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Saturated Metabolism of Voriconazole *N*-Oxidation Resulting in Nonlinearity of Pharmacokinetics of Voriconazole at Clinical Doses

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Metabolic saturation of voriconazole based on the trough plasma concentrations of voriconazole and its major metabolite *N*-oxide were evaluated according to CYP2C19 genotypes in 58 Japanese patients receiving voriconazole (median dose; 200 mg twice daily) for prophylaxis or treatment. Predose trough plasma concentrations of voriconazole and *N*-oxide were monitored on day 5d or later after initiation of voriconazole treatment. Large interindividual variations in trough plasma concentrations of voriconazole and *N*-oxide were observed. Dose-normalized trough plasma concentrations of voriconazole were strongly correlated with its absolute trough concentrations, and the straight regression line between them intersected close to the origin of the coordinates. No significant correlation was observed between the trough plasma concentrations of voriconazole and *N*-oxide. The inverse value of the metabolic ratio of *N*-oxide to voriconazole was strongly correlated with the absolute trough voriconazole concentrations. No significant differences in the trough plasma concentrations of voriconazole and *N*-oxide or the metabolic ratio of *N*-oxide to voriconazole between the CYP2C19 genotypes were observed. Saturated metabolism of voriconazole *N*-oxidation rather than CYP2C19 genotypes contributed to the nonlinear pharmacokinetics. The metabolic process converting voriconazole to *N*-oxide was saturated at the clinical dose.

Key words voriconazole; metabolite; nonlinear pharmacokinetics; *N*-oxidation; CYP2C19

Voriconazole is a second-generation triazole antifungal agent. Compared with other azole antifungal agents, it has potent activity against a broader spectrum of clinically significant fungal pathogens, including *Aspergillus*, *Candida*, and some unusual organisms.¹⁾ In clinical settings, voriconazole is initially administered for the treatment of invasive pulmonary aspergillosis and empirical antifungal therapy in patients with persistent fever and neutropenia and in non-neutropenic patients.^{2,3)}

Therapeutic drug monitoring is recommended for voriconazole in order to ensure its safety and efficacy. An association between successful therapeutic outcome in fungal infections and plasma voriconazole concentrations has been reported.⁴⁾ The lowest effective concentration on trough of voriconazole was reported as 1–2 µg/mL.^{4–8)} In contrast, a higher plasma concentration on trough of voriconazole was associated with the incidence of adverse effects, such as ocular, neurological, or hepatic toxicity. These adverse effects were observed in patients whose plasma concentration on trough of voriconazole exceeded 4 µg/mL.^{4,7,9,10)} However, there is large interindividual variability in the plasma concentration on trough of voriconazole. The prediction of voriconazole concentration on trough remains extremely difficult in clinical settings.

Voriconazole is available as intravenous and tablet formulations in Japan. The standard maintenance doses of voriconazole are 3 or 4 mg/kg for injection and 150, 200 or 300 mg twice daily for oral administration. Voriconazole exhibits a nonlinear pharmacokinetic profile at the clinical dose.¹¹⁾ Voriconazole is metabolized mainly in the liver to the major metabolite voriconazole *N*-oxide (*N*-oxide). *N*-Oxide accounts for 72% of all circulating metabolites in the plasma.¹²⁾ Although voriconazole *N*-oxide has minimal antifungal activity compared with voriconazole, it has been found to inhibit the

metabolic activity of CYP3A4 and CYP2C19 *in vitro*.¹²⁾ Because voriconazole is mainly metabolized *via* CYP2C19 or CYP3A4, the *N*-oxide may have an effect on the metabolic profile of voriconazole. The determination of *N*-oxide together with voriconazole can be useful in evaluating the metabolic process of voriconazole.

