**In Vitro** Study of the Variable Effects of Proton Pump Inhibitors on Voriconazole

Krista L. Niece,1 Natalie K. Boyd,2,3 Kevin S. Akers1,4

Department of Extremity Trauma and Regenerative Medicine, United States Army Institute of Surgical Research, JBSA Ft. Sam Houston, Texas, USA; College of Pharmacy, The University of Texas at Austin, Austin, Texas, USA; Pharmacotherapy Education and Research Center, The University of Texas Health Sciences Center, San Antonio, Texas, USA; Infectious Disease Service, Department of Medicine, San Antonio Military Medical Center, JBSA Ft. Sam Houston, Texas, USA

Voriconazole is a broad-spectrum antifungal agent used for the treatment of severe fungal infections. Maintaining therapeutic concentrations of 1 to 5.5 μg/ml is currently recommended to maximize the exposure-response relationship of voriconazole. However, this is challenging, given the highly variable pharmacokinetics of the drug, which includes metabolism by cytochrome P450 (CYP450) isotypes CYP2C19, CYP3A4, and CYP2C9, through which common metabolic pathways for many medications take place and which are also expressed in different isoforms with various metabolic efficacies. Proton pump inhibitors (PPIs) are also metabolized through these enzymes, making them competitive inhibitors of voriconazole metabolism, and coadministration with voriconazole has been reported to increase total voriconazole exposure. We examined the effects of five PPIs (rabeprazole, pantoprazole, lansoprazole, omeprazole, and esomeprazole) on voriconazole concentrations using four sets of human liver microsomes (HLMs) of different CYP450 phenotypes. Overall, the use of voriconazole in combination with any PPI led to a significantly higher voriconazole yield compared to that achieved with voriconazole alone in both pooled HLMs (77% versus 59%; P < 0.001) and individual HLMs (86% versus 76%; P < 0.001). The mean percent change in the voriconazole yield from that at the baseline after PPI exposure in pooled microsomes ranged from 22% with pantoprazole to 51% with esomeprazole. Future studies are warranted to confirm whether and how the deliberate coadministration of voriconazole and PPIs can be used to boost voriconazole levels in patients with difficult-to-treat fungal infections.

Voriconazole is a broad-spectrum antifungal agent with activity against *Aspergillus*, *Candida*, *Scedosporium*, and *Fusarium* spp. and is predominantly used in settings of invasive fungal infections (1–3). Voriconazole is extensively metabolized in the liver, primarily through the cytochrome P450 (CYP) enzyme CYP2C19 and, to a lesser extent, through CYP enzymes CYP3A4 and CYP2C9. Its high bioavailability (>90%) and extensive tissue distribution are advantageous characteristics of voriconazole. On the other hand, voriconazole is also known for exhibiting wide inter- and intradividual variability in plasma concentrations, depending on an individual’s age, liver function, CYP450 polymorphisms, plasma albumin levels, and concomitant medications (4–8). Furthermore, nonlinear pharmacokinetics complicate the dose-concentration relationship of voriconazole, potentially leading to unpredictable exposures after incremental dose changes (9, 10). Trough concentrations between 1 and 5.5 μg/ml have correlated with improved clinical responses as well as decreased incidences of adverse events (4, 5, 10–15). Therapeutic drug monitoring (TDM) is therefore recommended to ensure optimal systemic voriconazole exposure (5, 16, 17).

