ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2010, p. 852–859 Vol. 54, No. 2
0066-4804/10/$12.00 doi:10.1128/AAC.00429-09

Voriconazole Pharmacokinetics in Liver Transplant Recipients▽

H. J. Johnson,[1,2] K. Han,[1] B. Capitano,[1,3] D. Blisard,[2] S. Husain,[1] P. K. Linden,[1,2] A. Marcos,[2] E. J. Kwak,[1,2] B. Potoski,[1,2] D. L. Paterson,[1] M. Romkes,[1] and R. Venkataramanan[1]*

University of Pittsburgh[1] and University of Pittsburgh Medical Center,[2] Pittsburgh, Pennsylvania 15232, and Pfizer Global Pharmaceuticals, New York, New York 10019[3]

Received 30 March 2009/Returned for modification 9 July 2009/Accepted 29 September 2009

The objective of this study was to evaluate the pharmacokinetics of voriconazole and the potential correlations between pharmacokinetic parameters and patient variables in liver transplant patients on a fixed-dose prophylactic regimen. Multiple blood samples were collected within one dosing interval from 15 patients who were initiated on a prophylactic regimen of voriconazole at 200 mg enterally (tablets) twice daily starting immediately posttransplant. Voriconazole plasma concentrations were measured using high-pressure liquid chromatography (HPLC). Noncompartmental pharmacokinetic analysis was performed to estimate pharmacokinetic parameters. The mean apparent systemic clearance over bioavailability (CL/*F*), apparent steady-state volume of distribution over bioavailability (V_{ss}/F), and half-life ($t_{1/2}$) were 5.8 ± 5.5 liters/h, 94.5 ± 54.9 liters, and 15.7 ± 7.0 h, respectively. There was a good correlation between the area under the concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$) and trough voriconazole plasma concentrations. $t_{1/2}$, maximum drug concentration in plasma (C_{max}), trough level, $AUC_{0-\infty}$, area under the first moment of the concentration-time curve from 0 h to infinity ($AUMC_{0-\infty}$), and mean residence time from 0 h to infinity ($MRT_{0-\infty}$) were significantly correlated with postoperative time. $t_{1/2}$, λ, $AUC_{0-\infty}$, and CL/*F* were significantly correlated with indices of liver function (aspartate transaminase [AST], total bilirubin, and international normalized ratio [INR]). The C_{max}, last concentration in plasma at 12 h (C_{last}), $AUMC_{0-\infty}$, and $MRT_{0-\infty}$ were significantly lower in the presence of deficient *CYP2C19*2* alleles. Donor characteristics had no significant correlation with any of the pharmacokinetic parameters estimated. A fixed dosing regimen of voriconazole results in a highly variable exposure of voriconazole in liver transplant patients. Given that trough voriconazole concentration is a good measure of drug exposure (AUC), the voriconazole dose can be individualized based on trough concentration measurements in liver transplant patients.

Due to chronic immunosuppression, infections are common life-threatening complications in organ transplant patients (7). Invasive aspergillosis is one of the most dreaded complications after organ transplantation (21) due to its high mortality rate, which can range up to 88.1% (18).

Voriconazole (V-Fend [Pfizer]; formerly known as UK-109496), (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1,2,4-triazol-1-yl)butan-2-ol, is a novel broad-spectrum triazole systemic antifungal agent and an ideal drug to prevent invasive aspergillosis. Compared with other azole antifungal agents, it has potent activity against a broader spectrum of clinically significant fungal pathogens, including *Aspergillus*, *Candida*, *Cryptococcus neoformans*, and some unusual organisms, such as *Fusarium* and *Pseudallescheria boydii* (11, 24, 37, 39, 43).

Voriconazole is extensively metabolized hepatically, primarily via the cytochrome P450 (CYP) isoenzymes CYP2C19 and, secondarily, CYP2C9 and CYP3A4 (15, 25, 39) to inactive metabolites. Large inter- and intraindividual variabilities in voriconazole plasma concentrations regardless of the route of administration or the type of patient population have been documented and discussed in the literature (2, 17, 19, 22, 23, 33, 38, 40, 42, 45). Factors associated with interindividual variability of voriconazole exposure include liver dysfunction, alcohol abuse in the past (47), concomitant use of potent CYP450 inducers or inhibitors (10, 12), *CYP2C19* genetic polymorphisms (including poor as well as ultrarapid metabolizers) (13, 20, 46), gastrointestinal abnormalities (e.g., mucositis or diarrhea) (38) impairing drug absorption, and intake with or without food (15).

Voriconazole is approved at our institution for prophylaxis in all liver transplant patients. The pharmacokinetics of voriconazole in liver transplant patients has not been evaluated, and there is limited information about the pharmacokinetics of voriconazole in other solid organ transplant patient populations (3). We hypothesized that the use of a fixed-dosing regimen of voriconazole would lead to a large degree of variability in voriconazole exposure in liver transplant patients. Given that a low voriconazole plasma level of less than 0.25 μg/ml is associated with a poor outcome in patients with aspergillosis (4, 8, 22, 31, 38, 40) and with ultimately death of the patients, while a high voriconazole plasma concentration of over 5.5 μg/ml is correlated with an increased risk for toxicity, including visual disturbances, elevated transaminase levels, central nervous system (CNS) disorders (e.g., encephalopathy), and electrolyte disturbances (2, 4, 38, 41), it is important to optimize the use of voriconazole in this patient population.

The objective of this prospective single-center observational study was to characterize the pharmacokinetics of voriconazole in liver transplant patients on a fixed-dose prophylactic regimen in order to determine the extent of interpatient variability in voriconazole exposure among liver transplant patients and to evaluate the potential correlations between pharmacoki-

* Corresponding author. Mailing address: 718 Salk Hall, Pittsburgh, PA 15232. Phone: (412) 648-8547. Fax: (412) 383-7436. E-mail: rv@pitt.edu.

▽ Published ahead of print on 23 November 2009.

netic parameters and certain patient variables that could potentially explain the large interindividual variability in voriconazole pharmacokinetics in liver transplant patients.

MATERIALS AND METHODS

Patients. Between January 2007 and March 2007, liver transplant recipients who were initiated on a prophylactic voriconazole regimen (200-mg tablets twice daily orally or via a nasogastric tube) immediately posttransplant as part of their standard clinical care and who gave informed consent were enrolled in this prospective study. Children under age 18, patients who were receiving any medications known to influence the pharmacokinetics of voriconazole, and patients receiving voriconazole to treat an active fungal infection were excluded from this study. Demographic data, including age, gender, height, weight, race, laboratory results, and current medication use were recorded. All patients received tacrolimus as their primary immunosuppressive agent. The protocol was approved by the Institutional Review Board at the University of Pittsburgh.

Blood sample collection. Serial blood samples (3 ml) were collected from each patient just prior to (0 h) and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 h following administration of a minimum of five oral doses (range, 5th to 15th dose; mean, 7th dose). Blood samples were collected into heparinized Vacutainer tubes and centrifuged at 3,000 rpm for 10 min, and plasma was separated and stored at −70°C until analysis.

