

In Vivo Microdialysis To Determine Subcutaneous Interstitial Fluid Penetration and Pharmacokinetics of Fluconazole in Intensive Care Unit Patients with Sepsis

Mahipal G. Sinnollareddy,^{a,b} Michael S. Roberts,^{a,b} Jeffrey Lipman,^{c,d} Melissa Lassig-Smith,^c Therese Starr,^c Thomas Robertson,^{a,b,*} Sandra L. Peake,^e Jason A. Roberts^{c,d,f}

School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia^a; Therapeutics Research Centre, Basil Hetzel Institute for Translational Health Research, The Queen Elizabeth Hospital, Adelaide, Australia^b; Burns, Trauma and Critical Care Research Centre, The University of Queensland, Brisbane, Australia^c; Department of Intensive Care Medicine, Royal Brisbane and Women's Hospital, Brisbane, Australia^d; Department of Intensive Care Medicine, The Queen Elizabeth Hospital, Adelaide, Australia^e; Pharmacy Department, Royal Brisbane and Women's Hospital, Brisbane, Australia^f

The objective of the study was to describe the subcutaneous interstitial fluid (ISF) pharmacokinetics of fluconazole in critically ill patients with sepsis. This prospective observational study was conducted at two tertiary intensive care units in Australia. Serial fluconazole concentrations were measured over 24 h in plasma and subcutaneous ISF using microdialysis. The concentrations in plasma and microdialysate were measured using a validated high-performance liquid chromatography system with electrospray mass spectrometer detector method. Noncompartmental pharmacokinetic analysis was performed. Twelve critically ill patients with sepsis were enrolled. The mean *in vivo* fluconazole recovery rates \pm standard deviation (SD) for microdialysis were 51.4% \pm 16.1% with a mean (\pm SD) fluconazole ISF penetration ratio of 0.52 \pm 0.30 (coefficient of variation, 58%). The median free plasma area under the concentration-time curve from 0 to 24 h (AUC_{0–24}) was significantly higher than the median ISF AUC_{0–24} (340.4 versus 141.1 mg · h/liter; $P = 0.004$). There was no statistical difference in median fluconazole ISF penetration between patients receiving and not receiving vasopressors (median, 0.28 versus 0.78; $P = 0.106$). Both minimum and the maximum concentrations of drug in serum (C_{\max} and C_{\min}) showed a significant correlation with the fluconazole plasma exposure (C_{\max} , $R^2 = 0.86$, $P < 0.0001$; C_{\min} , $R^2 = 0.75$, $P < 0.001$). Our data suggest that fluconazole was distributed variably, but incompletely, from plasma into subcutaneous interstitial fluid in this cohort of critically ill patients with sepsis. Given the variability of fluconazole interstitial fluid exposures and lack of clinically identifiable factors by which to recognize patients with reduced distribution/exposure, we suggest higher than standard doses to ensure that drug exposure is adequate at the site of infection.

Infections and related sepsis are among the most important reasons for admission to intensive care units (ICUs). *Candida* sp. infections are responsible for up to 30% and 38% of fungal sepsis and septic shock episodes, respectively (1–4), and currently are the third leading cause of infections in ICUs globally (5). The attributable mortality associated with candidiasis in ICU patients is estimated to be 28.3% (6).

Most *Candida* infections are extravascular in origin, with interstitial fluid (ISF) of tissues and other body fluids representing the actual target site for antifungal therapy to treat the vast majority of *Candida* infections (7–9). While antibacterial treatment failures have been attributed to impaired target site penetration in critically ill patients with sepsis and shock (9, 10), data on antifungals are limited. However, it is highly likely that successful treatment with antifungal agents relies on the achievement of adequate concentrations at the site of infection, i.e., the ISF.

Fluconazole is a frequently used triazole antifungal agent in ICUs. In addition to delay in initiating fluconazole therapy, inadequate fluconazole dosing is an independent predictor of mortality in ICU patients with *Candida* infections (11). Given increasing evidence on the fluconazole exposure-response relationship (12–15), knowledge of plasma and ISF fluconazole concentrations is important to optimize exposure and hence response to fluconazole treatment in this cohort of critically ill patients.

In the present study, we investigated the pharmacokinetic (PK) profile of fluconazole in plasma and subcutaneous (SC) ISF in ICU patients with sepsis.

MATERIALS AND METHODS

This prospective observational study was conducted at two tertiary ICUs in Australia between May 2012 and January 2014. The study protocol was approved by the respective local human ethics committees (2012039 and HREC/13/QRBW/75). Prior informed written consent was obtained from either the patient or a substitute decision maker for the patient.