Voriconazole interacts with an exhaustive list of medications, many of which can significantly impact plasma concentrations. Proton pump inhibitors (PPIs) are of particular interest, as they are among the most widely used medications and also undergo CYP450-dependent metabolism, primarily through CYP2C19, CYP3A4, and CYP2C9 (18), making these drugs competitive inhibitors of voriconazole. Both voriconazole and PPIs are also vulnerable to the significant pharmacokinetic variability associated with CYP2C19 polymorphisms. Unsurprisingly, increased plasma voriconazole concentrations during coadministration with a PPI have been reported (5, 12, 19, 20). Despite the widespread use of PPIs and the potential for CYP450-mediated interactions with voriconazole (12, 17), the net effect of PPIs on voriconazole pharmacokinetics has not been delineated. Although drug-drug interactions are generally perceived to be sources of adverse events, moderate inhibition of voriconazole metabolism may be clinically beneficial if it can lead to expedited target attainment. Intentional boosting of voriconazole concentrations may be advantageous when treating individuals harboring ultrarapidly metabolizing CYP450 enzymes, particularly when coupled with TDM. To investigate this issue in finer detail, we explored the effects of PPI exposure on voriconazole concentrations using a cell-free system of pooled and single-donor human liver microsomes (HLMs) with various CYP450 enzyme activity profiles.

**MATERIALS AND METHODS**

**Chemicals and reagents.** Voriconazole and itraconazole were purchased from Selleck Chemicals (Houston, TX). Lansoprazole, esomeprazole, and omeprazole were obtained from Sigma (St. Louis, MO). Rabeprazole and pantoprazole were obtained from the U.S. Pharmacopeia (Rockville, MD). Sulobutylether cyclodextrin (Captisol; used in pharmaceutical-grade intravenous voriconazole) was obtained from Cydex Pharmaceuticals (Lexena, KS). A RapidStart NADPH-generating system was obtained from Bioluminescence (Ann Arbor, MI). Sulfobutylether cyclodextrin (Captisol; used in pharmaceutical-grade intravenous voriconazole) was obtained from Cydex Pharmaceuticals (Lexena, KS). A RapidStart NADPH-generating system was obtained from Bioluminescence (Ann Arbor, MI).

**Received 22 April 2015 Returned for modification 22 May 2015 Accepted 20 June 2015 Accepted manuscript posted online 29 June 2015**


Address correspondence to Kevin S. Akers, kevin.s.akers.mil@mail.mil.

Copyright © 2015, American Society for Microbiology. All Rights Reserved. doi:10.1128/AAC.00884-15
TABLE 1 Relative CYP450 enzyme activities of HLMs

<table>
<thead>
<tr>
<th>HLM type</th>
<th>Relative CYP450 content</th>
<th>Ratio of CYP450 enzyme metabolic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMG1</td>
<td>1.6</td>
<td>2C19 33 2C9 1.2 3A4 NA 3.6</td>
</tr>
<tr>
<td>PMG2</td>
<td>2.1</td>
<td>2C19 4.4 2C9 2.5 3A4 34.6 8</td>
</tr>
<tr>
<td>Individual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C19-WT</td>
<td>1.4</td>
<td>2C19 1 1 1.9 1 1</td>
</tr>
<tr>
<td>2C9-WT</td>
<td>1.4</td>
<td>2C9 1 1 6.1 3.9</td>
</tr>
</tbody>
</table>

a Relative CYP450 content was calculated as the ratio of the total CYP concentration (in nanomoles per milligram of total protein) to the concentration of the HLM type with the lowest concentration, 2C19-WT. The metabolic activity ratios were calculated using the apparent activity of that enzyme in each HLM type on the basis of metabolism of a marker substrate (in picomoles per milligram per minute). All lot-specific information is from data sheets kindly provided by XenoTech, LLC. NA, not available.

from XenoTech LLC (Lenexa, KS). Acetonitrile and methanol were high-performance liquid chromatography (HPLC) grade and purchased from VWR (Radnor, PA). Ultrapure deionized water was generated using an Aqua Solutions type I water purification system (resistivity, ≥18.2 MΩ) and used in all applications.

Pantoprazole and rabeprazole stock solutions were prepared at 1 mg/ml in water. Voriconazole and esomeprazole were solubilized at 1 mg/ml with 40% (wt/vol) sulfobutylether cyclodextrin and rocked overnight at 4°C. The resulting clear solution was passed through a 0.2-µm-pore size syringe filter to remove insoluble material and then aliquoted and stored at −80°C. Itraconazole was prepared daily at 5 mg/ml in dimethyl sulfoxide and diluted in methanol to the desired final concentration. Lansoprazole and omeprazole were solubilized at 19.2 mM in methanol.