Genotyping. A 1-ml sample of whole blood was collected and immediately stored at −80°C for genetic analysis. Additionally, whenever available, allograft biopsy tissue was also collected and stored at −80°C for future genetic analysis. Genetic analysis was conducted through isolation of genomic DNA using the PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN). Determination of a panel of *CYP2C9*, *CYP2C19*, and *CYP3A4* and *CYP3A5* allelic variants was performed by TaqMan allele discrimination analyses. The genotyping of *CYP2C9*2*, *CYP2C9*3*, *CYP2C19*2*, *CYP2C19*3*, *CYP3A4*1B*, *CYP3A5*3*, and *CYP3A5*6* was performed for all 15 patients (blood) and for 7 donors (liver) using the Applied Biosystems drug metabolism genotyping assay kits to genotype for C3608T, A42614C, G19154A, G17948A, A-392G, A6986G, and G14690A, respectively. Positive and negative PCR controls were included with each amplification reaction. Blinded duplicate sample analyses were also performed for all genotyping assays. An additional 10% of samples were subjected to repeat analysis to avoid further misclassification and to verify the reproducibility of the assay. All results were interpreted independently by two laboratory personnel, and no discordant results were obtained.

Analytical assay. Plasma voriconazole concentrations were measured using a validated high-pressure liquid chromatography (HPLC) method that was modified based on previously published assays (9, 26, 27). Sixty microliters of 6% perchloric acid (Fisher Scientific, Fair Lawn, NJ) was added to 120 μl of plasma, vortexed, and centrifuged (13,000 rpm) for 4 min at room temperature. Fifty microliters of supernatants was injected onto a HPLC system consisting of a Waters model 510 HPLC pump, a Waters model 717 plus automatic sampler, and a Waters model 2487 UV tunable absorbance detector set to 255 nm. Separation was performed at ambient temperature on a 5-μm, 4.6- by 250-mm Waters C_{18} symmetry analytical column. The mobile phase consisted of HPLC-grade acetonitrile and water (68:32, vol/vol; Fisher Scientific, Fair Lawn, NJ). The total run time was 10 min at a flow rate of 0.8 ml/min. Chromatographic data were collected and analyzed using Empower chromatography software (Waters, version 5.0). The assay precision (intraday variability) was 1.3% to 9.0% (0.2 to 9 μg/ml), and the assay bias (interday variability) was 0.7% to 3.1% (0.5 to 9 μg/ml). The linearity range was 0.2 to 9 μg/ml ($r^2 = 0.9998$).

Pharmacokinetic analysis. Various pharmacokinetic parameters were calculated using noncompartmental analysis with WinNonlin software (version 4.1; Pharsight Corporation, Mountain View, CA). The parameters calculated after enteral administration of voriconazole included the terminal disposition rate constant (λ_z), terminal disposition half-life ($t_{1/2}$), area under the curve (AUC), apparent systemic clearance over bioavailability (CL/*F*), apparent steady-state volume of distribution over bioavailability (V_{ss}/F), mean residence time (MRT), peak concentration in plasma (C_{max}), time to reach peak concentration (T_{max}), last concentration in plasma at 12 h (C_{last}), and area under the first moment of the concentration-time curve (AUMC). λ_z and $t_{1/2}$ were derived from data points during the terminal disposition phase only when at least three data points were available, and the $AUC_{0-\infty}$ and $AUMC_{0-\infty}$ specific for the dose evaluated were calculated using the reverse superposition principle. The projected trough voriconazole plasma concentration (C_{last}) was used for three patients (no. 11, 15, and 16) because C_{last} was missing for these three patients. Each of these parameters is presented as mean and standard deviation. Statistical comparison of different parameters was made using a paired two-tailed Student *t* test (SPSS software, Windows-based version 14.0; SPSS, Chicago, IL). A *P* value of <0.05 was considered statistically significant.

Statistical analysis. The relationship between various pharmacokinetic parameters and patient variables and biochemical indices was examined by simple linear regression analysis. A relationship was considered to be statistically significant at a *P* value of <0.05 for the deviation of the coefficient from zero in the linear regression analysis. The difference between trough concentrations (C_0 and C_{12}) was tested using a paired two-tailed Student *t* test. The effect of dichotomous variables (such as gender, race, and concomitant medication) on various pharmacokinetic parameters of voriconazole was tested using an unpaired two-tailed Student *t* test except, for the effect of the *CYP2C19* genotype, which was tested using an unpaired one-tailed Student *t* test since carriers of *CYP2C19*2* and *CYP2C19*3* alleles have been identified as poor metabolizers (20). The effect of feeding methods on various pharmacokinetic parameters of voriconazole was tested using one-way analysis of variance (ANOVA). A *P* value of <0.05 was considered to be statistically significant in all the statistical tests. The relationship between CL/*F*, V_{ss}/F, and body weight were evaluated using both a simple linear model and an allometrical model, parameter $= A \times (WT/\overline{WT})^B$, where parameter includes CL/*F* and V_{ss}/F, WT denotes actual body weight, and *A* and *B* are coefficients and exponents to be estimated using nonlinear regression.

The 95% confidence bands and 95% prediction bands were calculated and plotted using GraphPad Prism (version 4.03; GraphPad Software, Inc.) to evaluate the precision of parameter estimation and predictive performance.

RESULTS

A total of 15 patients were enrolled in this study. The characteristics of the patients, including the primary diagnosis, days posttransplantation on the day of study, methods of feeding at time of study, concomitant medications, MELD (model for end-stage liver disease) score, age, gender, and race; the characteristics of the donors, including the cold ischemic time, warm ischemic time, age distribution, and type of liver donation; the laboratory biochemical and hematological profiles of the study patients before transplantation and on the day of pharmacokinetics study; and the pharmacogenomic profiles of patients and donors are shown in Table 1.

The mean and individual plasma concentrations of voriconazole over time after enteral voriconazole administration are shown in Fig. 1. Among all 15 patients who completed the study, one patient (no. 13) had an undetectable concentration of voriconazole in all of the samples and could not be evaluated (no particular reason was identified); three patients (no. 1, 3, and 7) had extremely atypical profiles with fewer than three data points during the terminal disposition phase, and noncompartmental pharmacokinetic analysis could therefore not be readily performed. Complete pharmacokinetic data could be calculated for 11 patients. The pharmacokinetic parameters of voriconazole after enteral administration are shown in Table 2. There was a wide variation in various pharmacokinetic parameters of voriconazole in liver transplant patients after enteral voriconazole administration (Fig. 1).