Study subjects. All ICU patients were screened, and eligible patients were identified for participation in the study. Patients were eligible for inclusion if the following criteria were met: at least 18 years old, admitted to the ICU, presence of sepsis, arterial line *in situ* or planned insertion, indwelling urinary catheter *in situ* or planned insertion, ability to obtain prior informed consent from the patient or person responsible, and prescription of intravenous fluconazole. Sepsis was defined as the presence of a suspected or confirmed infection and two or more systemic inflamma-

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Address correspondence to Jason A. Roberts, j.roberts2@uq.edu.au.

* Present address: Thomas Robertson, CPR Pharma Services Ltd., Adelaide, Australia.

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tory response syndrome criteria documented in the previous 24 h: a core temperature of $<36.0^{\circ}\text{C}$ or $>38.0^{\circ}\text{C}$; a heart rate of >90 beats/minute; a respiratory rate of >20 breaths/minute, partial pressure of carbon dioxide in the arterial blood (PaCO_2) of <32 mm Hg, or the patient being mechanically ventilated for an acute process; and a white cell count (WCC) of $>12.0 \times 10^9$ or $<4.0 \times 10^9$ /liter or $>10\%$ immature bands (16). Patients who met one or both of the following criteria were excluded: (i) known or suspected allergy to triazole antifungal agents or penicillins and (ii) pregnancy.

Drug administration. Fluconazole initiation and dose were at the discretion of the treating physician based on the clinical requirements.

Microdialysis procedure to measure ISF concentrations and *in vivo* calibration. Microdialysis was used to measure the free (or unbound) fluconazole concentration in subcutaneous-tissue ISF. The principles and details of microdialysis have been described previously (17). In this study, a microdialysis probe (CMA 60; Microdialysis AB, Stockholm, Sweden) with a molecular-mass cutoff of 20 kDa, an outer diameter of 0.6 mm, and a membrane length of 30 mm was aseptically placed in the subcutaneous tissue of the upper arms of the patients following the local injection of 0.5% lidocaine solution (<1.5 ml). The probe was then perfused with flucloxacillin (10 mg/liter; internal standard, perfusate) in 0.9% sodium chloride at a flow rate of 1.5 $\mu\text{l}/\text{min}$. Flucloxacillin was chosen as the internal standard for analytical purposes. The perfusion was initiated at least 60 min prior to the sampling time to establish equilibrium. After commencement of the fluconazole infusion, microdialysis samples were collected (the sampling frequency is described below). The *in vivo* recovery of fluconazole in the microdialysate was interpolated from the loss of the internal standard (flucloxacillin) across the microdialysis membrane into the subcutaneous ISF (18–22) using the following equation: percent *in vivo* fluconazole recovery = $100 \times (C_{\text{in}} - \text{mean } C_{\text{out}})/C_{\text{in}}$, where C_{in} is flucloxacillin at 10 mg/liter (perfusate) and C_{out} is the measured flucloxacillin concentration in the microdialysate.

Sample collection and processing. Arterial-blood samples were collected during one dosing interval from an indwelling arterial line just before commencing the fluconazole infusion (0 h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h after commencement. Microdialysate samples were collected at 30-min intervals between 0 and 6 h, hourly between 6 and 12 h, and then at 24 h. A 24-h urine sample was collected to measure creatinine clearance. Blood samples were centrifuged at $2,000 \times g$ for 10 min, and plasma was collected. The plasma and microdialysate samples were stored at -80°C until analysis. Sample transport was carried out by using dry ice and adhering to cold-chain procedures.

Data collection. Demographic and clinical data were collected, including age, gender, height, weight, admission diagnosis, acute physiological and chronic health evaluation (APACHE) II score, sequential organ failure assessment (SOFA) score, receipt of mechanical ventilation, vasoactive treatment, and response to fluconazole therapy (defined as cessation of fluconazole therapy without antifungal recommencement within 14 days).

