A pharmacokinetic comparison of two voriconazole formulations and the effect of CYP2C19 polymorphism on their pharmacokinetic profiles

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Introduction

Voriconazole is a triazole antifungal agent, indicated for the treatment of invasive aspergillosis, candidiasis, and serious infection caused by *Scedosporium apiospermum* and *Fusarium* spp.1 Voriconazole is extensively metabolized in the liver, with only 2% excreted in urine unchanged.1 An in vitro study revealed that CYP2C19 enzyme plays a key role in the N-oxidation of voriconazole, with CYP2C9 and CYP3A4 involved to a lesser extent.2 Furthermore, the genetic polymorphism of CYP2C19 influences the pharmacokinetics of voriconazole. The *2* and *3* alleles are deficient alleles.3,4
whereas the *17 allele, a recently identified allelic variant, is associated with ultra-rapid metabolism of voriconazole.\(^5\)

Because voriconazole is poorly dissolved in aqueous media, Vfend\(^\text{®}\) (Pfizer Inc, New York, NY, USA), the currently marketed intravenous formulation of voriconazole, contains sulfobutyl ether beta cyclodextrin sodium (SBECD) as a solubilizer. As the clearance (CL) of SBECD is decreased in a linear fashion as renal function is diminished, SBECD is accumulated in renally impaired patients.\(^6\)

In a preclinical study, repeated doses of intravenous SBECD resulted in dose-dependent histologic changes such as renal tubule vacuolation and pulmonary foam cell foci.\(^7\) Although there is some evidence that these histologic changes do not exert toxic clinical effects in humans,\(^8\)–\(^10\) the accumulation of SBECD in patients with impaired renal function may limit the use of intravenous voriconazole in this population.\(^1\)

SYP-1018 is a novel lyophilized polymeric nanoparticle formulation of voriconazole for intravenous administration developed by Samyang Biopharmaceuticals Corporation (Seoul, Republic of Korea). SYP-1018 is composed of a low molecular weight, biodegradable, amphiphilic diblock copolymer, and methoxy-poly(ethylene glycol)-block-poly(D,L-lactide) (mPEG-PDLLA). Additionally, sodium salt of polylactic acid (D,L-PLACOONa) is used as a solubilizer instead of SBECD. Based on several preclinical studies, drugs containing mPEG-PDLLA appear to be safe.\(^11\)–\(^13\)

Furthermore, a polymeric micelle formulation of paclitaxel (Genexol-PM\(^\text{®}\)) was approved in Korea in 2006, and the clinical data showed that the polymeric micelle formulation was safe and well tolerated.\(^14\)–\(^16\)

Based on this understanding, the present study compared the pharmacokinetic and tolerability profiles of SYP-1018 with those of Vfend\(^\text{®}\) after a single intravenous administration in healthy subjects. Furthermore, the effect of CYP2C19 polymorphism on the pharmacokinetics and bioequivalence of the two formulations of voriconazole was evaluated.

**Materials and methods**

**Subjects and study design**

A randomized, open-label, two-treatment, two-period, two-sequence, crossover study was performed with a washout period of 7 days. In each period, subjects randomly received a single intravenous dose of SYP-1018 (voriconazole-loaded polymeric nanoparticle) or Vfend\(^\text{®}\), a reference voriconazole formulation currently available in the market. After written informed consent was obtained, healthy male volunteers of 20–45 years underwent screening, which included medical history, physical examination, laboratory tests (hematology, blood chemistry, coagulation, and urinalysis), 12-lead electrocardiogram (ECG), and vital signs. Subjects were excluded if their alanine aminotransferase (ALT) or aspartate aminotransferase (AST) exceeded 1.25 times the upper limit of normal.

Eligible subjects were admitted to the Clinical Trials Center at Seoul National University Hospital (SNUH), Seoul, Korea, a day before study drug administration in each period. On the 1st day in each period, subjects received SYP-1018 or Vfend\(^\text{®}\) at 200 mg intravenously for 1.5 hours according to their randomized sequence. Serial blood samples of 4 mL for pharmacokinetic analysis were taken at 0 (ie, pre-dose), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours post dose. Additionally, 4 mL of blood was collected for genotyping (period 1 only). Physical examination, vital signs, laboratory tests, and 12-lead ECGs were conducted at the study protocol-specified times. Adverse events were collected during the entire study period.

This study was conducted in compliance with the declaration of Helsinki, Good Clinical Practices, and other regulatory guidelines (ClinicalTrials.gov: NCT01657201). The study protocol and informed consent form were approved by the institutional review board at SNUH.