The trough concentrations prior to dosing (C_0) and at 12 h after dosing (C_{12}) are not significantly different ($P = 0.2794$), and the difference between the trough concentrations ($C_{12} - C_0$)/C_{12} averaged 6.4%, indicating that steady state had been reached in most of the patients at the time of study. There was a good correlation ($r^2 = 0.75$) between the trough voriconazole plasma concentrations and the corresponding $AUC_{0-\infty}$ (Fig. 2) ($n = 11$). Thirty-three percent of the patients had a trough level lower than 1 μg/ml, and the rest of the patients had a trough level of between 1 μg/ml and 6 μg/ml.

TABLE 1. Characteristics of patients and donors

Characteristic[a]	Value[b]
Gender (no. male/no. female)	11/4
No. with diagnosis:	
Viral hepatitis (HBV/HCV)	1/5
HCV + alcohol	2
Nonalcoholic steatohepatitis	3
Primary sclerosing cholangitis	2
Autoimmune hepatitis	1
Wilson's disease	1
MELD score[c]	20.6 ± 11.3 (8–43)
Age (yr)	56.3 ± 10.3 (41–76)
Wt (kg)	84.1 ± 17.7 (56–121)
Race (Caucasian/Asian)	13/2
Days posttransplantation on day of study	3.7 ± 1.4 (2–7)
Feeding at time of study (tube/clear liquid/regular food)	3/11/1
Anastomosis (T-tube present/T-tube absent/Roux-en-Y)[d]	6/8/1
Concomitant drug (pantoprazole/famotidine)	10/5
Cold ischemic time (min)	538.9 ± 266.6 (86–935)
Warm ischemic time (min)	27.2 ± 5.1 (16.8–37.8)
Donor age (yr)	47.9 ± 21.6 (14–84)
Cadaveric/Living donor (*n*)	13/2
Total bilirubin (mg/dl)	6.4 ± 5.9 (1.9–25.4)/6.1 ± 6.4 (0.5–22.9)
AST (U/liter)	1,088.9 ± 726.2 (180–2405)/294.8 ± 204.2 (33–620)
ALT (U/liter)	682.6 ± 444.8 (144–1569)/346.1 ± 263.4 (53–792)
INR	1.76 ± 0.35 (1.1–2.3)/1.3 ± 0.16 (1–1.6)
SCr (mg/dl)†	1.6 ± 0.8 (0.6–2.9)/1.7 ± 1.3 (0.5–5.2)
Baseline plasma albumin (g/liter)	3.1 ± 0.5 (2.3–4.1)
*CYP2C9*2* (C3608T)	15:0:0 (6:1:0)
*CYP2C9*3* (A42614C)	12:3:0 (5:2:0)
*CYP2C19*2* (G19154A)	12:3:0 (6:1:0)
*CYP2C19*3* (G17948A)	15:0:0 (6:0:0)
*CYP3A4*1B* (A-392G)	15:0:0 (6:0:0)
*CYP3A5*3* (A6986G)	0:0:15 (7:0:0)
*CYP3A5*6* (G14690A)	15:0:0 (7:0:0)

[a] HBV, hepatitis B virus; ALT, alanine aminotransferase; SCr, serum creatinine.
[b] Values are expressed as mean ± standard deviation (range) and were measured for patients unless specified as measurements for donors. For bilirubin, AST, ALT, INR, SCr, and plasma albumin, values are displayed as baseline measurement/measurement on the day of study. For the *CYP3* alleles, the donor genotype (liver) is given in parentheses. The three values displayed represent wild-type homozygous extensive metabolizers (−/−):heterozygous extensive metabolizers (−/+):poor metabolizers (+/+).
[c] MELD (model for end-stage liver disease) score = 3.78(ln serum bilirubin [mg/dl]) + 11.2(ln INR) + 9.57(ln serum creatinine [mg/dl]) + 6.43.
[d] Duct-to-duct anastomosis; the T-tube had been removed from some of the patients at the time of study.

There were significant correlations between various estimated pharmacokinetic parameters and patient variables and various biochemical indices (the linear regression coefficient differed significantly from zero). All of the correlations are summarized in Table 3 and Fig. 3. The 95% confidence bands and 95% prediction bands were calculated and plotted (Fig. 3). The correlation between body weight and the two independent pharmacokinetic parameters CL/*F* and V_{ss}/F was very poor using both simple linear regression ($r^2 = 0.1345$ for CL/*F* and 0.0308 for V_{ss}/F) and the principle of allometry ($r^2 = 0.0990$ for CL/*F* and 0.1350 for V_{ss}/F).

Despite the small number of subjects in this study, the presence of deficient *CYP2C19*2* alleles and race were significantly associated with some pharmacokinetic parameters of voriconazole. Compared to those in homozygous extensive metabolizers (*CYP2C19*1/*1*), C_{max}, C_{last}, $AUMC_{0-\infty}$, and $MRT_{0-\infty}$ were significantly higher in heterozygous extensive metabolizers (CYP2C19*1/*2), i.e., by 1.9-fold, 2.0-fold, 5.1-fold, and 3.9-fold, respectively. Compared to that in Caucasian patients ($n = 9$), T_{max} was significantly higher ($P = 0.016$), by 3-fold, and C_{max} was significantly lower ($P = 0.0402$), by 2.6-fold, in Asian patients ($n = 2$) (Table 3). In addition, V_{ss}/F was 2.1-fold higher ($P = 0.0513$), CL/*F* was 2.5-fold higher ($P = 0.1023$), and $AUC_{0-\infty}$ was 2.7-fold lower ($P = 0.0711$) in Asian patients than in Caucasian patients, although these differences did not reach statistical significance.

Interestingly, concomitant treatment with pantoprazole and oral voriconazole was associated with a statistically significant decrease in voriconazole exposure. The voriconazole half-life, C_{max}, C_{last}, $AUC_{0-\infty}$, $MRT_{0-\infty}$, and $AUMC_{0-\infty}$ were significantly lower, by 37% to 70%, in patients receiving concomitant pantoprazole treatment compared than in those not on pantoprazole (Table 3). The CL/*F* was 3.5-fold higher in patients on concomitant pantoprazole treatment than in those not on pantoprazole, although this difference did not reach statistical significance ($P = 0.0533$). Feeding methods (regular diet, clear liquids, or tube feedings) had no effect on the pharmacokinetic parameters of voriconazole.

It is also worth mentioning that all of the donor variables, including cold ischemic time, warm ischemic time, donor age, and type of liver donation, poorly correlated with all of the estimated pharmacokinetic parameters ($r^2 < 0.4$).

