aspergillosis</td>
<td>Stable</td>
<td>5 iv</td>
<td>NS</td>
<td>6.14</td>
<td>NS</td>
<td>2.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>Disseminated S. apiospermum infection</td>
<td>Complete</td>
<td>4.3 po</td>
<td>NS</td>
<td>0.39</td>
<td>NS</td>
<td>0.16 (2)</td>
<td>0.41</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>S. apiospermum brain abscess</td>
<td>None</td>
<td>4 iv</td>
<td>ND</td>
<td>1.47</td>
<td>2</td>
<td>0.76</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 iv</td>
<td>ND</td>
<td>9</td>
<td>0.67</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 iv</td>
<td>ND</td>
<td>11</td>
<td>0.13 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>5 iv</td>
<td>NS</td>
<td>1.29</td>
<td>9</td>
<td>0.65 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.81</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>5 iv</td>
<td>ND</td>
<td>NS</td>
<td>1.04 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>Cerebral aspergillosis</td>
<td>None</td>
<td>4 iv</td>
<td>NS</td>
<td>0.31</td>
<td>10</td>
<td>0.23</td>
<td>0.74</td>
</tr>
<tr>
<td>9</td>
<td>58</td>
<td>Disseminated H. capsulatum infection</td>
<td>Partial</td>
<td>4 iv</td>
<td>ND</td>
<td>2</td>
<td>0.72</td>
<td>NA</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 iv</td>
<td>ND</td>
<td>3</td>
<td>0.67</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 iv</td>
<td>ND</td>
<td>4</td>
<td>0.63 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 iv</td>
<td>ND</td>
<td>11</td>
<td>0.19 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>46</td>
<td>Phaeohyphomycosis (cerebral abscess)</td>
<td>Stable</td>
<td>3.9 iv</td>
<td>12</td>
<td>0.04</td>
<td>12</td>
<td>0.04</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>S. apiospermum encephalitis</td>
<td>Complete</td>
<td>3.8 iv</td>
<td>1</td>
<td>1.08</td>
<td>6</td>
<td>0.27</td>
<td>0.25</td>
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<tr>
<td>12</td>
<td>18</td>
<td>Disseminated fusariosis</td>
<td>None</td>
<td>3.7 po</td>
<td>ND</td>
<td>ND</td>
<td>NS</td>
<td>0.33</td>
<td>NA</td>
</tr>
<tr>
<td>13</td>
<td>28</td>
<td>Cryptococcal meningitis</td>
<td>Stable</td>
<td>3.1 po</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>0.08 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>21</td>
<td>Cryptococcal meningitis</td>
<td>Partial</td>
<td>2.5 po</td>
<td>3</td>
<td>0.37</td>
<td>3</td>
<td>0.22</td>
<td>0.59</td>
</tr>
</tbody>
</table>

**NOTE.** C. immitis, Coccidioides immitis; H. capsulatum, Histoplasma capsulatum; ND, not done; NS, not stated; S. apiospermum, Scedosporium apiospermum.

<sup>a</sup> After administration of Vor.

<sup>b</sup> More than 1 sample was obtained at the same time. Data are median µg/mL (no. of samples obtained). Samples were obtained via intraventricular catheters (patients 7, 9, and 13) or lumbar puncture (remaining patients).

<sup>c</sup> Blood and CSF samples were obtained at the same time.
Concentrations of voriconazole in plasma (solid markers) and CSF (empty markers) samples obtained from guinea pigs. Animals received oral doses of 4 mg/kg (squares) or 10 mg/kg (circles) q8h for 5 days. On day 5, 1 animal per timepoint was killed for analysis of CSF and blood samples with validated high-performance liquid chromatography.

was not detected in the CSF [16]. These findings have been supported by clinical studies. Despite the negligible concentrations of itraconazole detectable in the CSF, high doses have successfully been used to treat nonneutropenic patients with cerebral aspergillosis [17] and patients with cryptococcal meningitis [18]. Eradication of infection in the CNS may depend not only on drug concentrations in the CSF but also on the presence of biologically active drug concentrations in brain tissue. The moderate lipophilicity and large-volume distribution of voriconazole may well contribute to the higher concentrations detected in brain tissue, compared with those detected in the CSF or blood.

Voriconazole has been successfully used in the treatment of CNS aspergillosis and scedosporiosis in patients who did not respond to other available antifungal therapies [19–22]. In the compassionate-use program, 6 (54.4%) of 11 children demonstrated partial or complete response to therapy [23]. The outcome associated with voriconazole therapy administered to adult patients with CNS aspergillosis was less favorable. However, a success rate of 16% is still better than the 8.8% reported elsewhere [1, 24].

In conclusion, voriconazole penetrates into the CSF and brain tissue and, thus, is a promising option for patients with CNS fungal infections. Additional studies are warranted to describe the pharmacokinetic profile of voriconazole in the CSF and to examine the association between CSF drug concentrations and treatment efficacy.

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References

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732 • CID 2003:37 (1 September) • BRIEF REPORT