Relationship between Trough Plasma and Epithelial Lining Fluid Concentrations of Voriconazole in Lung Transplant Recipients

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Trough (predose) voriconazole concentrations in plasma and pulmonary epithelial lining fluid (ELF) of lung transplant recipients receiving oral voriconazole preemptive treatment were determined. The mean (± standard deviation [SD]) ELF/plasma ratio was 12.5 ± 6.3. A strong positive linear relationship was noted between trough plasma and ELF voriconazole concentrations ($r^2 = 0.87$), suggesting the feasibility of using trough plasma voriconazole concentration as a surrogate to estimate the corresponding concentration in ELF of lung transplant recipients.

Invasive fungal infections (IFIs) are a major cause of morbidity and mortality in lung transplant recipients. While IFIs caused by Aspergillus spp. are relatively uncommon (6 to 16%) (1), noninvasive Aspergillus colonization occurs in approximately one-third of lung transplant recipients (2). These colonized patients may be predisposed to a high risk of IFIs due to failure of anastomosis healing, neutrophil dysfunction, and, to a lesser degree, T-cell immunosuppression. Even in the absence of fungal invasion, Aspergillus colonization and bronchitis may lead to graft dysfunction and increase the risk of bronchiolitis obliterans syndrome in lung transplant recipients (2). A preemptive antifungal approach is an attractive strategy where antifungal treatment is administered to high-risk patients with pre- and/or posttransplant bronchial Aspergillus colonization (3). Voriconazole (VRC) is widely employed (4), but its oral bioavailability is significantly lower in lung transplant recipients (24 to 64%) than healthy adults (96%) (5). The risk of suboptimal VRC exposure is of concern, as pre-dose (trough) serum concentrations below 1.5 μg/ml have been correlated with increased likelihood of breakthrough IFIs and fungal airway colonization (6). For treatment of established IFIs, a higher therapeutic range (1.5 to 4.5 μg/ml in plasma) is required for >85% probability of clinical success (7). As ~78% of IFI cases are limited to the lungs (8), it is imperative to ensure adequate antifungal concentrations at the airway interface for eradication of the colonizing fungal isolate(s), or at least to prevent fungal invasion.

The concentration of VRC within the lung epithelial lining fluid (ELF) may provide a better estimate of antifungal exposure for prophylaxis and treatment of the early stage of pulmonary IFI (9); however, routine monitoring of VRC concentration in the ELF via bronchoscopy is not practical. The use of plasma VRC concentration as a surrogate for the ELF concentration is therefore an attractive option. A study investigated the bronchopulmonary penetration of VRC following intravenous administration for 3 days to healthy adults; bronchoalveolar lavage (BAL) fluid and plasma samples were collected at 4, 8, and 12 h (10). The relevance of this study to lung transplant patients with pulmonary colonization or IFIs is unclear. Another study reported the concentration of VRC in ELF and plasma (samples collected simultane-ously) of lung transplant recipients, but only two out of 12 patients had trough concentrations measured (11). The current study explored the relationship between trough plasma and ELF VRC concentrations in lung transplant recipients.

This was a prospective observational pilot study. Participants aged ≥18 years, receiving oral VRC for preemptive treatment and undergoing scheduled bronchoscopy with BAL after lung transplantation, were enrolled, and their consent was obtained. Every patient was sampled predose after at least 1 week of oral VRC therapy and followed up to 3 months post-BAL. Human ethics committees of Alfred Hospital and Monash University approved the study. Fiber-optic bronchoscopy was performed per standard procedure at the Alfred Hospital. Sterile 20-ml aliquots of normal saline were sequentially instilled into the right middle lobe and immediately aspirated into collection traps. The total volume of pooled BAL fluid aspirates was measured and recorded. The BAL fluid sample was placed on ice prior to centrifugation (800 x g, 5 min, 4°C), as described previously (10). A blood sample (3 ml) was collected during bronchoscopy and centrifuged (1,505 x g, 10 min, 4°C). The BAL fluid supernatant and plasma samples were stored at ~80°C and ~20°C, respectively, until analysis.

VRC concentrations in plasma and BAL fluid were measured using a high-performance liquid chromatography–fluorescence method (12), modified slightly for plasma in the sample preparation and chromatographic conditions. Normal saline (surrogate matrix) was used to prepare the calibration curves for the BAL fluid assay and validated using VRC-free blank BAL fluid from lung transplant recipients. The calibration curves were linear from 2.5 to 500 ng/ml and 50 to 10,000 ng/ml for BAL fluid and plasma assays, respectively. Urea concentrations in the BAL fluid super-
natant and plasma were determined using the QuantiChrom urea assay kit. The volume of ELF and the VRC concentration in the ELF were calculated using the urea dilution method as previously reported (13,14). The relationship between plasma and ELF VRC concentrations was examined using linear regression.

Between October 2011 and July 2012, 12 consecutive patients were enrolled (Table 1). BAL fluid samples from three patients were excluded from analysis due to either gross contamination with blood or interference in the analytical measurement of VRC concentration. All except one patient reported full adherence to VRC during the previous 7 days. An average (standard deviation [SD]) of 78 (32) ml of saline was instilled, with 36/11006 ml fluid recovered. The calculated volume (mean SD) of ELF recovered was 0.45/11006 0.21 ml, or 1.3% of total BAL fluid recovered.