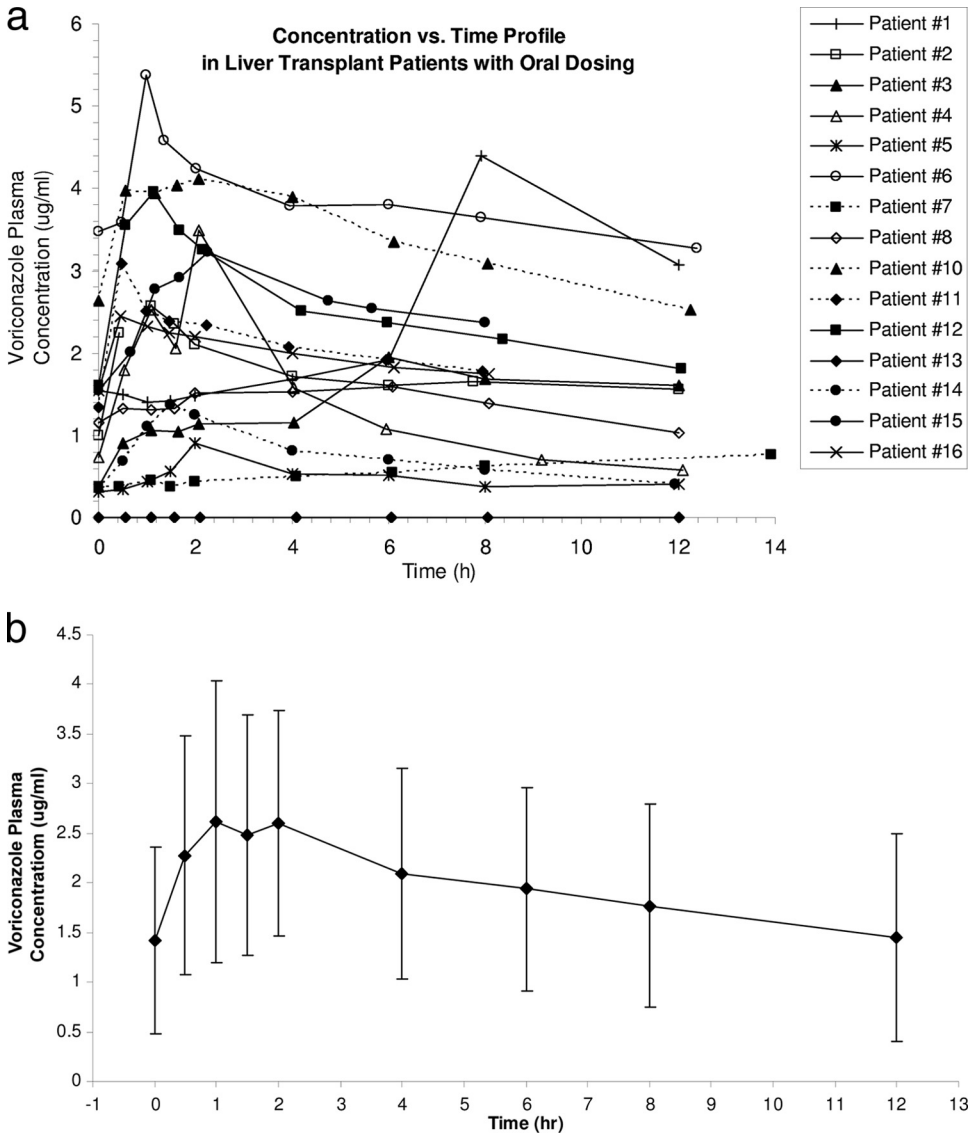

FIG. 1. (a) Plasma concentration profiles of voriconazole during one dosing interval. Large interindividual variability can be observed. Patients 1, 3, 7, and 13 had extremely atypical profiles. (b) Mean (± standard deviation) plasma concentrations of voriconazole during one dosing interval, with patients 1, 3, 7, and 13 excluded (see text).

DISCUSSION

Limited pharmacokinetic data on voriconazole in transplant patients exist in the literature. To date this is the first study to evaluate the pharmacokinetics of voriconazole in liver transplant patients.

This study involved intense blood sampling (nine data points for each patient in a single dosing interval) in the immediate posttransplant period (within 7 days) in a small group of relatively homogenous liver transplant patients (n = 15). The pharmacokinetic profiles of voriconazole are characterized by an early and sharp increase of voriconazole concentration, with the peak concentration being reached around 1 to 2 h after dosing. These profiles were consistent with rapid absorption of voriconazole. This observation is similar to what has been reported for nontransplant patients (30).

Despite the relative homogeneity of the population studied, a large interindividual variability both in plasma concentrations of voriconazole over time and in individual pharmacokinetic parameters calculated using a noncompartmental model has been demonstrated. In fact, the existence of large inter- and intraindividual variabilities in voriconazole plasma levels regardless of the route of administration or the type of patient population has been widely discussed (2, 17, 19, 22, 23, 33, 38, 40, 42, 45). Studies with healthy volunteers revealed a 90 to 100% variation in exposure with a fixed-dose voriconazole regimen. Unpublished results from our research group have shown that nearly 15.2% of the patients on recommended doses do not have any measurable trough plasma concentration, and nearly 42.5% of the patients have trough plasma concentrations of less than 1 μg/ml (our clinical observations). We have observed a 35-fold variation in exposure as indicated by the area under the plasma concentration-time curve (AUC) among lung transplant patients receiving a fixed-

TABLE 2. Pharmacokinetic parameters of voriconazole after enteral administration[a]

Patient	λ_z (h^{-})	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/ml)	C_{last} (μg/ml)[b]	$AUC_{0-\infty}$ (h · μg/ml)	AUMC $_{0-\infty}$ (h · h · μg/ml)	*V*/*F* (liters)	CL/*F* (liters/h)	$MRT_{0-\infty}$ (h)
2	0.04	16.1	1.1	2.6	1.6	34.3	1,662.8	81.1	3.5	48.5
4	0.12	5.6	2.1	3.5	0.6	15.2	145.7	76.8	9.5	9.6
5	0.07	9.5	2.0	0.9	0.4	7.2	182.9	239.1	17.5	25.4
6	0.02	30.0	1.0	5.4	3.3	37.7	12,945.6	46.0	1.1	343.5
8	0.07	9.4	6.1	1.6	1.0	14.7	480.2	89.7	6.6	32.7
10	0.05	14.9	2.1	4.1	2.5	38.1	2,610.8	45.3	2.1	68.6
11	0.04	17.8	0.5	3.1	1.52	27.9	2,089.1	82.4	3.2	74.9
12	0.04	15.5	1.2	4.0	1.8	34.4	1,940.9	63.6	2.8	56.3
14	0.09	7.4	1.5	1.4	0.4	9.2	135.5	165.2	15.5	14.8
15	0.03	21.3	2.3	3.2	2.08	45.7	3,826.1	65.8	2.1	83.7
16	0.04	17.7	0.5	2.4	1.49	20.6	2,113.1	84.8	3.3	102.4
Mean	0.06	15.0	1.8	2.9	1.6	25.9	2,557.5	94.5	6.1	78.2
SD	0.03	7.0	1.5	1.3	0.9	13.1	3,643.6	57.6	5.7	92.7
Coefficient of variation (%)	52.69	46.6	84.8	45.4	58.3	50.7	142.5	61.0	92.6	118.6
Median	0.04	15.5	1.5	3.1	1.7	27.9	1,940.9	81.1	3.3	56.3
95% Confidence interval	0.04–0.08	10.9–19.1	0.9–2.74	2.1–3.7	1.0–2.1	18.1–33.7	404.3–4,710.7	60.5–128.6	2.8–9.5	23.4–133.0

[a] C_{max}, maximum concentration; T_{max}, time to reach maximum concentration; C_{last}, concentration at 12 h; λ_z, disposition rate constant; $t_{1/2}$, apparent half-life; $AUC_{0-\infty}$, area under the concentration-time curve 0 h to infinity; CL/*F*, clearance/bioavailability; *V*/*F*, volume of distribution/bioavailability; $MRT_{0-\infty}$, mean residence time; $AUMC_{0-\infty}$, area under the first moment of the concentration-time curve from 0 h to infinity.