Sample analysis. Plasma fluconazole concentrations and microdialysate fluconazole and flucloxacillin concentrations were analyzed with a high-performance liquid chromatography (HPLC) system with electrospray mass spectrometer detector (Applied Biosystems [Foster City, CA, USA] API2000; Shimadzu [Kyoto, Japan] HPLC). Briefly, for plasma samples, the method was as follows. Plasma (300 μl) was mixed with 50 μl internal standard (voriconazole; 1 mg/liter) and precipitated with 150 μl of 10% trichloroacetic acid. The samples were centrifuged at $12,000 \times g$ for 6 min at 4°C after being thoroughly vortexed for 30 s. The analytes were separated through an Agilent Zorbax Eclipse XDB-c18 (2.1 by 150 mm; 3.5- μm particle size) by gradient elution using water containing 0.1% formic acid and methanol containing 0.1% formic acid with a total flow rate of 0.3 ml/min and detected by an electrospray positive-ionization mode of tandem mass spectrometry. The mass-to-charge ratios (m/z) in multiple-reaction monitoring were 307.3/127.0 for fluconazole and 349.9/224.0 for the internal standard. Microdialysate samples (10 μl) were

assayed directly without any sample preparation under the same conditions used for the plasma samples. The calibration curve was linear over the concentration range of 0.1 to 20 mg/liter for both plasma and microdialysate samples. The intra- and interday coefficients of variation were validated to be within 10% at low, medium, and high concentrations of the calibration range. Assays were validated and conducted using criteria from the U.S. Food and Drug Administration guidance on bioanalysis (23).

PK analysis. Plasma and ISF PK parameters were estimated using noncompartmental methods. The apparent terminal elimination rate constant (k_{el}) was determined from log-linear least-squares regression. The minimum and maximum concentrations of drug in serum for the dosing period (C_{min} and C_{max}) were the observed values. The half-life ($t_{1/2}$) was calculated as $\ln(2)/k_{\text{el}}$. The area under the plasma concentration-time curve from 0 to 24 h (AUC_{0-24}) was calculated using the linear trapezoidal approximation. The area under the plasma concentration-time curve from time zero to infinity ($\text{AUC}_{0-\infty}$) was calculated by the log-linear trapezoidal rule until the time of the last quantifiable plasma concentration and then extrapolated to infinity by using the quotient of the last measurable concentration to k_{el} . Clearance (CL) was calculated using the following formula: $\text{dose}/\text{AUC}_{0-\infty}$. Protein binding of fluconazole was assumed to be 12% to determine free AUC_{0-24} ($f\text{AUC}_{0-24}$) (24). An $f\text{AUC}_{0-24}/\text{MIC}$ ratio of ≥ 100 was chosen as the target PK/pharmacodynamic (PD) index associated with efficacy based on the EUCAST guidelines (25). The current EUCAST MIC breakpoints for fluconazole are as follows: susceptible, ≤ 2 mg/liter, and resistant, >4 mg/liter. The current CLSI MIC breakpoints are as follows: susceptible, ≤ 2 mg/liter; susceptible dose dependent, 4 mg/liter; and resistant, ≥ 8 mg/liter. These breakpoints apply to *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis*.

The estimated concentrations from microdialysis samples were corrected, using the *in vivo* recovery calculated for fluconazole, prior to pharmacokinetic analyses with the following equation: $C_{\text{ISF}} = 100 \times (C_{\text{microdialysate}}/\text{percent recovery } in vivo)$, where C_{ISF} is the drug concentration in ISF and $C_{\text{microdialysate}}$ is fluconazole concentration in the microdialysate. ISF penetration ratios were calculated using the AUC_{0-24} for subcutaneous ISF concentrations and the $f\text{AUC}_{0-24}$ in plasma ($f\text{AUC}_{0-24 \text{ plasma}}$) as follows: ISF penetration ratio = $\text{AUC}_{\text{ISF}}/f\text{AUC}_{0-24 \text{ plasma}}$. A two-sided *t* test was used to compare the means of concentrations in plasma and subcutaneous ISF and PK parameters. The Mann-Whitney U test was used to compare the ISF penetration between patients receiving and not receiving vasopressors. All analyses were done using GraphPad Prism 5.03 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Twelve patients were included in this study; six were female. All patients were treated with 400 mg fluconazole (5.1 mg/kg of body weight) administered once daily as an intravenous infusion over 60 min. All sampling occurred between days 3 and 5 after initiation of fluconazole treatment. Seven (58.3%) patients received fluconazole for the directed treatment—six (50%) patients for intra-abdominal sepsis and one (8.3%) patient for lower respiratory tract infection. Three (25%) patients received empirical fluconazole for intra-abdominal sepsis, one (8.3%) patient received empirical fluconazole for lower respiratory tract infections, and one (8.3%) patient received fluconazole to treat *C. albicans* in a submandibular abscess. An overview of the patient characteristics is shown in Table 1.