Table 1 showed that VRC concentrations in ELF were higher than the reported MIC90 (i.e., 0.5/11006 g/ml) against most Aspergillus and yeast isolates in all patients (15). A strong positive linear relationship was noted between ELF and plasma VRC concentrations (r2 = 0.868, P < 0.001) (Fig. 1), which can be described by the regression equation [VRC]ELF = 15.44[VRC]plasma - 1.16, where [VRC]ELF was the VRC concentration in ELF and [VRC]plasma was the plasma VRC concentration. The VRC ELF/plasma ratio (mean SD) was 12.5/11006 6.3 (coefficient of variation [CV], 50%).

In this pilot study, we have observed a strong linear relationship between trough plasma and ELF VRC concentrations, which provides a basis to support the use of trough plasma VRC concentration as a surrogate for the corresponding concentration in ELF. Despite differences in study design (trough versus random sampling, early versus late postoperative time) between the present and previous study in lung transplant recipients (11), the mean ELF/plasma ratio of VRC noted in this study was similar to, but less variable than, that in the earlier study (mean ± SD, 11 ± 8; CV, 73%). The exact mechanism for the higher VRC concentration in ELF than in plasma is not fully understood. One possible mechanism relates to ion trapping of VRC, which is a weak organic base (pKa, 1.76), in the lower-pH environment of lung fluid (16). However, ion trapping will have its greatest impact on a weak organic base with a pKa in the range of 7.5 to 10.5 and is therefore unlikely to be a major contributor to our observed high ELF/plasma concentration ratio. Second, higher binding to proteins in ELF than in plasma.

**TABLE 1**: Characteristics and outcome of lung transplant recipients receiving VRC.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Type of transplant</th>
<th>Underlying disease</th>
<th>Timing of bronchoscopy posttransplant (days)</th>
<th>VRC dose regimen (mg b.i.d.)</th>
<th>Timing of sampling after last dose (h)</th>
<th>Treatment duration of VRC prior to BAL (mo)</th>
<th>Plasma (g/ml)</th>
<th>ELF (g/ml)</th>
<th>ELF/plasma ratio</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Single</td>
<td>IPF</td>
<td>54</td>
<td>1.0</td>
<td>0.75</td>
<td>42.3</td>
<td>12.2</td>
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<td>24.3</td>
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<td>2</td>
<td>Single</td>
<td>IPF</td>
<td>197</td>
<td>3.5</td>
<td>0.15</td>
<td>11.9</td>
<td>11.8</td>
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<tr>
<td>3</td>
<td>Single</td>
<td>COPD</td>
<td>14</td>
<td>12</td>
<td>3.77</td>
<td>11.6</td>
<td>3.77</td>
<td>0.3</td>
<td>11.6</td>
<td>Failure</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Double</td>
<td>Sarcoidosis</td>
<td>3,285</td>
<td>14.9</td>
<td>2.3</td>
<td>15.4</td>
<td>2.3</td>
<td>0.3</td>
<td>15.4</td>
<td>Failure</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Single</td>
<td>Double</td>
<td>3,285</td>
<td>14.9</td>
<td>2.3</td>
<td>15.4</td>
<td>2.3</td>
<td>0.3</td>
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<td>2.3</td>
<td>15.4</td>
<td>2.3</td>
<td>0.3</td>
<td>15.4</td>
<td>Failure</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Double</td>
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<td>99</td>
<td>10.0</td>
<td>0.38</td>
<td>10.4</td>
<td>0.38</td>
<td>0.3</td>
<td>10.4</td>
<td>Failure</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Double</td>
<td>Emphysema</td>
<td>60</td>
<td>16.7</td>
<td>2.0</td>
<td>12.7</td>
<td>2.7</td>
<td>0.3</td>
<td>12.7</td>
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<td>0.76</td>
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<tr>
<td>12</td>
<td>Double</td>
<td>Emphysema</td>
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<td>12.7</td>
<td>0.3</td>
<td>8.66</td>
<td>0.76</td>
<td>0.3</td>
<td>8.66</td>
<td>Failure</td>
<td></td>
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</tbody>
</table>

**FIG 1** Linear model predicting the relationship between VRC concentrations in plasma ([VRC]plasma) and ELF ([VRC]ELF); P < 0.001.
could, in theory, also contribute to an ELF/plasma concentration ratio greater than unity. However, the albumin concentration in ELF has been reported to be only ~9% of that in plasma (14), and there is a paucity of information about the types and concentrations of other proteins. Thus, differential protein binding in ELF and plasma is not likely to be an explanation for the high concentration ratio. Third, a relatively high intracellular concentration of VRC within alveolar macrophages (10), likely to be important in treating fungal infections, could result in in vitro efflux of VRC from these cells during BAL fluid sample collection and processing, thereby contributing to the high ELF/plasma ratio (17), an effect that is inherent with the BAL procedure.

In conclusion, the current study found a close relationship between trough plasma and ELF VRC concentrations, suggesting that trough plasma VRC concentration could serve as a potential surrogate of the concentration in ELF. Prospective evaluation of the use of trough plasma VRC concentration as an aid to clinical decision making around VRC dosage regimens is under way.

ACKNOWLEDGMENTS
We thank the participants who provided BAL fluid and plasma samples, the clinicians and nursing staff in the Lung Transplant Service of The Alfred Hospital for assistance in sample collection, and Lauren Mitchell for help in flagging eligible participants.

An Endeavor Postgraduate Award to S.-C. Heng is gratefully acknowledged.

REFERENCES