HLMs. Two pooled and two individual HLM preparations were obtained from XenoTech LLC (Lenexa, KS). Pooled microsomes were derived from mixed gender populations: the first (PMG1) consisted of 8 donors unselected on the basis of CYP450 enzyme activity, while the second (PMG2) consisted of 15 donors and represented a population with notably higher levels of CYP3A4/5 activity. The remaining two preparations consisted of HLMs prepared from two individual, genotyped donors: (i) a 45-year-old Caucasian male with the 2C9*1/*1 genotype, or a wild-type (WT), homozygous extensive metabolizer (2C9-WT), and (ii) a 59-year-old Caucasian female with the 2C9*1/*1 genotype, or a wild-type, homozygous extensive metabolizer (2C9-WT). The manufacturer provided the CYP enzyme metabolic profiles (in picomoles per milligram of protein per minute) for each HLM, based on standard CYP isoform-specific probe substrate reactions (5-methylenothionin for CYP2C19, diclofenac for CYP2C9, midazolam for CYP3A4, and testosterone for CYP3A4/5). These predetermined HLM CYP enzyme rates (in picomoles per milligram of protein per minute) are summarized as relative enzyme activities in Table 1. Notably, the 2C19 enzyme activity rates were similar between the genotyped single-donor HLMs, 2C19-WT and 2C9-WT, but the 2C9 enzyme activity rates of 2C19-WT and 2C9-WT were significantly lower than those of the pooled HLMs (PMG1 and PMG2).

CYP enzyme activity ratios also revealed variable CYP3A4 and CYP3A4/5 activity rates for PMG1 versus PMG2 and 2C19-WT versus 2C9-WT.

Voriconazole-PPI interaction studies. Voriconazole (2 µM, or 0.7 µg/ml) was combined with esomeprazole, omeprazole, lansoprazole, rabeprazole, or pantoprazole at 12 µM (4.1 to 4.6 µg/ml) each in 0.1 M phosphate buffer (pH 7.4) containing 3 mM magnesium chloride. HLMs were added at a final concentration of 1 mg/ml protein. These reaction conditions were selected semiempirically to enable in vitro measurement of the differences in voriconazole metabolism between PPI and microsome types. In preliminary studies (data not shown), lower voriconazole concentrations were difficult to quantify by HPLC after a 60-min incubation, and higher concentrations were minimally metabolized by microsomes; 2 µM is also the concentration used in previously published studies (21, 22). A 1:1 molar ratio of PPI to voriconazole had no quantifiable influence on voriconazole metabolism, leading to the selection of a 12 mM PPI concentration (6:1 molar ratio of PPI to voriconazole). The Rapid-Start NADPH-generating system was diluted according to the manufacturer’s recommendations and added to the incubation mixture to give an expected NADPH concentration of 1.4 to 1.8 mM. At time zero and 60 min, 200-µl aliquots were removed from the reaction mixture and quenched in 1 ml ice-cold methanol containing 1 µg itraconazole (internal standard). For each PPI tested, a control mixture containing the solubilization vehicle (water, sulfobutylether cyclodextrin, or methanol) at the same concentration at which it was used in the test mixture was prepared and assayed in parallel. Samples were centrifuged at 10,000 × g for 10 min to precipitate the microsomal proteins. The supernatant was dried under a stream of air (−15 liters/min) and reconstituted in 150 µl of HPLC mobile phase A (5 mM ammonium acetate, pH 4). Each interaction study was performed three to four times.