Several studies investigated factors related to the nonlinear pharmacokinetics of voriconazole.^{11,13)} The saturation of metabolic clearance is believed to cause nonlinear pharmacokinetics because voriconazole is eliminated predominantly by metabolism. However, there are no clinical reports on the relationship of voriconazole and its major *N*-oxide metabolite that take into consideration CYP2C19 gene variants. As previously described, CYP molecular species participate in the metabolism of voriconazole and CYP2C19 plays the most dominant role. The genetic variants of CYP2C19 exhibited a large interindividual variation in voriconazole exposure.^{14–16)} The genetic variants of CYP2C19 may affect the nonlinear pharmacokinetics of voriconazole in the metabolic process. The relationship between the metabolic process of voriconazole to *N*-oxide and genetic variants of CYP2C19 has not been fully investigated in clinical settings.

There is a growing interest in the monitoring of plasma concentrations on trough of voriconazole in patients immunocompromised or infected with severe fungi. The aim of this study was to evaluate the metabolic saturation of voriconazole based on the plasma concentrations on trough of voriconazole and its *N*-oxide. The influence of CYP2C19 genetic variants on metabolic saturation was also investigated.

MATERIALS AND METHODS

Ethics The present study was performed in accordance

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with the Declaration of Helsinki and its amendments. The Ethics Committee of Hamamatsu University Hospital approved the protocol. Each patient obtained information about the scientific aim of the study and provided written informed consent.

Study Population and Schedule The present study was an observation study (UMIN-CTR UMIN000011499) conducted at a single site (Hamamatsu University Hospital) in Japanese patients who orally or intravenously received voriconazole (Vfend®, Pfizer Japan Inc., Tokyo, Japan) treatment. Exclusion criteria were as follows: patients (1) on hemodialysis or peritoneal dialysis; (2) who had hepatopathy (total bilirubin >2.0 mg/dL); (3) were being co-treated with rifampicin, ritonavir, carbamazepine, and/or a long-acting barbiturate; and (4) with poor compliance with respect to their medications. Blood was sampled just before dosing on the 5th day after initiation of therapy or later. Clinical laboratory values such as total protein, serum albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase, blood urea nitrogen, serum creatinine, C-reactive protein, and white blood cell count were obtained from routine laboratory tests just before starting the administration. The estimated glomerular filtration rate was calculated using the following formula; estimated glomerular filtration rate = $194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287} \times 0.739$ (if female).¹⁷⁾

Determination of Voriconazole and *N*-Oxide Voriconazole and *N*-oxide were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). Disodium hydrogenphosphate, papaverine hydrochloride as an internal standard, HPLC-grade acetonitrile, and methanol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were commercially available. Voriconazole and *N*-oxide concentrations on trough in plasma were determined using a previously validated HPLC-UV method.¹⁸⁾ The concentrations of both voriconazole and *N*-oxide were linear ($r > 0.999$) over the concentration range of 0.1–8.0 $\mu\text{g/mL}$. The lower limit of quantitation was 0.1 $\mu\text{g/mL}$ for both voriconazole and *N*-oxide. Small intra- and inter-assay coefficients of variation with respect to the precision of voriconazole and *N*-oxide determination were obtained (<12.6%). Likewise, similar intra- and inter-assay coefficients of variation with respect to the accuracy of their determination were obtained (96.2–112.9%).

Genotyping Procedures for CYP2C19 DNA was extracted from peripheral venous whole blood with a DNA Extractor WB Kit (Wako Pure Chemical Industries, Ltd.). CYP2C19 genetic variants (G636A and G681A on exon 4 and exon 5) were determined using polymerase chain reaction-restriction fragment length polymorphism amplification techniques previously described by Tanigawara *et al.*,¹⁹⁾ with some modification. *CYP2C19*1* as the wild type was identified if no *2 or *3 alleles were detected. To determine genotypes of CYP2C19, patients were classified into the following three groups: extensive metabolizer (EM), *1/*1; intermediate metabolizer (IM), *1/*2 and *1/*3; and poor metabolizer (PM), *2/*2, *2/*3 and *3/*3. Although the impact of the *CYP2C19*17* allele as enzymatic activator on the pharmacokinetics of voriconazole is controversial, we did not analyze that allele because of its low frequency in Japanese.²⁰⁾ The genetic analysis was not performed in patients with insufficient blood cells and who were undergoing chemotherapy or

bone marrow transplantation. Any missing data were deleted in a pairwise manner.