[b] The C_{last} values for patients 1, 3, 7, and 13 were 3.07 μg/ml, 1.6 μg/ml, 0.76 μg/ml, and 0 μg/ml (unmeasurable), respectively. Projected C_{last} values for patients 11, 15, and 16 were used (see text).

dose prophylactic regimen (3). Factors associated with interindividual variability of voriconazole exposure have been suggested to include liver dysfunction or alcohol abuse in the past (47), concomitant use of potent CYP450 inducers or inhibitors (10), *CYP2C19* genetic polymorphisms including poor as well as ultrarapid metabolizers (13, 20, 46), and intake with or without food (15).

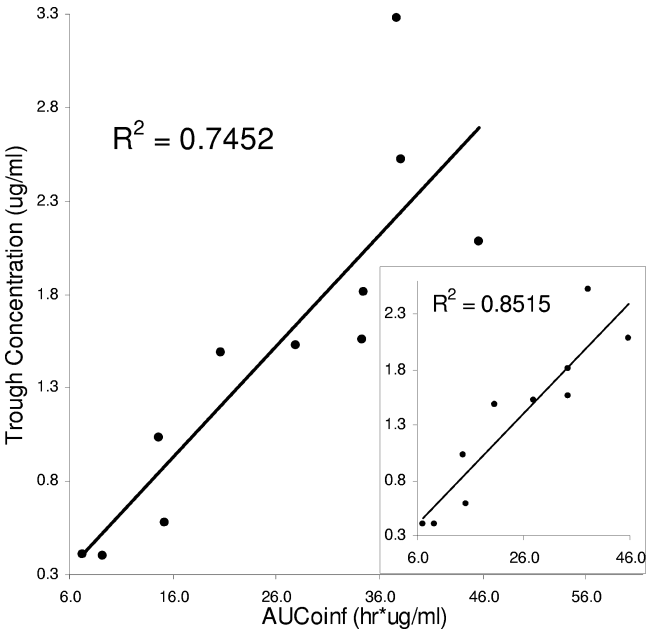

FIG. 2. Correlation between $AUC_{0-\infty}$ and trough concentrations (C_{12}). Main figure, $r^2 = 0.745\%$ when $AUC_{0-\infty}$ and trough concentrations are correlated for all 11 patients who had typical profiles. Inset, $r^2 = 0.852\%$ when $AUC_{0-\infty}$ and trough concentrations are correlated for 10 patients who had typical profiles, with patient 6 omitted.

The large variability observed in liver transplant patients may be explained by variations in absorption, elimination and drug-drug interactions. Variability in absorption with oral voriconazole administration may cause the large interindividual variability of voriconazole exposure. First, a decrease in the motility of the gastrointestinal tract after liver transplant surgery is a common physiological change that is unique to this patient population. The magnitude of the decrease in the gastrointestinal motility is quite different from patient to patient and therefore may be a source of variability and could potentially alter the rate of absorption of voriconazole. Second, voriconazole is highly lipophilic, and therefore its absorption is likely dependent on secretion of bile. Variation in bile flow between patients and variable dissolution of voriconazole may be another source of variability and could potentially lead to altered bioavailability. Finally, administration of voriconazole with food has a significant influence on voriconazole absorption, and therefore the feeding method is also a source of variability. Although this study involved only enteral administration of voriconazole and bioavailability could not be estimated, unpublished results from our research group have shown that voriconazole bioavailability varied from 57% to 94% in lung transplant patients.

Variability in elimination may be another factor responsible for the large interindividual variability of voriconazole exposure. First, the most relevant physiological factor that can lead to the large variability of voriconazole exposure may be the differences in liver function caused by physiological characteristics unique to this patient population, because voriconazole is extensively metabolized in the liver, with less than 2% of the administered dose being excreted unchanged in urine and feces (28, 29, 30, 36). There are no clinically relevant effects of renal impairment on the pharmacokinetic profile of oral or intravenous voriconazole (28, 29, 30). Second, voriconazole has demonstrated nonlinear pharmacokinetics due to satura-

TABLE 3. Correlations between estimated pharmacokinetic parameters and patient variables

Patient variable[a]	Correlation[b] with:									
	λ	$t_{1/2}$	T_{max}	C_{max}	C_{last}	$AUC_{0-\infty}$	*V*/*F*	CL/*F*	$AUMC_{0-\infty}$	$MRT_{0-\infty}$
HPT		0.7415 (+)		0.6132 (+)	0.6256 (+)	0.4564 (+)			0.7444 (+)	0.7004 (+)
ASTo										0.4395 (+)
Bild		0.4746 (+)								
INRo	0.6510 (−)	0.4214 (+)						0.4490 (−)		
INRd	0.5639 (−)					0.4555 (+)				
RACE			0.016	0.0402		0.0711	0.0513	0.1023		
PAN				0.0066	0.0112	0.0939			0.0629	0.0868
T-tube						0.0841				
CYP2C19				0.0136	0.0352				0.0131	0.0154

[a] HPT, hours posttransplantation; ASTo, baseline AST; Bild, total bilirubin on day of study; INRo, baseline international normalized ratio; INRd, international normalized ratio on day of study; PAN, pantoprazole; T-tube, T-tube present or absent at time of study; *CYP2C19*, heterozygous extensive metabolizers (*CYP2C19*1/*2*).
[b] For HPT, ASTo, Bild, INRo, and INRd, r^2 for linear regression between the two variables is shown, and symbols in parentheses indicate a positive (+) or negative (−) association. For RACE, PAN, T-tube, and *CYP2C19*, the *P* value is shown.

tion of metabolism (28, 29, 30), especially in patients with decreased liver function. Voriconazole metabolism may be saturated in some patients. Finally, genetic polymorphism of *CYP2C19* (encoding the major metabolizing enzyme for voriconazole) among patients can result in interindividual variability in metabolism (28, 29, 30, 13, 20, 46).

Potential drug-drug interactions may also contribute to the large interindividual variability of voriconazole exposure. Transplant patients simultaneously receive many therapeutic agents for treatment and prophylaxis. *In vitro* studies have shown that voriconazole has the greatest affinity for CYP2C19, lower affinity for CYP2C9, and limited affinity for CYP3A4 (28, 29, 30). Inhibitors and/or inducers of these enzymes may change the pharmacokinetics of voriconazole (28, 29, 30), but in this study, patients were not receiving any drugs known to be inhibitors or inducers of voriconazole metabolism.