HPLC conditions. Calibration standards were prepared using heat-inactivated (45°C for 30 min) microsomal proteins and contained between 0.29 and 2.9 µM voriconazole. Calibration samples were quenched in methanol-itraconazole and treated as described above. Validation standards prepared in the mobile phase were used to verify instrument performance between calibrations and determine the percent analyte recovery. Each PPI was prepared at 10 µg/ml in mobile phase A and assayed by HPLC to determine the elution time and potential interference with the voriconazole assay. HPLC was performed as described by Yanni et al. (21), with minor modifications, using an Agilent 1200 series instrument equipped with a C18 (2) Luna column (150 by 4.6 mm; 100 Å; Phenomenex, Torrance, CA). Detection was by UV at 265 nm.

The mobile phase components were 5 mM ammonium acetate, pH 4 (mobile phase A), and acetonitrile (mobile phase B), both of which contained 0.1% trifluoroacetic acid. A linear gradient of 95% mobile phase A to 10% mobile phase B over 13 min at a flow rate of 1 ml/min was used. Voriconazole and itraconazole (the internal standard) eluted at 11.1 and 12.1 min, respectively. Voriconazole concentrations were quantitated by comparing the peak area ratios (voriconazole/internal standard) to the peak area ratios generated with a standard curve. Standard curve correlation coefficients (r² values) were greater than 0.99.

Data analysis. Voriconazole concentrations at time zero and after 60 min were calculated for each sample using the slope of the HPLC calibration curve as described above. The percentage of the original voriconazole concentration remaining at the end of each experiment was calculated by dividing the concentration at 60 min by the average concentration (n = 3 to 4) under the same conditions at time zero. The percent change in voriconazole stability observed due to PPI addition was calculated as ([M\text{ppi} - M]/M) · 100, where M\text{ppi} and M represent the fraction of voriconazole remaining in the presence and absence of PPI, respectively. The magnitude of these differences was compared to the overall inhibition by all PPIs (n = 12 to 14) or by individual PPIs (n = 3 to 4) using one-way or two-way analysis of variance (ANOVA) with Tukey post hoc multiple comparisons. Data were analyzed using GraphPad Prism software (version 5.0; GraphPad Software, San Diego, CA). P values of <0.05 were considered statistically significant.

RESULTS Characterization of baseline voriconazole concentration in HLMs and mean PPI treatment effect. The percentage of voriconazole remaining after 60-min incubations with microsomes was determined for voriconazole alone and voriconazole in combination with PPIs (Fig. 1). Variability in the remaining voriconazole percentages was notable between certain HLM groups, even before PPIs were introduced into the incubation mixtures. The voriconazole concentrations remaining at the baseline (i.e., with-
out PPI) in single-donor HLMs averaged 75.8% ± 10.7% of the starting concentrations. The baseline percentage of voriconazole remaining in 2C19-WT microsomes was notably high at 85.8% ± 2.8%, indicating minimal voriconazole loss during incubation, whereas it was 66.6% ± 5.2% in 2C9-WT microsomes. The consumption of voriconazole was markedly greater in pooled microsomes, with mean baseline percentages averaging 59.1% ± 7.7% of the initial voriconazole concentrations. The differences in the remaining voriconazole concentrations between pooled and individual HLMs observed at the baseline are consistent with the ratios of precharacterized CYP450 enzyme activities reported by the manufacturer (Table 1).

The addition of a PPI increased the percentage of voriconazole remaining for all microsome types. For PGM1 microsomes, addition of a PPI increased the remaining voriconazole percentage from 64.5% ± 5.6% to 86.0% ± 8.0%. For PGM2 microsomes, addition of a PPI increased the remaining voriconazole percentage from 53.3% ± 4.7% to 70.0% ± 7.0%. For 2C19-WT microsomes, addition of a PPI increased the remaining voriconazole percentage from 85.8% ± 2.8% to 94.0% ± 5.0%. Finally, for 2C9-WT microsomes, addition of a PPI increased the remaining voriconazole percentage from 66.6% ± 5.2% to 78.0% ± 8.0%.

All comparisons resulted in a statistically significant difference ($P < 0.001$).