**Determination of voriconazole plasma concentrations**

Blood samples were centrifuged at 2,000×g for 10 minutes and the separated plasma were stored below −70°C until voriconazole concentration was determined using high performance liquid chromatography (1260 series; Agilent Technologies, Santa Clara, CA, USA) with mass spectrometer (API4000 QTRAP; SCIEX, Framingham, MA, USA). Voriconazole-d3 (Santa Cruz Biotechnology Inc, Dallas, TX, USA) was used as an internal standard, and the sample was protein precipitated with acetonitrile. Kinetex 2.6 μm C18, 100×2.10 mm (Phenomenex, Torrance, CA, USA) was used as the analytical column, and the mobile phase consisted of 0.1% formic acid in 10 mM ammonium formate and 100% acetonitrile. The flow rate was 0.3 mL/min. The lower limit of quantification (LLOQ) was 25 ng/mL, and the calibration range was 25 ng/mL–4,000 ng/mL. The intrabatch and interbatch coefficient of variation (CV) was less than 2.911% and 5.609%, respectively. Likewise, the intrabatch and interbatch accuracy ranged between 91.38% and 105.1%, and 87.83% and 97.17%, respectively.

**Pharmacokinetic analysis**

Pharmacokinetic parameters were calculated using a noncompartmental method implemented in Phoenix\(^\text{®}\)-WinNonlin\(^\text{®}\) 6.3 (Certara, St Louis, MO, USA). Maximum plasma concentration (C\(_{\text{max}}\)) and time to reach C\(_{\text{max}}\) (T\(_{\text{max}}\)) were determined directly...
from the observed individual time–concentration profiles. Terminal elimination half-life ($t_{1/2}$) was calculated as the natural logarithm of 2 divided by $\lambda_z$, which is the terminal elimination rate constant estimated in the linear decline portion of the natural logarithmic transformed individual plasma concentrations. Area under the concentration–time curve (AUC) from dosing to the last quantifiable concentration ($AUC_{last}$) was calculated using the linear-up/log-down trapezoidal method, and AUC from dosing to infinity ($AUC_{inf}$) was calculated as the sum of $AUC_{last}$ and $C_{last}/\lambda_z$, where $C_{last}$ was the last measurable concentration. CL was also derived as the administered dose divided by $AUC_{inf}$.

**Genotyping**

Various allelic variants for CYP2C19, such as the *2 (681G>A, rs4244285, assay ID: C_25986767_70), *3 (636G>A, rs4986893, assay ID: C_27861809_10), and *17 (-806C>T, rs12248560, assay ID: C_469857_10) alleles were genotyped using the TaqMan allelic discrimination assays on an ABI Prism 7500 Sequence Detection System (Thermo Fisher Scientific). Based on the genotype analysis, subjects were identified as extensive metabolizers (EMs) for *1/*1, intermediate metabolizers (IMs) for *1/*2 and *1/*3, or poor metabolizers (PMs) for *2/*2, *2/*3, and *3/*3. Subjects with allelic variant *17 were not classified into specific phenotype.

**Statistical analysis**

It was estimated that enrolling 52 subjects would yield an 80% power that the 90% confidence interval (CI) of the geometric mean ratio (GMR) between the two treatments, a CI that falls in the range of (0.8–1.25) at a significance level of 0.05, based on an intrasubject CV of 35% obtained from a previous study in a Korean population (Clinicaltrials.gov, NCT01080651). The demographic characteristics of the 52 subjects who completed the study, 51 subjects were genotyped: EM (N=19), IM (N=19), PM (N=10), and unclassified (N=3), the latter of which included *1/*17 (N=1) and *2/*17 (N=2). The demographic characteristics of the 52 subjects who completed the study are summarized in Table 1, and there were no differences between the two sequences of study drug administration.

**Pharmacokinetics of voriconazole**

The pharmacokinetic parameters of voriconazole were similar between SYP-1018 and Vfend® when analyzed separately for CYP2C19 genotype. The pharmacokinetics of SYP-1018 and Vfend® were considered comparable if the 90% CI fell entirely within the conventional bioequivalence range of 0.80–1.25 for both $C_{max}$ and $AUC_{last}$

The number of subjects with adverse events and frequency of adverse events were compared between treatments using the chi-square test. All the statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA), and P-values ≤0.05 were regarded as statistically significant. Using the intrasubject variability by genotypes, the number of subjects required to meet the conventional bioequivalence criteria was calculated based on a two-sided tests procedure.17

**Results**

**Subjects and genotyping**

This study enrolled 59 subjects, six of whom were dropped prior to drug administration in period 1 due to withdrawal of consent (N=2), pre-treatment events (N=3, two chest discomforts, one eye swelling), and a no-show (N=1). The remaining 53 subjects received at least one study drug, with 52 subjects completing the study. One subject was dropped because of an adverse event that had occurred in period 1, after administration of Vfend®. Age, height, and body weight did not significantly differ between the two sequences. Of the 52 subjects who completed the study, 51 subjects were genotyped: EM (N=19), IM (N=19), PM (N=10), and unclassified (N=3), the latter of which included *1/*17 (N=1) and *2/*17 (N=2). The demographic characteristics of the 52 subjects who completed the study are summarized in Table 1, and there were no differences between the two sequences of study drug administration.