Pharmacokinetic Analyses The correlations of plasma concentrations on trough of voriconazole and its *N*-oxide and the metabolic ratio of *N*-oxide to voriconazole according to voriconazole dose were evaluated. The association between the dose-normalized and absolute concentration on trough of voriconazole was regarded as an indicator for the linearity of voriconazole pharmacokinetics. The correlation between the plasma concentration on trough of voriconazole and its *N*-oxide was considered as an indicator of the metabolic process of voriconazole. The correlation between the voriconazole concentration on trough and the inverse value of the metabolic ratio of *N*-oxide to voriconazole was evaluated in order to determine if the metabolic process to *N*-oxide was saturated. The influences of CYP2C19 genetic variants on the dose-normalized concentrations on trough of voriconazole and its *N*-oxide and the metabolic ratio were analyzed. The regression lines obtained in the above correlations were compared between the EM, IM, and PM groups to assess the impact of CYP2C19 genetic variants on the pharmacokinetics of voriconazole.

Statistical Analyses Data are presented as the median and interquartile range (IQR). All statistical analyses were performed using SPSS 15.0J (SPSS Japan Inc., Tokyo, Japan). $p < 0.05$ was considered to indicate statistical significance. Pearson's correlation coefficient test was subsequently carried out to test for overall heterogeneity within individual regions. The influences of CYP2C19 genetic variants on the concentrations on trough and metabolic ratio were analyzed using the two-sided Mann–Whitney *U* test. Pearson's correlation coefficient test and simple linear regression were used to assess the relations between the CYP2C19 EM, IM, and PM groups. The significance of the relationship between *x* and *y* was decided by testing the null hypothesis that the coefficient/intercept was zero.

Differences in the correlation coefficients of the regression lines obtained from each group of CYP2C19 were determined by testing the *t*-value.

Stepwise multiple linear regression analysis ($p < 0.05$ to enter and $p > 0.10$ to remove) was performed to assess the dependence between the dose-normalized concentration on trough of voriconazole or the inverse value of the metabolic ratio of *N*-oxide to voriconazole and 13 predictor values (gender (man: 0 and woman: 1), age, total protein, serum albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase, blood urea nitrogen, estimated glomerular filtration rate, and genotypes of the CYP2C19 (EM group: (0, 0), IM group: (0, 1), PM group: (1, 1)), voriconazole concentration on trough). Standardized partial regression coefficient (β) and variance inflation factor (VIF) were calculated.

RESULTS

Patient Characteristics A total of 58 Japanese patients receiving voriconazole (median and IQR of dose, 200 mg and 150–200 mg twice daily) were enrolled in the study. Underlying diseases were pulmonary aspergillosis ($n=25$), acute myelocytic leukemia ($n=10$), possible fungus infection ($n=7$), lymphoma malignum ($n=6$), candida esophagitis ($n=3$), and 6

other diseases ($n=7$). Table 1 shows the demographic characteristics of the patients in this study population.

Plasma Concentrations on Trough of Voriconazole and *N*-Oxide A large interindividual variation was observed in the voriconazole concentration on trough (median and IQR, 2.49 and 1.49–5.38 $\mu\text{g/mL}$), *N*-oxide concentration on trough (median and IQR, 1.58 and 1.01–2.00 $\mu\text{g/mL}$), and the metabolic ratio (median and IQR, 0.579 and 0.320–1.06). No correlations were found between the dose adjusted with body weight and plasma concentrations on trough of voriconazole and *N*-oxide and the metabolic ratio (Fig. 1).