Differential effects of individual PPIs. Relative PPI treatment effects on voriconazole are illustrated in Fig. 2. Overall, PPI exposure increased the amount of voriconazole remaining in pooled microsomes compared with that at the baseline by an average of 32%. Esomeprazole demonstrated the largest effect, producing a 51% ± 7.8% mean percent increase in the voriconazole concentration from that at the baseline. Rabeprazole and lansoprazole were a distant second, producing 32% and 31% mean increases, respectively, followed by omeprazole and pantoprazole, each producing a mean increase of 22%. The magnitude of the effect from each PPI was similar (within 5%) between PMG1 and PMG2, with the exception of that from omeprazole, which showed a greater impact on voriconazole in PMG1 than PMG2 (33% versus 15%).

The overall PPI effect on the voriconazole concentration in single-donor HLMs was lower and more variable, producing a mean increase of 13% ± 10% compared to that in pooled HLMs. Combination of voriconazole with esomeprazole, rabeprazole, or lansoprazole increased the voriconazole yield by 18% compared to that at the baseline. Contrary to the findings with pooled HLMs, the addition of pantoprazole did not significantly alter the remaining voriconazole quantities in single-donor HLMs (mean change, –2.6%; $P > 0.05$).

The degree to which each PPI impacted voriconazole differed between 2C19-WT and 2C9-WT (a 9% ± 16% versus a 16% ± 13% increased voriconazole yield), despite the presence of nearly equivalent CYP2C19 enzyme activities (Fig. 3). This difference

FIG 1 Percentage of voriconazole (VOR) remaining after incubation for 60 min with the four HLM types. Bars and error bars represent the means and standard deviations from 11 to 12 (no PPI) or 17 to 20 (with PPI) experiments, respectively. Significance was determined using a one-way ANOVA with Tukey’s test. *, $P < 0.001$.

FIG 2 Mean changes in voriconazole (VOR) levels relative to the level at the baseline for pooled HLMs (A) and individual HLMs (B) for each of the five PPIs tested. Bars and error bars represent the means and standard deviations from 7 or 8 experiments, respectively. OMEP, omeprazole; PANT, pantoprazole; LANS, lansoprazole; RABE, rabeprazole; ESOM, esomeprazole. Significance was determined using a one-way ANOVA with Tukey’s test. *, $P < 0.001$.

FIG 3 Relative effects of PPIs on voriconazole (VOR) for individual HLM groups. Bars and error bars represent the means and standard deviations from 3 or 4 experiments, respectively.
grew larger when the negligible effects from pantoprazole exposure were excluded (12% ± 4% versus 22% ± 6%). PPI treatment effects were similar for esomeprazole, lansoprazole, omeprazole, and rabeprazole with 2C9-WT microsomes (range of mean change in the voriconazole yield from that at the baseline, 21% to 25%) but were lower and more variable with 2C19-WT (mean change, 8% to 16%). Differences in individual PPI treatment effects between 2C19-WT and 2C9-WT were most notable for rabeprazole (an 8% versus a 25% increased voriconazole yield) and omeprazole (an 8% versus a 22% increased voriconazole yield).

**DISCUSSION**

In this study, we used an *in vitro* model to examine the influence of five different PPIs on voriconazole using four phenotypically distinct HLMs. Two pooled HLMs were included, one of which was derived from patients unselected for CYP450 enzyme activity (PMG1) and another representing HLMs from a population with high CYP3A4/5 enzyme activity (PMG2), were included. Pooled HLMs provide a means for determining the average expected effect from a drug interaction, given that the HLMs are derived from a combination of several individual donors. Individual HLMs, typically used for characterizing CYP enzymes during drug metabolism, are known for significant interindividual variability in CYP enzyme activity but were included nonetheless to examine the effects of a voriconazole-PPI interaction in two individual genotypes. The results obtained with pooled HLMs revealed a potential for any one of the five PPIs to influence the voriconazole concentration. Esomeprazole demonstrated the greatest effect on voriconazole, with postincubation voriconazole yields exceeding those at the baseline by more than 50%. Although the propensity for other PPIs to influence the voriconazole yield was lower than that of esomeprazole, the effect was appreciable nevertheless in pooled HLMs, with a mean 22% increase in the voriconazole yield being achieved with pantoprazole and omeprazole and mean 31% and 32% increases being achieved with lansoprazole and rabeprazole, respectively. These data are valid for the tested concentrations of voriconazole (2 mM) and PPI (12 mM) and may vary considerably depending on both the absolute and the relative amounts of the two drugs. As the product of an *in vitro* test, these results do not account for the complexity of organism-level physiology, and so it is difficult to correlate the concentrations of voriconazole and PPI used here with those that would be required to achieve similar effects *in vivo*. However, case studies indicate that the interaction between these drugs is both tolerated and measurable at clinically achievable concentrations (5, 12, 23), and the findings of further clinical studies to systematically determine these interactions would be informative when used in conjunction with the data from the present study.