Microbiology. Abdominal fluid was positive for *C. albicans* in five patients and for *Candida glabrata* in one patient. Fluconazole was changed to caspofungin in the last patient after identification to the species level. Lower respiratory tract secretions were positive for *C. albicans* in one patient, and in one patient, the submandibular abscess aspirate was positive for *C. albicans*. No other *Can-*

TABLE 1 Patient characteristics

Characteristic ^a	Median value (IQR)
Age (yr)	53 (43–67)
Wt (kg)	75 (70–87)
Height (m)	1.7 (1.6–1.8)
CrCL _{measured} (ml/min)	117.2 (74.0–143.3)
APACHE II score	18 (17.7–20.5)
SOFA score	3.5 (3.0–6.5)
Invasive ventilation [<i>n</i> (%)]	9 (75)
Vasopressor/inotropic support [<i>n</i> (%)]	5 (42)

^a CrCL_{measured}, measured creatinine clearance using 24-h urine collected on the sampling day. APACHE and SOFA scores were calculated using the data obtained over the preceding 24 h in relation to the sampling day. Vasopressor/inotropic support was defined as the patient having received one of the following to maintain clinician-targeted mean arterial pressure: noradrenaline, adrenaline, dopamine, vasopressin, and dobutamine.

did spp. were isolated in this patient cohort. The MIC values were reported for only two patients (0.25 and 0.6 mg/liter), both of which were below EUCAST and CLSI breakpoints for fluconazole.

Microdialysis. The mean *in vivo* fluconazole recovery rate for microdialysis \pm standard deviation (SD) was 51.4% \pm 16.1%. The fluconazole ISF penetration was 0.52 \pm 0.30 (coefficient of variation, 58%). The median (interquartile range [IQR]) fluconazole ISF penetrations in patients receiving and not receiving vasopressors were 0.28 (0.17 to 0.57) and 0.70 (0.48 to 0.94), respectively ($P = 0.106$). The ISF PK parameters are presented in Table 2.

Fluconazole PK. The concentration-time profile of fluconazole in plasma and ISF of subcutaneous soft tissue in critically ill patients with sepsis is shown in Fig. 1. The free median plasma AUC_{0–24} was significantly higher than the median ISF AUC_{0–24} (340.4 versus 141.1 mg · h/liter; $P = 0.004$) (Fig. 2). Both C_{max} and C_{min} showed significant correlation with the fluconazole plasma exposure (C_{max} , $R^2 = 0.86$, $P < 0.001$; C_{min} , $R^2 = 0.75$, $P < 0.001$). Of the patients with *Candida* sp. isolates, those that responded to treatment did not seem to have a fluconazole exposure different from that of the patients who did not respond to the treatment; the respective median (IQR) plasma exposures were 366 mg · h/liter (257.4 to 485.7 mg · h/liter) and 454 mg · h/liter (391.9 to 516.1 mg · h/liter), and the respective median (IQR) ISF exposures were 96.6 mg · h/liter (65.2 to 179.4 mg · h/liter) and 352.1 mg · h/liter (254.6 to 449.6 mg · h/liter).

TABLE 2 Plasma and ISF pharmacokinetics of fluconazole in critically ill patients^a

Parameter ^b	Median value (IQR)	
	Plasma	ISF
C_{max} (mg/liter)	20.3 (17.2–26.4)	9.8 (6.3–16.0)
T_{max} (h)	1.4 (1–1.4)	3.8 (1.2–4.8)
C_{min} (mg/liter)	11.6 (8.1–16.4)	3.5 (1.4–6.7)
$t_{1/2}$ (h)	33.7 (24.7–74.5)	
$fAUC_{0–24}$ (mg · h/liter)	340.4 (263.5–523.7)	141.1 (68.2–267.0)
CL _{obs} (liters/h)	0.5 (0.2–0.6)	
V_{ss} (liters)	20.4 (15.5–26.7)	

^a Pharmacokinetics after receiving a 400-mg dose.

^b C_{max} , maximum concentration observed; T_{max} , time to reach C_{max} ; C_{min} , minimum concentration observed; CL_{obs}, observed clearance; V_{ss} , steady-state volume of distribution.