Linearity of Voriconazole Pharmacokinetics The dose-

normalized concentration on trough of voriconazole had a strong correlation with its absolute concentration on trough ($r^2=0.90$, $p<0.001$). A straight line passing through close to the origin of the coordinates was obtained as the regression line between the dose-normalized and absolute concentration on trough of voriconazole (Fig. 2). The correlation between the plasma concentrations on trough of voriconazole and its *N*-oxide was not significant ($r^2=0.030$, $p=0.194$) (Fig. 3(A)). When data for the relationship between the voriconazole concentration on trough and inverse value of the metabolic ratio of *N*-oxide to voriconazole was fitted by linear approximation, the regression formula obtained was $y=0.675x+0.096$ ($r^2=0.690$, $p<0.001$) (Fig. 3(B)).

Influence of CYP2C19 Genetic Variants The numbers of patients with *CYP2C19**1/*1, *1/*2, *1/*3, and *2/*3 were 16 (34.0%), 12 (25.5%), 13 (27.7%), and 6 (12.8%), respectively. No patients had *CYP2C19**2/*2 and *3/*3. No significant difference was observed in the dose-normalized concentrations on trough of voriconazole and *N*-oxide, or the metabolic ratio between the CYP2C19 genotypes (Table 2). The formulas of the regression line between the dose-normalized and absolute plasma concentrations on trough of voriconazole in the CYP2C19 EM, IM, and PM groups were $y=0.260x+0.040$, $y=0.277x+0.064$, and $y=0.246x+0.275$, respectively. Strong positive correlations were found in the CYP2C19 EM group ($r^2=0.893$, $p<0.01$), IM group ($r^2=0.922$, $p<0.01$), and PM group ($r^2=0.638$, $p=0.035$). Slight differences in the regression coefficients of the regression line were observed between groups of CYP2C19 (EM vs. IM: $t=0.502$, $p=0.619$; EM vs. PM: $t=0.231$, $p=0.820$; and IM vs. PM: $t=0.536$, $p=0.597$). Strong positive correlations for the formula of the regression

Table 1. Patient Demographics

Number of patients [male/female]	58, 40/18
Dose (mg twice daily)	200 (150–200)
Age (years)	70 (64–75)
Body weight (kg)	52.0 (44.0–58.6)
Total protein (g/dL)	6.2 (5.1–6.8)
Serum albumin (g/dL)	3.0 (2.6–3.4)
Total bilirubin (mg/dL)	0.6 (0.5–0.8)
Aspartate aminotransferase (IU/L)	25 (18–37)
Alanine aminotransferase (IU/L)	20 (14–41)
γ -Glutamyl transpeptidase (IU/L)	79 (35–155)
Blood urea nitrogen (mg/dL)	16.0 (12.7–22.5)
Estimated glomerular filtration rate (mL/min/1.73 m ²)	72.5 (53.3–91.0)
Serum creatinine (mg/dL)	0.74 (0.57–1.00)
C-Reactive protein (mg/dL)	2.06 (0.605–5.01)
White blood cell (counts/ μL)	5900 (3400–8140)

Data are median and IQR unless otherwise stated.

Fig. 1. Independencies of Plasma Concentrations on Trough of Voriconazole (A), *N*-Oxide (B), and the Metabolic Ratio of *N*-Oxide to Voriconazole (C) According to Dose of Voriconazole ($n=58$)

line between the inverse value of the metabolic ratio of *N*-oxide to voriconazole and absolute plasma concentrations on trough of voriconazole were found in the CYP2C19 groups (Fig. 4). Significant differences in the regression coefficients

of the regression line were observed between the CYP2C19 groups (EM vs. IM: $t=5.56$, $p<0.01$; EM vs. PM: $t=7.24$, $p<0.01$; IM vs. PM: $t=2.33$, $p=0.027$).

