Intracellular Concentrations of Posaconazole in Different Compartments of Peripheral Blood

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Therapeutic drug monitoring (TDM) of antifungal plasma concentrations is increasingly recommended. However, data on antifungal concentrations in the other compartments of the peripheral blood are limited. Hence, we collected 23 blood samples from 14 patients receiving posaconazole for prophylaxis of fungal infections. These samples were separated by double-discontinuous Ficoll-Hypaque density centrifugation. The intracellular posaconazole concentrations of the obtained cells, i.e., the peripheral blood mononuclear cells (PBMCs), polymorphonuclear leukocytes (PMNs), and red blood cells (RBCs), were determined by liquid chromatography-tandem mass spectrometry. The intracellular concentrations of the PBMCs and PMNs were significantly higher than those of surrounding media (P < 0.001). The ratios between the intracellular and extracellular concentrations (C/E) were 22.5 ± 21.2, 7.66 ± 6.50, and 0.09 ± 0.05 for the PBMCs, PMNs, and RBCs, respectively. Posaconazole reaches high concentrations within human PBMCs and PMNs and is, to a lesser extent, present in RBCs. The high intracellular concentrations might contribute to posaconazole efficacy and distribution.

Posaconazole is an orally administered broad-spectrum triazole antifungal which has recently been approved for prophylaxis and treatment of opportunistic invasive fungal infections in immunocompromised patients. Posaconazole exposure depends on prandial status and drug-drug interactions, leading to intra- and interindividual variability (7, 12). Depending on the dosing group, a 38 to 68% interindividual variability of the pharmacokinetic parameters has been observed (14). The clinical significance of this finding is currently unclear (15).

The intestinal absorption is likely to be influenced by graft-versus-host disease and mucositis. Ullmann et al. also described a reduced bioavailability of posaconazole in neutropenic patients (16). Due to the extensive pharmacokinetic variability of posaconazole, therapeutic drug monitoring (TDM) has been discussed as a possible means of ensuring a sufficient exposure (9, 13). While TDM usually is performed by measurement of serum or plasma concentrations, little is known about the concentrations in other compartments of the peripheral blood, i.e., peripheral blood mononuclear cells (PBMCs), polymorphonuclear leukocytes (PMNs), and red blood cells (RBCs). PBMCs and PMNs are important pillars of peripheral blood, i.e., peripheral blood mononuclear cells (PBMCs), polymorphonuclear leukocytes (PMNs), and red blood cells (RBCs). PBMCs and PMNs are important pillars of host defense against invasive fungal diseases (IFD). Interactions with these cells might well influence the efficacy of antifungal drugs.

In a recently published trial by Conte et al., a 33-fold increase of the maximum concentration (Cmax) and area under the concentration-time curve (AUC) of posaconazole in alveolar macrophages (AM) compared to those of plasma was shown (4). However, up to now, no data on the intracellular concentration of posaconazole in PBMCs, PMNs, and RBCs have been published.

The aim of the present work was to determine the intracellular concentrations of posaconazole in different compartments of the peripheral blood using a previously published liquid chromatography-tandem mass spectrometric assay (6).

In keeping with the established literature, we use the term “intracellular” throughout this article, although we are aware that the term “cell associated” may be more accurate.

MATERIALS AND METHODS

Patients. Whole blood obtained from patients (n = 14) receiving posaconazole at 200 mg three to four times per day was collected as part of the Cologne biobank protocol (08-160; University of Cologne Ethics Committee) on improving diagnosis of severe infections in immunocompromised patients (ISI) (5). All patients had hematological malignancies, were hospitalized in the University Hospital of Cologne, and required treatment with posaconazole for the prevention of invasive fungal infections. Patients were approached in the first half of 2009 upon recovery from severe neutropenia. Written informed consent was obtained prior to blood sampling. All samples were at trough levels and collected during the posaconazole steady-state phase.

Posaconazole assay. Blood samples were processed and analyzed as described previously (6). In brief, whole blood was collected in two EDTA salt-containing tubes (2 by 8 ml) and separated by double-discontinuous Ficoll-Hypaque density gradient (Histopaque 1077 and 1119; Sigma-Aldrich, Munich, Germany) centrifugation. The cells were collected at the corresponding layers and washed twice with phosphate-buffered saline (PBS). During the second washing step, a hyper-tonic lysis was implemented for the PBMC and PMN fractions in order to eliminate contaminating red blood cells (RBCs). Afterwards, the cells were counted in a Neubauer chamber. The volume of each pellet was calculated based on the total cell count and the mean cell volume; i.e., 0.4 pl for PBMCs, 0.334 pl for PMNs, and 0.09 pl for RBCs. The isolated PBMCs and PMNs were extracted...
with 100 μl acetonitrile containing itraconazole as an internal standard by sonication for 10 min, vortexing for 1 min, and centrifugation for 10 min at 4°C and 13,000× g. The RBCs (500 μl) were treated analogously with 1,000 μl acetonitrile containing the internal standard. The samples, i.e., the supernatants, were analyzed by liquid chromatography-tandem mass spectrometry, facilitating a method developed for the quantitation of antifungals in different compartments of the peripheral blood as previously described (6). The lower limit of quantitation (LLOQ) and the limit of detection (LOD) for posaconazole were 10 ng/ml and 3.3 ng/ml, respectively. The calibration range was 10 ng/ml to 6,200 ng/ml. For the calibration standard, the accuracy (percent bias) ranged from −10.9% to 1.1%, and the precision (percentage of coefficient of variation; relative standard deviation [RSD]) ranged from 2.4% to 12.8%.