Voriconazole is a safe and effective antifungal agent, provided that plasma concentrations are maintained within a therapeutic range to maximize the exposure-response relationship. The large intra- and interindividual variability in voriconazole pharmacokinetics creates difficulties with achieving adequate exposures. Recent literature suggests that current dosing regimens result in plasma voriconazole concentrations that are frequently below the expected therapeutic concentration (12, 24, 25). One recent review noted that of 14,370 untimed plasma samples from patients on voriconazole, only 50% had concentrations in the therapeutic range of 1 to 5.5 μg/ml. An additional 39% of samples had levels below 1 μg/ml, and the levels were undetectable (<0.2 μg/ml) in more than 10% of subjects. Achievement of voriconazole target concentrations is especially critical when treating severe, life-threatening infections. Voriconazole was a first-line treatment in the 2012 outbreak of invasive fungal infections, primarily from *Exserohilum rostratum*, that resulted from epidural injections of contaminated methylprednisolone acetate. Many of these infections invaded the central nervous system (26, 27), and cerebrospinal fluid voriconazole concentrations of 1 to 2 μg/ml were recommended for treatment of the E. rostratum infection (26). Since the penetration of voriconazole across the blood-brain barrier is approximately 50% of the plasma concentration (27, 28), maintenance of a 6-μg/ml plasma level was recommended. At this level, side effects may be increased (27), but this concentration was achieved in less than 50% of patients with standard dosing (29). Likewise, invasive soft tissue fungal infections have previously been described in immunocompromised patients (30–32) and have more recently been described to be an emerging problem in civilian and military trauma patients (33–36). Several clinical studies evaluating voriconazole therapeutic drug monitoring and factors affecting plasma concentrations have reported an association between concomitant PPI use and elevated voriconazole concentrations (5, 12, 23). Furthermore, case reports of the occurrence of increased plasma voriconazole concentrations after coadministration of voriconazole with a PPI have also been reported; two of the reports were distinct, in that the studies involved the deliberate use of a PPI to boost voriconazole concentrations into the therapeutic range (19, 20, 37). For patients in whom higher plasma voriconazole levels are required to improve tissue penetration or to overcome rapid metabolism, PPI-mediated inhibition could be a reasonable solution for achieving target voriconazole concentrations, provided that TDM is available to guide therapy.

Of the five PPIs tested herein, esomeprazole produced the greatest increase in voriconazole levels in our assay. This observation is consistent with the *in vitro* findings of Zvyaga et al. (38), in which esomeprazole was identified to be one of the most potent inhibitors of CYP2C19. The *in vitro* inhibitory effects of esomeprazole on voriconazole appear to translate clinically as well. A post hoc analysis from a retrospective clinical study of voriconazole TDM revealed a statistically significant association between increased voriconazole concentrations and esomeprazole use (*P* < 0.0001) but not between increased voriconazole concentrations and pantoprazole or rabeprazole use (23). Pantoprazole appeared to have the smallest effect on voriconazole yield in our study, although a sizeable increase of 22% was still observed in pooled HLMs, and the effect of pantoprazole was similar to that of omeprazole. However, the effect of pantoprazole exposure on the voriconazole yield was essentially nonexistent in individual HLMs. Pantoprazole appears to consistently have the lowest propensity of the PPIs tested to inhibit voriconazole concentrations *in vivo*. Linear regression analysis from a multicenter retrospective clinical study (12) revealed that higher median voriconazole concentrations occurred during coadministration with esomeprazole, pantoprazole, omeprazole, or rabeprazole (lansoprazole was not included in that study). However, although the effect of pantoprazole was statistically significant (*P* < 0.01), a low regression coefficient indicated reduced effects from pantoprazole compared to those of other PPIs. Hoening et al. found a statistically significant association between subtherapeutic voriconazole concentra-
tions and concomitant PPI treatment ($P < 0.01$), with 88% of the concomitant PPI treatments consisting of pantoprazole (39).