Stepwise Multiple Regression Analysis for Dose-Normalized Concentration on Trough of Voriconazole and the Inverse Value of the Metabolic Ratio of *N*-Oxide to Voriconazole Voriconazole concentration on trough and estimated glomerular filtration rate were predictors accounting for the variability of dose-normalized concentration on trough of voriconazole ($p<0.01$). Their β values were 0.939 and -0.103 , respectively. The equation acquired with multiple regression analysis accounted for 91.6% of the interindividual variability in dose-normalized concentration on trough of voriconazole ($p=0.021$). The inverse value of the metabolic ratio of *N*-oxide to voriconazole was significantly correlated with voriconazole concentration on trough and CYP2C19 genotypes with two variants in multiple regression analysis ($\beta=0.847$, $p<0.001$ and $\beta=0.215$, $p=0.011$). The acquired equation accounted for 70.2% of the interindividual variability in the inverse value of the metabolic ratio of *N*-oxide to voriconazole ($p<0.01$) (Table 3).

DISCUSSION

The current clinical study has demonstrated that the nonlinear pharmacokinetics of voriconazole arise most likely during the metabolic process. The dose-normalized concentration on trough of voriconazole had a strong correlation with its absolute concentration on trough. The inverse value of the metabolic ratio of *N*-oxide to voriconazole was strongly correlated with the voriconazole concentration on trough. In addition,

Fig. 2. Correlation between Absolute and Dose-Normalized Plasma Concentration on Trough of Voriconazole ($n=58$)

Fig. 3. Relationship between Voriconazole Concentration on Trough and Its *N*-Oxide Concentration on Trough (A) and between Voriconazole Concentration on Trough and the Inverse Value of the Metabolic Ratio of *N*-Oxide to Voriconazole (B) ($n=58$)

Table 2. Influence of CYP2C19 Genetic Variants on Voriconazole Concentration, *N*-Oxide Concentration, and Metabolic Ratio of the *N*-Oxide to Voriconazole

CYP2C19 genotypes	EM (n=16)	IM (n=25)	PM (n=6)	<i>p</i>
Voriconazole concentration ($\mu\text{g/mL}$ per mg/kg)	0.51 (0.34–1.05)	0.85 (0.57–1.70)	0.78 (0.43–1.29)	0.197
<i>N</i> -Oxide concentration ($\mu\text{g/mL}$ per mg/kg)	0.42 (0.37–0.56)	0.47 (0.33–0.60)	0.28 (0.25–0.31)	0.083
Metabolic ratio	0.79 (0.48–1.60)	0.57 (0.27–0.84)	0.40 (0.33–0.78)	0.174

Data are expressed as median and interquartile range, which is in parentheses. The differences in voriconazole concentration, *N*-oxide concentration, and metabolic ratio of the *N*-oxide to voriconazole between EM (*1/*1), IM (*1/*2 or *1/*3), and PM (*2/*3) of CYP2C19 genotypes were tested using the Kruskal–Wallis test. The statistical results are shown as a *p* value. EM, extensive metabolizer. IM, intermediate metabolizer. PM, poor metabolizer. *p*, probability significance.

CYP2C19 genotypes did not significantly affect the nonlinear pharmacokinetics of voriconazole. These results indicate that metabolic saturation rather than CYP2C19 genotype contributes to the nonlinear pharmacokinetics of voriconazole. To the best of our knowledge, this is the first clinical report to evaluate voriconazole metabolic saturation based on the plasma concentrations on trough of voriconazole and its *N*-oxide classified according to CYP2C19 genotype.

A large interindividual variation was observed in the concentrations on trough of voriconazole in this study. Myrianthefs *et al.* and Ueda *et al.* also reported that standard doses of voriconazole resulted in highly variable concentrations on trough (less than 0.1 to 11.4 $\mu\text{g/mL}$ and 0.22–12.77 $\mu\text{g/mL}$).^{5,21} In addition, they reported there was no correlation between the voriconazole concentration on trough and its dose. The large variability of voriconazole concentration on trough was not thought to be due to dosage in clinical settings. The bioavailability of voriconazole is approximately 100%.¹² Patients enrolled in the present study did not have either severe renal or severe liver dysfunction. Therefore, factors responsible for the nonlinear pharmacokinetics most likely exist in the metabolic process.