**Table 1 Summary of demographic characteristics of subjects who completed the study**

<table>
<thead>
<tr>
<th></th>
<th>Total (N=52)</th>
<th>Sequence A (N=26)</th>
<th>Sequence B (N=26)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.9±4.9</td>
<td>26.7±5.5</td>
<td>27.1±4.4</td>
<td>0.7811</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.4±5.2</td>
<td>173.6±5.2</td>
<td>174.5±5.5</td>
<td>0.8959</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.7±7.8</td>
<td>70.4±7.8</td>
<td>71.1±8.1</td>
<td>0.7572</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.2±2.0</td>
<td>23.1±2.0</td>
<td>23.3±2.1</td>
<td>0.7529</td>
</tr>
</tbody>
</table>

Notes: Values are presented as arithmetic mean ± standard deviation; *Student’s t-test between sequence A and B; sequence A, SYP-1018 followed by Vfend® in order; sequence B, Vfend® followed by SYP-1018 in order.
90% CI for the GMR of SYP-1018 to Vfend® falling within the conventional bioequivalence range of 0.8–1.25 for both C\text{max} and AUC\text{last} (Table 2). The pharmacokinetic comparability between SYP-1018 and Vfend® was also seen when analyzed separately by CYP2C19 genotype, except for C\text{max} in the PM group, for which the upper bound of the 90% CI was still very close to 1.25 (ie, 1.26, Table 3). Furthermore, the mean plasma concentration–time profiles of the two formulations overlapped almost entirely when compared to each other (Figure 1).

The systemic exposure to voriconazole and its peak as assessed by AUC\text{int} and C\text{max}, respectively, were greatest in the PM group, followed by the IM group, with the EM group having the smallest values (Table 3). For example, the AUC\text{int} of both SYP-1018 and Vfend® (GMR and 90% CI) in the PM and IM groups were 2.35 (1.98–2.78) and 1.27 (1.10–1.46) fold greater, respectively, than that in the EM group. The AUC\text{last} and AUC\text{int} in the subject with *1/*17 allelic variant were close to those in the EM group (Table 3). On the other hand, subjects with *2/*17 had comparable mean AUC\text{last} and AUC\text{int} values to those in the IM group (Table 3).

The intrasubject CV for C\text{max} in the PM and IM groups was 44% and 22% larger, respectively, than that in the EM group (Table 4, Figure 2). Likewise, the intrasubject CV for AUC\text{last} in the PM and IM groups was 71% and 135% larger, respectively, than that in the EM group (Table 4, Figure 2). Consequently, the number of subjects required to meet the conventional bioequivalence criteria between SYP-1018 and Vfend® (ie, two-sided 90% CI for the GMR of SYP-1018 to Vfend® falling entirely within the range of [0.81–1.25]) with an 80% power at a significance level of 0.05 in a 2×2 crossover study was 80% and 40% greater in the PM and IM groups, respectively, for C\text{max} and 33% and 100% greater, respectively, for AUC\text{last} than in the EM group (Table 4).

### Table 2 Summary of voriconazole pharmacokinetic parameters by treatment after a single intravenous administration at 200 mg

<table>
<thead>
<tr>
<th></th>
<th>SYP-1018 (N=52)</th>
<th>Vfend® (N=52)</th>
<th>Geometric mean ratio* (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T\text{max} (hr)</td>
<td>1.50 (1.00–1.58)</td>
<td>1.52 (1.48–1.58)</td>
<td>NA</td>
</tr>
<tr>
<td>C\text{max} (µg/L)</td>
<td>2.120±472.3</td>
<td>2.141±466.1</td>
<td>0.99 (0.93–1.04)</td>
</tr>
<tr>
<td>AUC\text{last} (hr-µg/L)</td>
<td>7,848±3,445.2</td>
<td>8,125±3,539.1</td>
<td>0.97 (0.92–1.01)</td>
</tr>
<tr>
<td>AUC\text{int} (hr-µg/L)</td>
<td>9,708±6,426.7</td>
<td>10,127±7,120.4</td>
<td>0.97 (0.91–1.02)</td>
</tr>
<tr>
<td>CL (L/hr)</td>
<td>27±11.3</td>
<td>26±11.0</td>
<td>NA</td>
</tr>
<tr>
<td>t\text{1/2} (hr)</td>
<td>8.13±5.85</td>
<td>8.22±6.52</td>
<td>NA</td>
</tr>
</tbody>
</table>

Notes: Values are presented as arithmetic mean ± standard deviation, except for T\text{max}, which is presented as median (range); SYP-1018 to Vfend®.