It is important to identify patient factors that significantly contribute to this large inter- and intraindividual variability by exploring the correlations between pharmacokinetic parameters (especially drug exposure) and patient variables. This is particularly true for voriconazole, because simple efficacy measures for molds, to which the patient dose can be titrated, are not yet available. So far there have been only animal model data for *Candida* showing a predictive pharmacodynamic parameter (AUC/MIC) as a potential target value (1), with no equivalent data for molds. However, there is a simple HPLC/UV assay available to monitor voriconazole levels. In this study we have observed that patients with higher total bilirubin, international normalized ratio (INR), and aspartate transaminase (AST), which are indicative of hepatic dysfunction and hepatocellular injury, had higher voriconazole exposure, characterized by a lower λ_z (elimination rate constant), higher half-life, higher $AUC_{0-\infty}$, and lower CL/*F*. We have also identified a positive association between the hours posttransplantation and voriconazole exposure, characterized by an increased half-life, C_{max}, C_{last}, $AUC_{0-\infty}$, $AUMC_{0-\infty}$ and $MRT_{0-\infty}$. This suggested an increase in voriconazole exposure with increased time posttransplantation.

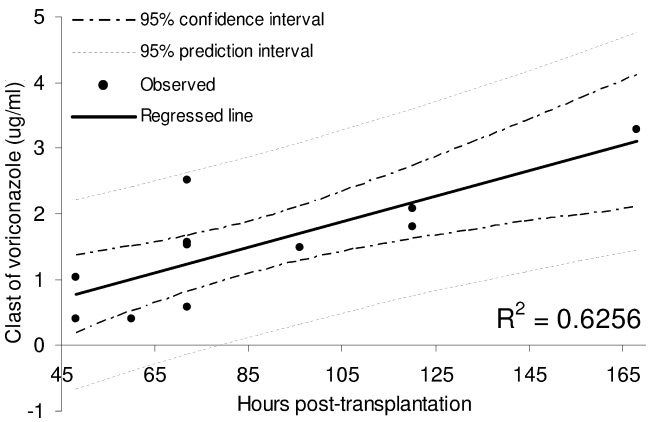

FIG. 3. Correlation between time (hours) posttransplantation and trough plasma concentrations of voriconazole for all 11 patients who had typical profiles.

The reason why poor (good) hepatic function is associated with a low (high) elimination rate constant, long (short) half-life, high (low) $AUC_{0-\infty}$, and low (high) CL/*F* of voriconazole is very likely that voriconazole is extensively metabolized in the liver, with less than 2% of the administered dose excreted unchanged in urine and feces (28, 29, 30, 36).

Voriconazole is a low- to intermediate-clearance drug. Voriconazole clearance is highly variable in different studies, ranging from 15 to 35.25 liters/h (14, 17, 28, 29, 30, 33, 35, 42), and oral clearance of voriconazole varies from 8.1 to 23.4 liters/h (16, 32, 34). Therefore, the hepatic extraction ratio should range from 0.09 to 0.39. For a low-clearance drug $CL_{apparent} \approx$ fraction unbound × intrinsic clearance. The fraction unbound depends on the liver function of the patient. Therefore, patients with higher total bilirubin, INR, and AST, indicative of hepatic dysfunction and hepatocellular injury, had higher voriconazole exposure and a lower CL/*F*.

The reason for the positive association between the time posttransplantation and voriconazole exposure as characterized by increased C_{max}, C_{last}, and $AUC_{0-\infty}$ is likely to be an increased bioavailability over time after transplantation. It has been observed in clinical settings that gastrointestinal motility is decreased immediately after transplantation surgery and is recovered gradually. Although the hepatic surgical damage is recovered and hepatic function is improved after transplantation, which could lead to increased metabolism and decreased exposure of voriconazole, recovery in gastrointestinal motility after the transplantation surgery is likely to contribute to a greater

extent, resulting in increased exposure to voriconazole (C_{max} and C_{last}). In addition, increased bile production and secretion may also contribute to an increased exposure to voriconazole.

A decreased unbound fraction in the blood caused by increased plasma protein synthesis with recovered hepatic function is not very likely to be responsible for the positive association between the time posttransplantation and voriconazole exposure because voriconazole is not very extensively bound to plasma proteins in blood, with an unbound fraction of less than 0.42 (28, 29, 30).

The presence of *CYP2C19*2* alleles resulted in a higher C_{max}, C_{last}, $AUMC_{0-\infty}$, and $MRT_{0-\infty}$. This observation in this study is in accordance with recently published data obtained with healthy volunteers (13, 20, 46). It has been reported that voriconazole exposure (AUC) is increased by 4-fold in poor metabolizers compared to homozygous extensive metabolizers. Nearly 15 to 20% of Asians and 3 to 5% of Caucasians are poor metabolizers (28, 29, 30). There is also an average 2-fold increase in exposure to voriconazole in heterozygous versus homozygous extensive metabolizers (28, 29, 30). The presence of deficient activity of *CYP2C19*2* alleles resulted in higher C_{max}, $AUMC_{0-\infty}$, and $MRT_{0-\infty}$. However, the *CYP2C19* genetic analysis in this study did not include the newly identified excessive allele *CYP2C19*17* (ultrarapid metabolizer) (46) and included only the deficient alleles *CYP2C19*2* and *CYP2C19*3*, which account for more than 85% of defective *CYP2C19* alleles in Caucasians (5). Therefore, the existence of excessive alleles and other defective alleles, and thus misclassification of patients, cannot be ruled out.

The possible effect of race on the pharmacokinetics of voriconazole observed in this study has never been reported before. It seems that Asian patients have a slower absorption process than Caucasian patients, characterized by a higher T_{max} and lower C_{max}, but this remains to be further investigated.

In addition, the possible effect of coadministered pantoprazole on the exposure of voriconazole might be due to decreased absorption of voriconazole caused by proton pump inhibition, since it has been reported that pantoprazole causes no apparent induction or inhibition of cytochrome P450 enzyme systems (28, 29, 30). Pantoprazole sodium is a proton pump inhibitor (PPI) that covalently binds to the (H^+,K^+)-ATPase enzyme system at the secretory surface of the gastric parietal cell. This action suppresses the final step in gastric acid production and leads to inhibition of both basal and stimulated acid secretion. Pantoprazole produces extensive and long-lasting inhibition of gastric acid secretion. PPI agents may reduce absorption of azoles by increasing gastric pH. However, this explanation is also questionable, because significant decrease in voriconazole exposure due to decreased absorption caused by proton pump inhibition has never been reported. In contrast, the PPI agent omeprazole has been reported to cause an increase in voriconazole exposure due to inhibition of metabolizing enzyme (28, 29, 30, 48). Therefore, further investigation is required to make any conclusion about the effect of coadministered pantoprazole on the exposure of voriconazole.