In theory, pharmacokinetics linearity can be determined by whether the fitted curve of the correlative relationship between the dose and blood concentration on trough of the drug is passing through the origin or not. The regression formula between dose and absolute drug concentration on trough usually has the intercept to some extent. How large of an intercept can be allowed for the determination of linearity is controversial. Meanwhile, nonlinear pharmacokinetics of phenytoin was explained using linearized transformation of the Michaelis–Menten equation.²² Michaelis–Menten equation can be converted to Hanes–Woelf equation, which was expressed as substrate concentration (*C*)/reaction velocity (*v*)=*C*/maximum reaction velocity (*V*_{max})+Michaelis constant (*K*_m)/*V*_{max}. In the steady state, *v* equals dose (*D*) per time (*t*). In this study, *D* per *t* instead of *v* was substitute for Hanes–Woelf equation and the equation expressed as $C/D = (1/V_{\max} \cdot t) \cdot C + K_m/V_{\max} \cdot t$ was obtained. The regression line between *C* and *C/D* draw linear (Fig. 2). The regression between dose-normalized drug concentration on trough and absolute drug concentration on trough is more practical method. This method enables the evaluation for linear pharmacokinetics visually, and resolves the problem mentioned above. For instance, a drug that has linear pharmacokinetics should exhibit a horizontal regression line in the correlation between its absolute and dose-normalized concentration on trough. Evaluation of dose–concentration relationship shown in Fig. 2 can lead to nonlinear pharmacokinetics of drugs which have narrow dose range and wide blood concentration. Minimum and maximum range of

voriconazole concentration was 0.02 to 7.89 $\mu\text{g/mL}$, and those of dose per body weight was 1.32 to 5.15 mg/kg. Thus, the range ratio of the dose per body weight to the voriconazole concentration are larger enough to consider.

In the present study, the dose-normalized plasma concentration on trough of voriconazole was strongly and positively correlated with its absolute concentration on trough in Fig. 2. Furthermore, the regression line between the absolute concentration on trough and the dose-normalized concentration on trough passes through the vicinity of the origin. As a result, voriconazole demonstrated a nonlinear pharmacokinetic profile in the clinical dose range. The nonlinear pharmacokinetics from the lower concentration of voriconazole implied the possibility of metabolic saturation or the impact of CYP2C19 genetic variants.

There was no significant correlation between the *N*-oxide concentration on trough and voriconazole concentration on trough. The concentration on trough of a drug with linear pharmacokinetics is positively correlated with the concentration on trough of its main metabolite. The inverse value of the metabolic ratio of *N*-oxide to voriconazole expresses voriconazole metabolic capacity. If the metabolic rate was constant and independent from the concentration on trough of voriconazole, a horizontal straight line indicating a constant value for x-axis should be drawn in the correlation between the voriconazole concentration on trough and the inverse value of the metabolic ratio of *N*-oxide to voriconazole. In the present study, the inverse value of the metabolic ratio of *N*-oxide to voriconazole was strongly correlated with the voriconazole concentration on trough. This correlation demonstrated that the metabolic saturation of voriconazole occurred mainly during metabolism to its *N*-oxide. The metabolic capacity of CYP2C19 for voriconazole seems to be insufficient for the clinical dosage. Roffey *et al.* and Purkins *et al.* reported the capacity-limited elimination kinetics of voriconazole.^{11,13} Because voriconazole is predominantly eliminated by metabolism, they attributed the nonlinearity pharmacokinetics of voriconazole to the metabolic saturation. Although our results proved the metabolic saturation of voriconazole, the genetic variants of CYP2C19 as the major metabolic enzyme might affect the evaluation of metabolism saturability.