Omeprazole demonstrated variable effects on the remaining voriconazole concentrations, which differed not only between 2C19-WT and 2C9-WT microsomes (8% versus 22% increased voriconazole yields) but also between PMG1 and PMG2 microsomes (33% versus 15%). Omeprazole was the least effective of the five PPIs at producing changes in voriconazole concentrations with PMG2 microsomes, which was unexpected. Given the higher CYP enzyme activities (CYP2C19, CYP2C9, CYP3A4/5) of PMG2 microsomes than PMG1 microsomes (Table 1), we anticipated a similar if not greater impact of omeprazole treatment of PMG2 microsomes, as was observed with the other PPIs. Another unex-
pected finding was the significant impact after exposure to rabe-
prazole, yielding an average of 32% excess voriconazole from that at the baseline in pooled microsomes. Rabeprazole also showed the greatest impact on voriconazole when incubated with the single-donor microsome 2C9-WT, although its impact was similar in magnitude to that of lansoprazole, omeprazole, and esomeprazole (25% versus 21 to 22% increased voriconazole yields). These ob-
servations with rabeprazole are in contrast to the findings of pre-
vious in vitro studies in which rabeprazole was identified to be a weak CYP2C19 and CYP3A inhibitor (38, 40). One possible ex-
planation for the results obtained with omeprazole and rabepra-
azole may be related to the disposition and metabolism of their enantiomers. Omeprazole, for example, exists as a racemic mi-
ture of S- and R-enantiomers, and the extent of inhibitory effects on CYP2C19 and CYP3A4 enzymes may vary depending on the S/R enantiomer ratios (41, 42). Rabeprazole is largely converted nonenzymatically to rabeprazole thioether, which has greater CYP2C19- and CYP3A4-inhibitory potency than rabeprazole (40): however, some rabeprazole also undergoes metabolism by CYP2C19 and CYP3A4 (43). Changes in enantiomer ratios and the extent of metabolite formation can modulate the extent of the inhibitory effects of PPIs on CYP450 enzymes. Further studies including a detailed in vitro analysis of parent and metabolite concen-
trations may provide more insight into the mechanisms taking place during the interaction.

CYP2C19 is known to be a high-affinity, low-capacity enzyme, whereas CYP3A4 is a low-affinity, high-capacity enzyme (44). Even though CYP2C19 is the primary enzyme responsible for voriconazole metabolism, CYP3A4 plays a larger role when the CYP2C19 enzyme functionality is low or null, and its role in poor metabolizers of CYP2C19 has been demonstrated (45, 46). In our study, the two individual HLMs possessed similar CYP2C19 en-
zyme activities but very different CYP3A4 activities (Table 1). Therefore, it is plausible that CYP3A4-mediated metabolism may have contributed to the differences in the remaining voriconazole quantities (both at the baseline and with PPI exposure) observed between the 2C19-WT and 2C9-WT microsomes, despite their nearly equal CYP2C19 enzyme metabolic rates.