Donor characteristics have been shown to have no effect on voriconazole pharmacokinetics in this study. If this observation is unbiased, the current voriconazole dosing regimen for liver transplant patients, without consideration of donor characteristics, should be an adequate dosing strategy. However, it is important to point out that exclusion of a factor does not necessarily mean that this covariate has no significant influence on the pharmacokinetic parameters, especially in this study with a small homogeneous group of patients in the immediate posttransplant period. There can be many reasons for exclusion of donor characteristics as a significant factor for voriconazole pharmacokinetics in this study. First, some of the donor characteristics are not variable in the population studied. Second, simple linear regression is not an adequate model to assess the correlation between donor characteristics and voriconazole pharmacokinetics. Third, some of the donor characteristics may have significant effects on voriconazole pharmacokinetics only when their values are above (or below) a certain threshold value. If the values of these donor characteristics in this study were all below (or above) this threshold value, these donor characteristics would be excluded as a significant factor influencing voriconazole pharmacokinetics, no matter how variable this covariate is. Finally, some of the donor characteristics may have significant effects on voriconazole pharmacokinetics only when evaluated with interaction and coeffects with other patient/donor factors together. When evaluated alone without interaction with other factors, a significant factor could be identified as insignificant, which is a limitation of this study that will be discussed below. Therefore, further investigation of the effects of donor characteristics on voriconazole pharmacokinetics is required.

A large variability in voriconazole exposure following a fixed dosing regimen necessitates individualizing voriconazole dosing to maximize therapeutic efficacy and minimize toxicity in liver transplant patients, especially considering that 33.3% of the patients in this study had a trough level below 1 μg/ml. There is no simple efficacy measure to which the patient dose can be titrated, but there is a simple HPLC/UV assay available to monitor voriconazole levels. Therapeutic monitoring may be important in optimizing therapy with this drug, which has been proposed (2, 4, 6, 44) and is currently performed in the routine clinical monitoring program at our institution, with a target trough concentration of 1 μg/ml to 6 μg/ml. However, trough plasma concentrations have never been documented as surrogate markers of voriconazole exposure in liver transplant recipients. The good correlation ($r^2 = 0.85$) observed in this study between the trough voriconazole plasma concentrations and the corresponding $AUC_{0-\infty}$ (Fig. 2 [$n = 10$]) indicates that trough voriconazole concentration is a good measure of voriconazole exposure (AUC) in patients.

These findings are likely to be clinically relevant because they suggest that the voriconazole dose should be relatively high immediately after transplantation, especially in patients with good liver function as measured by low AST, total bilirubin, or INR, in order to avoid ineffectiveness of prophylaxis/treatment and its consequences (fungal infections, especially invasive aspergillosis). It has been reported that a low voriconazole exposure of <0.25 or <1 μg/ml is associated with a poor outcome in patients with aspergillosis (4, 8, 22, 31, 38, 40) and in ultimately death of the patients. The voriconazole dose should be then gradually reduced, especially in patients with poor liver function as defined by high AST, total bilirubin, or INR, in order to avoid toxicity caused by high voriconazole exposure. It has been reported that high voriconazole plasma concentrations (>5.5 μg/ml) are correlated with an increased risk for toxicity,

including elevated transaminase levels, CNS disorders (e.g., encephalopathy), and electrolyte disturbances (2, 38, 41).

In conclusion, this study has demonstrated that there is a large interindividual variability in the pharmacokinetics of voriconazole in liver transplant patients. A fixed dosing regimen leads to widely variable exposure of voriconazole in liver transplant patients. Donor characteristics seem to have no significant influence on voriconazole pharmacokinetics, but further investigation is required due to the small number of subjects evaluated in this study. Postoperative time and poor liver function are positively associated with voriconazole exposure and half-life, which may be useful for dosage adjustment. CL/F and V_{ss}/F are not correlated with body weight, which does not support a weight-based dosing strategy. The trough concentration is a good measure of voriconazole exposure ($AUC_{0-\infty}$) and should be used in practice to individualize voriconazole dosage.

A fixed dosing regimen is not optimal for voriconazole therapy for prophylaxis and treatment in liver transplant patients. This evaluation will allow for an assessment of the adequacy of the prophylactic regimen in achieving therapeutic drug concentrations in all subjects and could potentially help identify patients at risk for extremes in voriconazole exposure.

ACKNOWLEDGMENTS

This work was supported by the Clinical Pharmacokinetics Laboratory at the University of Pittsburgh School of Pharmacy and by funds received from the Thomas E. Starzl Transplant Institute Young Investigator Award to Blair Capitano. Additional support was provided by funds received from NIH/NCRR/GCRC grant MO1-RR000056.

We acknowledge Shimin Zhang and Diana Pakstis for technical assistance.