The concentration on trough of voriconazole tended to be increased in the order of the CYP2C19 IM, EM, and PM group in this study. The metabolic ratio tended to be increased in the order of the CYP2C19 PM, IM, and EM group. Although few reports have evaluated the metabolic ratio between CYP2C19 genotypes, the above results seem reasonable. Our results indicated a higher median value for the metabolic ratio and greater IQR (0.579 and 0.320–1.06). In contrast, Eiden *et al.* reported the median value of the metabolic ratio was 1.7

Fig. 4. The Correlation between the Voriconazole Concentration on Trough and Inverse Value of the Metabolic Ratio of *N*-Oxide to Voriconazole in the CYP2C19 EM (A), IM (B), and PM Groups (C)

Table 3. Summary of Covariate Analysis Step

Coefficients ($p < 0.05$ to enter and $p > 0.10$ to remove)	B	β	p	VIF
Dose-normalized concentration of voriconazole (adjusted $R^2 = 0.916$, $p = 0.021$)				
Constant	0.189	—	0.023	
Voriconazole concentration ($\mu\text{g/mL}$)	0.280	0.939	< 0.001	1.021
Estimated glomerular filtration rate (mL/min/1.73 m^2)	-0.002	-0.103	0.021	1.021
Inverse value of metabolic ratio of <i>N</i> -oxide to voriconazole (adjusted $R^2 = 0.702$, $p < 0.05$)				
Constant	-0.153	—	0.608	
Voriconazole concentration ($\mu\text{g/mL}$)	0.698	0.847	< 0.001	1.018
CYP2C19 genotypes with two variants	1.314	0.215	0.011	1.018

Predictor values: gender, age, total protein, serum albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase, blood urea nitrogen, estimated glomerular filtration rate, CYP2C19 genotypes, and voriconazole concentration. B, partial regression coefficient. β , standard partial regression coefficient. p , probability significance. VIF, variance inflation factor.

(IQR 0.9–5.0).²³⁾ This difference in the data for median value and degree of variability is most likely due to genetic variants of CYP2C19. The participants in our study were all Japanese, while those in the Eiden *et al.* study were all Caucasian. The frequency of the wild type of CYP2C19 is much greater in Caucasians (70–75%) than in Asians (30–40%).²⁴⁾ The frequency of a *CYP2C19*17* allele was different between Asian and Caucasian. *CYP2C19*17* allele carriers are classified into ultrarapid metabolizer and has higher metabolic capacity than EM group. Although the frequency of *CYP2C19*17* allele in Japanese is only 1.3%,²⁰⁾ those of Polish or Germans reach 25%.^{25,26)} Moreover, the frequency of PM group in Japanese was 20% whereas that of Caucasian was a few percent.²⁷⁾ Differences in genetic frequency of *CYP2C19*17* between races may be responsible for lower metabolic ratio in this study than that in the previously reports. The genetic variants of CYP2C19 have a considerable impact on the *N*-oxidation metabolic pathway.

The regression coefficient of the formula for the linear approximation between the inverse value of the metabolic ratio of *N*-oxide to voriconazole and voriconazole concentration on trough, was the highest in the PM group and the lowest

in the EM group. The metabolic capacity of CYP2C19 made a contribution to the metabolic pathway from voriconazole to *N*-oxide. These results indicated that the metabolic saturation was independent on metabolic capacities of CYP2C19 on the voriconazole *N*-oxidation metabolic process at clinical doses. On the contrary, genetic variants of CYP2C19 had little impact on the formula for the linear approximation between the dose-normalized and absolute voriconazole concentrations on trough. This result indicated that the nonlinearity of voriconazole was hardly affected by the CYP2C19 genotype. The nonlinear pharmacokinetics of voriconazole could be more attributed to the metabolic pathway to other metabolites besides *N*-oxide via CYP2C19. Scholz *et al.* reported that *N*-oxide was the major circulating metabolite of voriconazole, however, the metabolic clearance of the hydroxylation pathway of voriconazole was 8-fold higher than that of the *N*-oxidation pathway.²⁸⁾ Alternative metabolic pathways by other metabolic enzymes, including CYP3A4, should be considered.