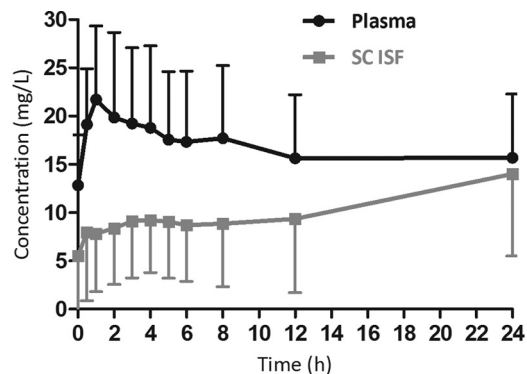


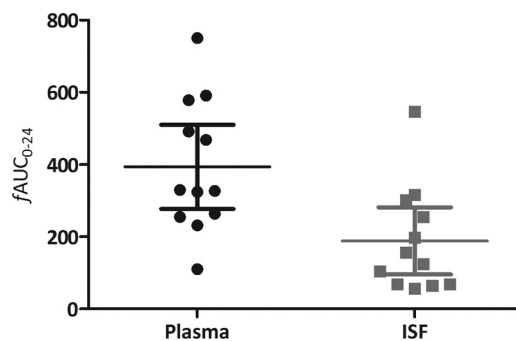
FIG 1 Free median (interquartile range [error bars]) plasma and SC ISF fluconazole concentration-time profile in critically ill patients (pharmacokinetic profile after receiving a 400-mg dose).

PK/PD. Fluconazole PK/PD target attainment in both plasma and ISF at EUCAST and CLSI breakpoints for *C. albicans* is shown in Fig. 3. Both patients with reported MICs in this cohort attained the PK/PD target ($fAUC_{0–24}/MIC \geq 100$) in both plasma and ISF. However, only one patient responded to the treatment as defined above.

DISCUSSION

Our data suggest that fluconazole was distributed incompletely from plasma into subcutaneous ISF in this cohort of critically ill patients with sepsis, with a mean penetration ratio \pm SD of 0.52 \pm 0.30. Peak concentrations in ISF were reached at 3.8 h compared to 1.4 h in plasma, suggesting delayed distribution into subcutaneous ISF. The variability in the observed fluconazole ISF penetration among patients was high. This was evident from the coefficient of variation (58%) observed among the patients. A large proportion of patients in the cohort would have achieved an $fAUC_{0–24}/MIC$ of ≥ 100 in plasma at EUCAST and CLSI breakpoints (Fig. 3). However, a majority of the patients would require higher doses in order to achieve an $fAUC_{0–24}/MIC$ of ≥ 100 in ISF (Fig. 3).

Sepsis- and septic-shock-related hemodynamic changes lead to tissue hypoperfusion, which results in alterations in antimicrobial pharmacokinetics (9, 26). During the initial stages of sepsis-related blood flow changes, hypoperfusion of vital organs occurs,

FIG 2 Comparison of the free area under the concentration-time curve from 0 to 24 h in plasma and subcutaneous interstitial fluid (means \pm SD in plasma and interstitial fluid were 393.3 \pm 183.7 and 188.6 \pm 146.6, respectively; $P = 0.006$).

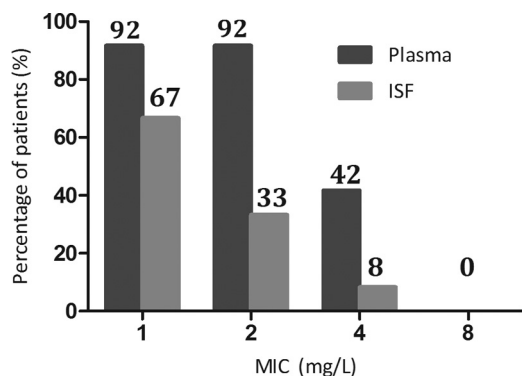


FIG 3 Percentages of patients who achieved the pharmacokinetic/pharmacodynamic target of an $fAUC_{0-24}/MIC$ of ≥ 100 in plasma and subcutaneous interstitial fluid at breakpoints ($n = 12$).

while peripheral tissues still receive high blood flow as a result of an increased cardiac workload and peripheral vasodilation, whereas peripheral tissue hypoperfusion occurs during the later stages of sepsis-related blood flow changes as a consequence of a physiological effort to increase blood perfusion to vital organs (27). Thus, using physiologically based PK simulations, Sasongko et al. (24) have shown that fluconazole uptake into subcutaneous ISF is dependent on blood flow to the subcutaneous tissue in healthy volunteers. Moreover, significant sustained compromised microcirculation in peripheral tissues was observed even after restoration of hemodynamics in sepsis patients (28, 29). Although not statistically significant, our results suggest that use of vasopressors, which result in peripheral vasoconstriction, may have an influence on peripheral subcutaneous ISF penetration of fluconazole.