REFERENCES

1. **Andes, D., K. Marchillo, et al.** 2003. In vivo pharmacokinetics and pharmacodynamics of a new triazole, voriconazole, in a murine candidiasis model. Antimicrob. Agents Chemother. **47:**3165–3169.
2. **Boyd, A. E., S. Modi, et al.** 2004. Adverse reactions to voriconazole. Clin. Infect. Dis. **39:**1241–1244.
3. **Capitano, B., B. Potoski, et al.** 2004. Pharmacokinetics of voriconazole in lung transplant patients, abstr. A-39. Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
4. **Denning, D. W., P. Ribaud, et al.** 2002. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. Clin. Infect. Dis. **34:**563–571.
5. **Desta, Z., X. Zhao, et al.** 2002. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin. Pharmacokinet. **41:**913–958.
6. **FDA.** 2001. Briefing document for voriconazole (oral and intravenous formulations). Antiviral Drugs Advisory Committee, FDA, Washington, DC.
7. **Fishman, J. A.** 2007. Infection in solid-organ transplant recipients. N Engl. J. Med. **357:**2601–2614.
8. **Freifeld, A., S. Arnold, et al.** 2007. Relationship of blood level and susceptibility in voriconazole treatment of histoplasmosis. Antimicrob. Agents Chemother. **51:**2656–2657.
9. **Gage, R., and D. A. Stopher.** 1998. A rapid HPLC assay for voriconazole in human plasma. J. Pharm. Biomed Anal. **17:**1449–1453.
10. **Geist, M. J., G. Egerer, et al.** 2007. Induction of voriconazole metabolism by rifampin in a patient with acute myeloid leukemia: importance of interdisciplinary communication to prevent treatment errors with complex medications. Antimicrob. Agents Chemother. **51:**3455–3456.
11. **George, D., P. Miniter, et al.** 1996. Efficacy of UK-109496, a new azole antifungal agent, in an experimental model of invasive aspergillosis. Antimicrob. Agents Chemother. **40:**86–91.
12. **Herbrecht, R., Y. Nivoix, et al.** 2005. Management of systemic fungal infections: alternatives to itraconazole. J. Antimicrob. Chemother. **56**(Suppl. 1)**:**i39–i48.
13. **Ikeda, Y., K. Umemura, et al.** 2004. Pharmacokinetics of voriconazole and cytochrome P450 2C19 genetic status. Clin. Pharmacol. Ther. **75:**587–588.
14. **Jeu, L., F. J. Piacenti, et al.** 2003. Voriconazole. Clin. Ther. **25:**1321–1381.
15. **Johnson, L. B., and C. A. Kauffman.** 2003. Voriconazole: a new triazole antifungal agent. Clin. Infect. Dis. **36:**630–637.
16. **Lazarus, H. M., J. L. Blumer, et al.** 2002. Safety and pharmacokinetics of oral voriconazole in patients at risk of fungal infection: a dose escalation study. J. Clin. Pharmacol. **42:**395–402.
17. **Leveque, D., Y. Nivoix, et al.** 2006. Clinical pharmacokinetics of voriconazole. Int. J. Antimicrob. Agents **27:**274–284.
18. **Lin, S. J., J. Schranz, et al.** 2001. Aspergillosis case-fatality rate: systematic review of the literature. Clin. Infect. Dis. **32:**358–366.
19. **Lipp, H. P.** 2008. Antifungal agents—clinical pharmacokinetics and drug interactions. Mycoses **51**(Suppl. 1)**:**7–18.
20. **Mikus, G., V. Schowel, et al.** 2006. Potent cytochrome P450 2C19 genotype-related interaction between voriconazole and the cytochrome P450 3A4 inhibitor ritonavir. Clin. Pharmacol. Ther. **80:**126–135.
21. **Panackal, A. A., A. Dahlman, et al.** 2003. Outbreak of invasive aspergillosis among renal transplant recipients. Transplantation **75:**1050–1053.
22. **Pascual, A., T. Calandra, et al.** 2008. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. Clin. Infect. Dis. **46:**201–211.
23. **Pascual, A., V. Nieth, et al.** 2007. Variability of voriconazole plasma levels measured by new high-performance liquid chromatography and bioassay methods. Antimicrob. Agents Chemother. **51:**137–143.
24. **Patterson, T. F.** 1999. Role of newer azoles in surgical patients. J. Chemother. **11:**504–512.
25. **Pearson, M. M., P. D. Rogers, et al.** 2003. Voriconazole: a new triazole antifungal agent. Ann. Pharmacother. **37:**420–432.
26. **Pennick, G. J., M. Clark, et al.** 2003. Development and validation of a high-performance liquid chromatography assay for voriconazole. Antimicrob. Agents Chemother. **47:**2348–2350.
27. **Perea, S., G. J. Pennick, et al.** 2000. Comparison of high-performance liquid chromatographic and microbiological methods for determination of voriconazole levels in plasma. Antimicrob. Agents Chemother. **44:**1209–1213.
28. **Pfizer.** 2007. Product information for Protonix delayed-release oral tablets, suspension. Pfizer, New York, NY.
29. **Pfizer.** 2008. VFend IV (voriconazole) for injection, VFend tablets, VFend for oral suspension. Pfizer, New York, NY.
30. **Pfizer.** 2005. Label: voriconazole for injection, tablets, oral suspension: LAB-0271-12. Pfizer, New York, NY.
31. **Potoski, B. A., and J. Brown.** 2002. The safety of voriconazole. Clin. Infect. Dis. **35:**1273–1275.
32. **Purkins, L., N. Wood, et al.** 2002. Pharmacokinetics and safety of voriconazole following intravenous- to oral-dose escalation regimens. Antimicrob. Agents Chemother. **46:**2546–2553.
33. **Purkins, L., N. Wood, et al.** 2003. Voriconazole, a novel wide-spectrum triazole: oral pharmacokinetics and safety. Br. J. Clin. Pharmacol. **56**(Suppl. 1)**:**10–16.
34. **Rengelshausen, J., M. Banfield, et al.** 2005. Opposite effects of short-term and long-term St John's wort intake on voriconazole pharmacokinetics. Clin. Pharmacol. Ther. **78:**25–33.
35. **Robatel, C., M. Rusca, et al.** 2004. Disposition of voriconazole during continuous veno-venous haemodiafiltration (CVVHDF) in a single patient. J. Antimicrob. Chemother. **54:**269–270.
36. **Roffey, S. J., S. Cole, et al.** 2003. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab. Dispos. **31:**731–741.
37. **Sanati, H., P. Belanger, et al.** 1997. A new triazole, voriconazole (UK-109,496), blocks sterol biosynthesis in Candida albicans and Candida krusei. Antimicrob. Agents Chemother. **41:**2492–2496.
38. **Scott, L. J., and D. Simpson.** 2007. Voriconazole: a review of its use in the management of invasive fungal infections. Drugs **67:**269–298.
39. **Sheehan, D. J., C. A. Hitchcock, et al.** 1999. Current and emerging azole antifungal agents. Clin. Microbiol. Rev. **12:**40–79.
40. **Smith, J., N. Safdar, et al.** 2006. Voriconazole therapeutic drug monitoring. Antimicrob. Agents Chemother. **50:**1570–1572.
41. **Tan, K., N. Brayshaw, et al.** 2006. Investigation of the potential relationships between plasma voriconazole concentrations and visual adverse events or liver function test abnormalities. J. Clin. Pharmacol. **46:**235–243.
42. **Theuretzbacher, U., F. Ihle, et al.** 2006. Pharmacokinetic/pharmacodynamic profile of voriconazole. Clin. Pharmacokinet. **45:**649–663.
43. **Tintillier, M., L. Kirch, et al.** 2005. Interaction between voriconazole and tacrolimus in a kidney-transplanted patient. Nephrol. Dial. Transplant. **20:**664–665.
44. **Touw, D. J., C. Neef, et al.** 2005. Cost-effectiveness of therapeutic drug monitoring: a systematic review. Ther. Drug Monit. **27:**10–17.
45. **Trifilio, S., G. Pennick, et al.** 2007. Monitoring plasma voriconazole levels may be necessary to avoid subtherapeutic levels in hematopoietic stem cell transplant recipients. Cancer **109:**1532–1535.
46. **Wang, G., H. P. Lei, et al.** 2009. The CYP2C19 ultra-rapid metabolizer genotype influences the pharmacokinetics of voriconazole in healthy male volunteers. Eur. J. Clin. Pharmacol. **65:**281–285.
47. **Weiler, S., H. Zoller, et al.** 2007. Altered pharmacokinetics of voriconazole in a patient with liver cirrhosis. Antimicrob. Agents Chemother. **51:**3459–3460.
48. **Wood, N., K. Tan, et al.** 2003. Effect of omeprazole on the steady-state pharmacokinetics of voriconazole. Br. J. Clin. Pharmacol. **56**(Suppl. 1)**:**56–61.