Voriconazole is mainly excreted by metabolism and its concentration seems to be not significantly affected by renal function. Multiple regression analysis revealed that the estimated glomerular filtration rate affected the pharmacokinetics of

the dose-normalized concentration on trough of voriconazole in the present study. However, the β value of the estimated glomerular filtration was so small that the impact of the estimated glomerular filtration was limited. The inverse value of the metabolic ratio was significantly correlated with the voriconazole concentration on trough and CYP2C19 genotypes with two variants. Because the voriconazole concentration on trough and CYP2C19 genotypes with two variants could explain only 70% of the variation of the inverse value of the metabolic ratio, other factors also may be considered as determinants. However, CYP2C19 gene variants, especially having two variants, may be the significant factor at clinical doses.

This study has several limitations. First, the patients enrolled received similar dosage, and the concomitant drugs were different between patients. Since most of the patients received voriconazole at doses close to 4mg/kg in this study, the data with a low or high dose might be insufficient to evaluate the dose–exposure relation. In order to perform a general evaluation of the pharmacokinetic aspects of the drug, it is necessary to perform the evaluation in patients administered any dosage beyond the adaptation range of voriconazole. Thus, a pharmacokinetic evaluation in which the dosage distribution is that for a healthy person should be conducted. However, the present study evaluated the pharmacokinetics of voriconazole in clinical settings. Accordingly, although the dosage was inevitably biased toward 4mg/kg, we believe that this study could adequately evaluate the dose–exposure relation of voriconazole in clinical settings. Patients receiving a drug or drugs listed in the exclusion criteria which can drastically induce or inhibit the metabolic enzymes of voriconazole were excluded. However, 9 patients were also receiving macrolide antibiotics and 12 patients omeprazole. These drugs may play a lesser role in voriconazole metabolism. These doses were unchanged during the study period, so their inhibitory effects on metabolic enzymes were most likely constant. Many patients had bacterial respiratory infections or stomach disorders. The current study has demonstrated the effects of CYP2C19 genotypes on voriconazole metabolism, however, more clear results from analysis of the voriconazole metabolic process in genetic variants of CYP2C19 would be obtained by excluding patients who received these drugs. Zonios *et al.* reported relationship between the metabolic ratios of both the *N*-oxide and hydroxylvoriconazole to voriconazole concentrations.²⁹⁾ The relationships of *N*-oxide and hydroxylvoriconazole to voriconazole concentrations seemed similar. Taking their report into consideration, the hydroxylation pathway was also saturable as well as *N*-oxidation pathway. Hydroxylvoriconazole and dihydroxyvoriconazole as hydroxylation metabolites of voriconazole were not measured in this study. Because the metabolic ratio with *N*-oxide could express only a limited part of the voriconazole metabolic capacity in this study, the evaluation of its hydroxides which have larger metabolic clearance is necessary.

Therapeutic drug monitoring is desirable also for the early prevention of adverse effects and for ascertaining sufficient antimicrobial activity of voriconazole. The plasma concentration on trough of voriconazole is difficult to predict because it exhibits nonlinear pharmacokinetics at the clinical dose. The prediction of voriconazole pharmacokinetics using only genetic variants of CYP2C19 was difficult based on our results. Although the metabolic ratio was hypothesized to be an index of

the metabolic capacity of voriconazole in this study, it was not enough to completely verify the nonlinearity of voriconazole. The detailed evaluation of voriconazole metabolism with the metabolic ratio using hydroxides and *N*-oxide might be more helpful for elucidation of its nonlinear pharmacokinetics and contribute to the prediction of voriconazole concentration on trough.

In conclusion, our findings showed that metabolic saturation rather than CYP2C19 genotype contributed to the nonlinear pharmacokinetics of voriconazole. The metabolic process that converts voriconazole to *N*-oxide is saturated at clinical doses.

Conflict of Interest The authors declare no conflict of interest.

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