Statistical analysis was performed using IBM SPSS Statistics 17.0 computer software (SPSS Inc., IL). Values were compared with analysis of variance (ANOVA), using a logarithmic approach to improve the homogeneity of variance; intergroup differences were confirmed by a Bonferroni post hoc test, with multiple test corrections, as well as a Dunnett T3 test.

### RESULTS

Twenty-three samples obtained from 14 subjects receiving posaconazole for the prophylaxis of fungal infections were analyzed. The mean age (±standard deviation [SD]) of the 14 subjects was 54.8 ± 14.3 years; 9 subjects were male, and 5 were female, with mean (±SD) leukocyte, neutrophil, and RBC counts of 5.4 ± 5.9 × 10⁹ cells/ml, 1.7 ± 1.9 × 10⁹ cells/ml, and 3.2 ± 0.5 × 10¹² cells/ml, respectively. All subjects had hematological malignancies, including acute myelogenous leukemia (7 patients), non-Hodgkin lymphoma (4 patients), acute lymphoblastic leukemia (1 patient), aplastic anemia (1 patient), and chronic myelogenous leukemia (1 patient). The mean (±SD) weight of the subjects was 73.7 ± 10.8 kg. All subjects received posaconazole at 200 mg, three times (n = 15) to four times (n = 8) per day, resulting in a mean daily dose of 8.9 ± 2.1 mg/kg.

The mean posaconazole concentrations in PBMCs, PMNs, RBCs, and plasma are shown in Table 1. While posaconazole concentrations within the PBMCs and PMNs were significantly increased compared to the plasma concentration (P < 0.001; ANOVA and post hoc tests), the concentration within the RBCs was significantly decreased compared to those in all other compartments (P < 0.001; ANOVA and post hoc tests). The posaconazole concentration in PBMCs was also significantly increased (P = 0.01; ANOVA and post hoc tests) compared to that in the PMNs. For mean ratios between intracellular and extracellular concentrations (C/E) for the PBMCs, PMNs, and RBCs, refer to Table 1. The intracellular posaconazole concentrations at different extracellular concentrations are shown in Fig. 1. There was only a moderate relationship between PMN and plasma levels, which may have been caused by an outlier among the four samples with plasma levels of >1,000 ng/ml.

### DISCUSSION

Posaconazole was present in all compartments of the peripheral blood; however, the concentrations within these compartments varied significantly. While the intracellular posaconazole concentrations within the PBMCs and PMNs was significantly increased (22.5-fold and 7.66-fold), the concentration within the RBCs was only 9% of that in the plasma.

The intracellular uptake of two representatives of theazole class, i.e., fluconazole (FLC) and voriconazole (VRC), by PMNs has been studied using radiometric assays (1, 10). Both drugs were characterized by a rapid intracellular uptake and...

### Table 1: Mean posaconazole concentrations and ratios between the intracellular and extracellular concentrations

<table>
<thead>
<tr>
<th>Blood compartment</th>
<th>Mean PSC concen ± SD (ng/ml)</th>
<th>C/E ratio ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMCs</td>
<td>12.764 ± 14.057</td>
<td>22.5 ± 21.2</td>
</tr>
<tr>
<td>PMNs</td>
<td>4.031 ± 3.692</td>
<td>7.66 ± 6.50</td>
</tr>
<tr>
<td>RBCs</td>
<td>50.8 ± 46.18</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>Plasma</td>
<td>603.2 ± 475.9</td>
<td></td>
</tr>
</tbody>
</table>

* PBMCs, peripheral blood mononuclear cells; PMNs, polymorphonuclear leukocytes; RBCs, red blood cells; PSC, posaconazole; C/E, ratio between intracellular and extracellular concentrations.

b P < 0.001 compared to plasma (ANOVA and Dunnett T3 test).