Abbreviations: CL, clearance; t\text{1/2}, terminal elimination half-life; NA, not applicable.

### Tolerability

There was no significant difference in the number of drug-related adverse events between the two treatments (five and nine cases after SYP-1018 and Vfend®, respectively, P=0.424, chi-square test). Likewise, the number of subjects with at least one drug-related adverse event were five and eight cases for SYP-1018 and Vfend®, respectively, which was not significantly different, either (P=0.555, chi-square test). One subject was dropped after the administration of Vfend® in period 1 because ALT was elevated to 2.7 times the upper limit of normal at 7 days post-dose.

All of the adverse events were mild except for one subject, who presented moderate intensity of multiple musculoskeletal pain accompanying erythema, heating sense, and pruritus after infusion of SYP-1018 in period 2. This case, and the other adverse events, however, resolved spontaneously without any medical intervention. Likewise, no clinically significant abnormality was noted in laboratory tests, 12-lead ECGs, vital signs, or physical examination throughout the entire study period.

### Discussion

This study demonstrated that the pharmacokinetic and tolerability profiles of SYP-1018 were comparable to those of Vfend® after a single intravenous administration in healthy subjects. The concentration–time profiles of the two formulations matched almost entirely (Figure 1), and the GMR and its 90% CIs for C\text{max} and AUC\text{last} satisfied the conventional regulatory criteria of bioequivalence (Table 2). The pharmacokinetic comparability for voriconazole between SYP-1018 and Vfend® was also noted even when analyzed separately by different CYP2C19 genotype (Table 3), except for C\text{max} in the PM group, which might have been due to the small sample size (N=10) relative to its large intrasubject CV of 20.8% (Table 4). Furthermore, both formulations were well tolerated without any serious adverse
Table 3 Summary of pharmacokinetic parameters of two voriconazole formulations by CYP2C19 genotype

<table>
<thead>
<tr>
<th>C_{max} (µg/L)</th>
<th>Vfend (µg/L)</th>
<th>AUC_{inf} (hr·µg/L)</th>
<th>Geometric mean ratio (90% CI)</th>
<th>AUC_{inf} (hr·µg/L)</th>
<th>Geometric mean ratio (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SYP-1018</td>
<td></td>
<td></td>
<td>SYP-1018</td>
<td></td>
</tr>
<tr>
<td>EM (N=19)</td>
<td>1,948±342.3</td>
<td>2,030±639.2</td>
<td>0.96 (0.87–1.05)</td>
<td>5,671±1,258.2</td>
<td>5,893±1,071.5</td>
</tr>
<tr>
<td>IM (N=19)</td>
<td>2,101±488.0</td>
<td>2,218±572.6</td>
<td>0.94 (0.86–1.03)</td>
<td>7,431±1,592.0</td>
<td>7,703±2,455.0</td>
</tr>
<tr>
<td>PM (N=10)</td>
<td>2,535±514.9</td>
<td>2,319±367.2</td>
<td>1.11 (0.97–1.26)</td>
<td>12,950±2,908.8</td>
<td>13,742±2,384.4</td>
</tr>
<tr>
<td>*1/*17 (N=1)</td>
<td>2,077.0</td>
<td>1,866.0</td>
<td>NA</td>
<td>4,619.1</td>
<td>4,317.4</td>
</tr>
<tr>
<td>*2/*17 (N=2)</td>
<td>1,869.0</td>
<td>1,935.5</td>
<td>NA</td>
<td>9,444.5</td>
<td>8,015.3</td>
</tr>
</tbody>
</table>

Notes: Values are presented as mean ± standard deviation except for *1/*17 and *2/*17, which is presented as individual value (*1/*17) or mean (*2/*17); SYP-1018 to Vfend.

Abbreviations: C_{max}, maximum plasma concentration; AUC_{inf}, area under the concentration–time curve (AUC) from dosing to the last quantifiable concentration; AUC_{inf}, AUC from dosing to infinity; CI, confidence interval; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; NA, not applicable; hr, hours.

