Adequate penetration to the infection/colonization site is crucial to attain optimal efficacy of posaconazole against Aspergillus fumigatus diseases. We evaluated posaconazole exposure in pulmonary epithelial lining fluid (ELF) in a murine model of invasive pulmonary aspergillosis. The posaconazole exposure (area under the plasma concentration-time curve from time zero to 24 h postinfusion [AUC0 –24]) in ELF was 20% to 31% of that in plasma for total drug after the third dose, and the relationship between plasma and ELF exposure was linear (r² = 0.97, P = 0.016).
partmental analysis with Phoenix, version 6.2 (Pharsight, Inc.). The area under the plasma concentration-time curve from time zero to 24 h postinfusion (AUC\textsubscript{0–24}) was determined by use of the log-linear trapezoidal rule. The elimination rate constant was determined by linear regression of the terminal points of the log-linear plasma concentration-time curve. The terminal half-life was defined as ln2 divided by the elimination rate constant. Clearance (CL) was calculated as dose/AUC\textsubscript{0–24}.

Concentrations of total posaconazole in BAL fluid from three mice per time point were determined as described previously (13). Urea in BAL aspirate and plasma was measured utilizing a modified enzymatic assay (QuantiChrom urea assay kit, DIUR-500; BioAssay Systems) (14,15). The concentration of posaconazole in ELF was then determined by using the ratio of urea concentration in BAL fluid to that in plasma as described previously (10, 11, 14–19): drug concentration\textsubscript{ELF} = drug concentration\textsubscript{BAL} × urea\textsubscript{plasma}/urea\textsubscript{BAL}.

All data analyses were performed using GraphPad Prism software, version 5.0, for Windows (GraphPad Software, San Diego, CA). A regression analysis was conducted to determine the linearity between posaconazole concentration in blood and ELF. The goodness of fit was checked by use of the \( r^2 \) value and visual inspection. Statistical significance was defined as a \( P \) value of <0.05 (two-tailed). The \( C_{\text{max}} \) and AUC\textsubscript{0–24} data were log\textsubscript{10} transformed to approximate a normal distribution prior to statistical analysis.

All animals were alive at the time of sample collection from 0 to 24 h postchallenge, and 192 blood and BAL samples were obtained.

The drug concentrations in plasma, including the \( C_{\text{max}} \) of posaconazole, were higher than those in ELF for all dosages (Fig. 1). A significant correlation between mean posaconazole concentrations in plasma and ELF was noted by linear regression analysis \( (r^2 = 0.61, P < 0.0001) \) (Fig. 2a). However, a better correlation was found for AUC\textsubscript{0–24} in plasma and ELF in a linear fashion for the dosages of 4 to 32 mg/kg \( (r^2 = 0.97, P = 0.016) \) (Fig. 2b).

The total AUC\textsubscript{0–24} in plasma and ELF is shown in Table 1. The penetration of posaconazole in ELF based on total drug was between 20.21 and 31.39%. At the highest dose (32 mg/kg), the total AUC\textsubscript{0–24}/MIC ratio was \( \frac{581}{157.56} \) in plasma and \( \frac{157.56}{581} \) in ELF for the isolate with an MIC of \( 0.5 \) mg/liter.

In the present study, we investigated the intrapulmonary posaconazole penetration in an immunosuppressed murine model of invasive pulmonary aspergillosis receiving oral prophylactic regimens. Our results indicate that posaconazole exposure (AUC\textsubscript{0–24}) in ELF was 20% to 31% of that in plasma for total drug, and the relationship between posaconazole and AUC\textsubscript{0–24} in plasma and ELF was significantly linear \( (r^2 = 0.97) \).

Our findings are comparable to the results of two clinical observations of Conte et al. (20, 21). In the first study, the plasma and intrapulmonary concentrations of posaconazole were evaluated in 25 healthy adult subjects receiving multiple doses (13 or 14) of oral posaconazole suspension 400 mg twice daily with a high-fat meal (20). The AUC\textsubscript{0–12} values in ELF and plasma were 18.3 and 21.9 mg · h/liter, respectively, corresponding to an ELF-to-plasma penetration ratio of 86% of total drug. However, the corresponding exposure in alveolar macrophages (AMs) was appreciably higher (range, 46.2 to 87.7 mg/liter; AUC\textsubscript{0–12} of 715 mg · h/liter). Similarly, in the second study of the above-mentioned group (21),
higher-than-predicted intrapulmonary penetration of posaconazole was observed in 20 adult lung transplant patients receiving posaconazole suspension 400 mg twice daily with a high-fat meal. The AUC0–12 values in plasma, ELF, and AMs were 10.99, 11.21, and 530.2 mg·h/liter, respectively.

Notably, the lipophilic nature and high intracellular (cell-associated) concentrations of posaconazole might contribute to distribution at the infection/colonization site, in this case ELF (22, 23). Campoli et al. demonstrated that lipophilic characteristics of posaconazole increase the penetration of posaconazole into mammalian host cell membranes, which can mediate its efficacy in prophylactic regimens and likely explains the observed discrepancy between posaconazole low serum level and high potency (23). They exposed intracellular cells to posaconazole and removed the extracellular drug prior to infection. Epithelial cells loaded with posaconazole were able to inhibit fungal growth for at least 48 h and were protected from damage caused by infection. Cell-associated posaconazole levels were 40- to 50-fold higher than extracellular levels, and the drug was predominantly detected in cellular membranes. On the other hand, Farowski et al. demonstrated that the increased 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.

The exposure-response relationships of posaconazole have been defined previously in experimental models of Aspergillus infections (4, 6, 24). Similar to the other triazoles, posaconazole displays exposure-dependent pharmacodynamic characteristics, for which a free AUC0–24/MIC ratio ranging from 1.67 to 1.78 was the value predictive of success associated with half-maximal efficacy. In general, it is accepted that only the unbound fraction of a drug in serum/plasma is pharmacologically active. Considering the high degree of posaconazole protein binding in plasma (98 to 99%) and negligible bounds in ELF, the results of the current study indicated that effective local concentrations might be achieved even at the lowest dose (4 mg/kg) with a free AUC0–24 and MIC ratio of 1.76 in plasma and 14.69 in ELF, which is probably high enough to prevent infection with A. fumigatus isolates with an MIC of ≤0.5. However, for the isolates with resistant phenotypes and higher MIC to posaconazole (≥16 mg/kg), the obtained free AUC0–24/MIC in plasma was ≤0.18 in plasma and ≤4.92 in ELF at the highest dose (32 mg/kg), which indicates the possibility of breakthrough IA.

We conclude that penetration of posaconazole in ELF is relatively high, which is consistent with lipophilic characteristics and its increased intracellular permeability. The high intrapulmonary penetration ratio of posaconazole indicates that this agent is an optimal therapeutic choice in prevention of IA due to azole-susceptible and/or azole-resistant A. fumigatus.

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