Mauric et al. (30) investigated fluconazole penetration into skeletal muscle of rats with severe sepsis using microdialysis and showed that free plasma fluconazole concentrations were superimposable with ISF concentrations in skeletal muscle. This finding is in contrast to the observed penetration in our study. However, the lipopolysaccharide-induced-sepsis rat model used by Mauric et al. does not mimic all forms of inflammation observed in humans. Moreover, inflammation and sampling were limited in duration to 6 h. It is possible that these differences— inflammatory changes and a shorter inflammatory period—might explain the incomplete distribution observed in our study.

Another important observation from this study was the heterogeneous distribution of fluconazole into subcutaneous ISF (Fig. 2). This could be a result of differences in individual PK, as well as the estimation of microdialysis probe recovery. It is generally recommended that the recovery rates be $>20\%$ to minimize the variability associated with sample handling and analysis (31). The mean *in vivo* recovery rate achieved in this study was 51.4%. Furthermore, the mean fluconazole recovery rates achieved in the current study were similar to those observed in healthy volunteers (57%) (24) and in an *in vivo* study using a *C. albicans*-infected rat model (50%) (32). Moreover, the observed *in vivo* recovery rate variability in this study was similar to those attained in other studies (coefficient of variation, 31% versus 28 to 37%) (24, 32, 33). Given the heterogeneous distribution and lack of readily identifiable clinical factors by which to recognize patients with reduced distribution into ISF, it is evident that care should be taken when

adjusting doses based only on the concentration in plasma. In plasma, as previously reported in a cohort of critically ill patients (34), variability in the observed mean fluconazole $fAUC_{0-24}$ and the C_{max} and C_{min} was higher than in surgical ICU patients (35) and healthy volunteers (36). The underlying causes of this variability could be 2-fold—physiological changes that occur during sepsis and patient-related factors, like weight and renal function. Thus, a recent study (34) has shown that a standard one-dose-fits-all approach, i.e., 400 mg without taking patient weight into account, would result in subtherapeutic fluconazole exposure in critically ill patients. This variability in plasma exposures might have also contributed to the incomplete distribution to ISF concentrations described above.

For fluconazole, an $fAUC_{0-24}/MIC$ of ≥ 100 has been shown to be the predictive PK/PD target associated with clinical success (25). A predictive PK/PD target in ISF has yet to be identified for fluconazole. In general, the percentage of patients who attained the PK/PD target decreased with increasing MICs in both plasma and ISF. Considering that similar PK/PD targets may apply to ISF, the percentage of patients attaining the PK/PD target was further reduced in ISF (Fig. 3). However, a different PK/PD target for ISF is highly possible. In patients infected with a susceptible *Candida* sp. with a MIC below the breakpoint, the PK/PD target in plasma would be attained in the majority of the patients with standard doses. However, higher than standard doses would be required to achieve similar targets in ISF. In patients infected with less-susceptible *Candida* spp., a lower exposure is highly likely to be a problem in both plasma and ISF. If the MIC is high or unknown, we recommend measuring fluconazole exposure or using higher than standard doses to ensure that the exposure is adequate.

This study had some limitations. We did not measure the unbound concentrations of fluconazole in plasma and used a published protein binding value (12%) to estimate free plasma fluconazole concentrations. Given the low protein binding of fluconazole, the difference between the measured and estimated fluconazole concentrations is unlikely to be significant (37). We used the breakpoint MICs determined by EUCAST and CLSI to estimate fluconazole PK/PD targets because of the lack of actual MIC data for 10 of 12 patients. We measured the fluconazole ISF concentration in the SC tissue of the upper arm in this study. The PK in this peripheral site may be different from that in other sites of infection, e.g., intra-abdominal or epithelial lining fluid, for the above-mentioned reasons. Considering the ethical constraints for critically ill patients, we chose to use SC tissue of the upper arm for this study. Therefore, the results should be interpreted cautiously, taking patient status and the site of infection into consideration.

In summary, fluconazole was distributed incompletely from plasma into subcutaneous ISF, with substantial variability between patients. In this cohort of critically ill patients, based on the PK/PD target, the observed exposures obtained in plasma should be sufficient to effectively treat susceptible *Candida* spp. Given the heterogeneous distribution and lack of clinically identifiable factors by which to recognize patients with reduced distribution/exposure, we recommend using higher than standard doses to ensure that the exposure is adequate at the site of infection. Decreased ISF penetration is one of the possible causes of treatment failure, and further studies are warranted.

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