We found that CYP2C19 polymorphism influenced the pharmacokinetics of voriconazole in a similar way as previously reported including Korean, Caucasian, and Oriental populations. However, the frequency of drug-related adverse events was not significantly different between the two formulations. There were no newly reported adverse events in the present study, and the frequency of drug-related adverse events was not significantly different between the two formulations. Voriconazole has highly variable pharmacokinetics, and the intrasubject variability in the CYP2C19 PM group was greater in the PM and EM groups than in the EM group, resulting in the conventional bioequivalence criteria at the same power and significance level (Table 4). Greater CV was greater in the PM and EM groups than in the EM group, and the CV in those with *2/*17 was more or less close to those in the IM group. However, no firm conclusion can be drawn about the effect of the *17 allele on the pharmacokinetic profiles of voriconazole in those with *2/*17 in the present study (N=21), however, no firm conclusion can be drawn about the effect of the *17 allele on the pharmacokinetic profiles of voriconazole in those with *2/*17 in the present study (N=21). However, no firm conclusion can be drawn about the effect of the *17 allele on the pharmacokinetic profiles of voriconazole in those with *2/*17 in the present study (N=21).
Table 4 Intrasubject coefficient of variation between SYP-1018 and Vfend® by CYP2C19 genotype

<table>
<thead>
<tr>
<th></th>
<th>EM (N=19)</th>
<th>IM (N=19)</th>
<th>PM (N=10)</th>
<th>Total (N=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrasubject coefficient, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cₘₚₙ</td>
<td>14.4</td>
<td>17.5</td>
<td>20.8</td>
<td>17.0</td>
</tr>
<tr>
<td>AUCₘₚₙ</td>
<td>7.2</td>
<td>6.9</td>
<td>12.3</td>
<td>12.4</td>
</tr>
<tr>
<td>AUCₘₚₙ</td>
<td>7.4</td>
<td>20.8</td>
<td>20.0</td>
<td>16.9</td>
</tr>
<tr>
<td>Number of subjects required</td>
<td>10</td>
<td>14</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>18</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

Notes: *Number of subjects required to meet the conventional bioequivalence criteria between SYP-1018 and Vfend® (ie, two-sided 90% confidence interval for a GMR of SYP-1018 to Vfend® falling entirely within [0.8–1.25]) with an 80% power at a significance level of 0.05 in a 2x2 crossover study.

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; Cₘₚₙ, maximum plasma concentration; AUCₘₚₙ, area under the concentration–time curve (AUC) from dosing to the last quantifiable concentration; AUCₘₚₙ, AUC from dosing to infinity; GMR, geometric mean ratio.

Intrasubject variability for mirtazapine. Because the relative frequency of CYP2C19 genotypes can be different among not only various ethnic groups, but also different study populations at hand, information on the composition of CYP2C19 polymorphism and the intrasubject variability by genotype in the study population could be of help in designing a future comparative pharmacokinetic study of voriconazole.

Although we could not conclude if the comparable pharmacokinetic profile for voriconazole between SYP-1018 and Vfend® will be maintained after repeated administration, it is very likely so given that the elimination phase pharmacokinetic parameters for voriconazole at steady state are not different from elimination phase pharmacokinetic parameters after a single dose. Furthermore, it is well known that plasma concentration of voriconazole is closely associated with clinical outcomes. Taken together, SYP-1018 is expected to show a similar efficacy profile to that of Vfend® after repeated administration in patients with fungal infections, and further studies may be warranted in various populations (eg, women, elderly, children, etc). Therefore, SYP-1018 can be further developed as an effective alternative to Vfend®, particularly for patients with decreased renal function.
Figure 2 Changes in the pharmacokinetic parameters of voriconazole after a single intravenous administration at 200 mg by CYP2C19 genotypes.

Notes: (A) Cmax, maximum plasma concentration; (B) AUClast, area under the concentration–time curve (AUC) from dosing to the last quantifiable concentration; (C) AUCinf, AUC from dosing to infinity.

Abbreviations: hr, hours; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.

Conclusion
In conclusion, the pharmacokinetic and tolerability profiles of SYP-1018 were comparable to those of Vfend® after a single intravenous infusion. CYP2C19 genotype affected not only the pharmacokinetics of voriconazole, but also affected its intrasubject variability. SYP-1018 can be further developed as a clinically effective alternative to Vfend®, particularly in patients with renal impairment.

Disclosure
This study was sponsored by Samyang Biopharmaceuticals Corporation (Seoul, Republic of Korea). Yong Jin Lee is an employee of Samyang Biopharmaceuticals Corporation. Otherwise, the authors declare no conflicts of interest in this work.

References