FIG. 1. PBMC-associated posaconazole (A; n = 23), PMN-associated posaconazole (B; n = 20), and RBC-associated posaconazole (C; n = 22) at different plasma concentrations.
elution. After in vitro incubation of PMNs with FLC (10) or VRC (1), the intracellular concentrations of these drugs were significantly increased (2.2-fold, and 8.5-fold, respectively) compared to those in the surrounding medium. The uptake was reversible, and independent of pH and temperature, suggesting a passive transport mechanism. The PMN C/E ratio of posaconazole was comparable with that of voriconazole (7.66 versus 8.5, respectively) (1), and higher than that observed for fluconazole (7.66 versus 2.2, respectively) (10).

It was previously suspected that the greater uptake of voriconazole compared to that of fluconazole was caused by its higher hydrophobicity (1). While the greater hydrophobicity of posaconazole compared to that of voriconazole should allow an even higher intracellular uptake, it is also a much larger molecule, with a molecular weight about twice as high as that of voriconazole. We observed an almost equal uptake of these two drugs (1), which might be explained by the different medium compositions that were examined. We obtained the cells from whole blood, whereas Ballesta et al. (1) and Pascual et al. (10) incubated the cells in Hanks’ balanced salt solution (HBSS). It may be speculated that highly liposoluble posaconazole accumulates in cell organelles and membranes without major involvement of active transport mechanisms; a similar model was discussed for the intracellular uptake of voriconazole (1).

The uptake of itraconazole by alveolar macrophages (AM) was previously studied using cells obtained from New Zealand White rabbits, which were infected with Mycobacterium bovis (11). The uptake was both rapid and substantial, with a C/E ratio of 87.9 ± 5.3 for the alveolar macrophages and 90.4 ± 10.1 for RBCs in serum-free medium. Beyond that, it was also shown that the uptake depended on the medium, with alveolar macrophage C/E ratios of 18 for 5% serum and 3 for 100% serum. The effect of different medium compositions on the uptake of itraconazole by RBCs was not studied (11). The observations on the uptake of itraconazole by alveolar macrophages are consistent with those made by Conte et al. (3), who studied intracellular levels of itraconazole in alveolar macrophages of healthy subjects. They demonstrated an overall itraconazole AM/plasma ratio of 3.4 ± 1.9 in healthy subjects after the administration of 10 doses of itraconazole (200 mg, twice daily).

In a study with 25 healthy subjects who received 14 doses of posaconazole (400 mg, twice daily) before undergoing bronchoscopy at different time intervals, the AM/plasma ratios ranged from 27.3 ± 18.0 to 44.3 ± 44.2 (4). In our study, the mean C/E ratio of the PMBCs was numerically lower than the mean AM/plasma ratio in the quoted study (22.5 versus 33, respectively) (4), although this difference was not significant.

To our knowledge, no data on the intracellular concentration of posaconazole exist, and apart from the above-cited animal study of itraconazole (11), no data on the intracellular concentration of azole antifungals in RBCs exist. The marked difference between the intracellular concentration of itraconazole in RBCs as reported by Perfect et al. (11) and the intracellular posaconazole concentration in our study might also have been caused by the different media used.

The various posaconazole concentrations within the different compartments of the peripheral blood might in part contribute to the various posaconazole concentrations that are observed in the plasma. The intracellular uptake of the azoles appears to be passive (1, 10) and dependent on the composition of the extracellular medium (11). Hence, the intracellular and, consequently, the extracellular concentrations would instantly change upon entering different body compartments with different extracellular medium compositions. For example, since the protein concentration within the lymphoid tissue (3 to 5 g/liter) is much lower than in the blood (60 to 80 g/liter), lymphocytes within lymphoid tissue, or the Peyer’s patches, might take up the azoles and release them at the sites of inflammation or within the peripheral blood. Furthermore, about 50% of the neutrophil granulocytes adhere to endothelial walls, especially those of the lung and spleen vessels, from where they may be rapidly mobilized (8).

Apart from possible effects on the pharmacodynamics and pharmacokinetics, the intracellular concentrations may well contribute to the intracellular killing of some fungi. It was shown that, e.g., intracellular voriconazole in monocyte-derived macrophages augments the killing of intracellular Candida glabrata, Candida krusei, and Candida parapsilosis (2). However, the intracellular voriconazole concentration was not assessed in that study. Further studies of this area may take heed of the varied uptake with respect to the surrounding medium and the different cells types. They may determine the intracellular concentrations by a quantitative assay.

We conclude that (i) administration of posaconazole oral solution results in significantly increased intracellular concentrations in PBMCs and PMNs compared to those in plasma and that (ii) posaconazole is, though to a lesser extent, present in RBCs. These intracellular concentrations might influence the distribution of posaconazole, possibly in part explaining its superior prophylactic efficacy compared to that of fluconazole and itraconazole. Future studies should explore a possible difference in plasma levels between neutropenic and nonneutropenic patients.

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