CYP450 genotyping has been suggested to be a means to guide voriconazole dosing (47–49). This follows the precedent of CYP450 genotyping to guide therapy in the cases of warfarin (50, 51) and clopidogrel (52, 53). Recent reports indicate that CYP2C19 polymorphisms account for up to 50% of the interindi-
vidual variability in voriconazole exposures (54). Although the CYP2C19 genotype is an important determinant of voriconazole pharmacokinetics, it has not been determined whether a correla-
tion between the CYP genotype and clinical outcomes exists (55) and CYP genotypes are not 100% predictive of the CYP enzyme phenotype (56, 57). Recently, Bouatou et al. reported on a patient receiving voriconazole who tested positive for the CYP2C19*17 variant (19), which is predictive of high CYP2C19 enzyme activity. However, while the patient was on esomeprazole, phenotype testing with a probe substrate revealed significantly reduced CYP2C19 enzyme activity, at which point voriconazole trough concentrations were therapeutic. Unsurprisingly, a brief interrup-
tion of the esomeprazole therapy resulted in subtherapeutic vori-
conazole trough concentrations. Therefore, drug-induced pheno-
type conversion may be very relevant in the current context of PPI interactions with voriconazole. This gives rise to an important, clinical translation question: could a PPI be utilized (or repur-
posed) to reduce the variability or increase the predictability of plasma voriconazole concentrations? Such measures may be important steps toward advancing precision medicine into clinical practice.

Although this report provides new details on the in vitro inter-
actions between PPIs and voriconazole, there are several limita-
tions and unanswered questions. First, we speculate that higher voriconazole concentrations resulted from CYP450 isoenzyme in-
hibition by PPIs, but we did not assess metabolite formation by voriconazole or probe substrates. Additionally, samples for vori-
conazole quantitative analysis were collected at one nonzero time point, as opposed to multiple time points, and our selection of PPI concentrations, at 12 µM each, reflected conditions optimizing a 60-min laboratory assay. Although the average expected peak (maximum) concentrations ($C_{\text{max}}$) with usual PPI doses are gen-
erally lower than the concentration selected for use in this study, ranging from ~1 to 6 µM (18), PPI dosing regimens are highly plau-
sible when alternative dosing regimens are considered. However, we caution that coadministration with voriconazole could in-
crease PPI levels as well, so lower doses may achieve similar effects. Furthermore, although current standards recommend the use of steady-state $C_{\text{max}}$ values for in vitro inhibitor concentrations (58), there is uncertainty as to whether $C_{\text{max}}$ accurately represent in vivo inhibitor concentrations at the site of drug-enzyme interaction (59). Other investigators used estimates representing presystemic exposures, such as liver or portal vein $C_{\text{max}}$ (38, 60). Furthermore, while liver microsome models are excellent tools for evaluating drug metabolism, no in vitro system can adequately capture the complexity and multiple pathways or account for patient-specific confounding factors influencing the disposition of voriconazole. Extrahepatic mechanisms, such as intestinal CYP transport sys-
tems, could also impact the voriconazole-PPI interaction, given the abundance of CYP3A4 enzymes and efflux transporters.

**Conclusions.** Although circumventing CYP450-mediated drug interactions is common practice for preventing toxicities and adverse events, we propose the deliberate exploitation of one to maximize the exposure-response relationship of voriconazole. In conjunction with TDM, intentional administration of PPIs con-
currently with voriconazole may be used to achieve therapeutic plasma voriconazole concentrations. Further studies should be pursued to confirm our findings obtained in this in vitro study.
REFERENCES

1. Fera MT, La Camera E, De Sarro A. 2009. New triazoles and echinocan-
dines: mode of action, in vitro activity and mechanisms of resistance. Ex-
3. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontovourkis DP,
Marr KA, Morrison VA, Segal BH, Steinbach WJ, Stevens DA, van Burik 
JA, Wingard JR, Patterson TF, Infectious Diseases Society of Amer-
ica. 2008. Treatment of aspergillosis: clinical practice guidelines of the 
http://dx.doi.org/10.1086/525258.
coses improves efficacy and safety outcomes. Clin Infect Dis 46:201–211. 
http://dx.doi.org/10.1086/524669.
6. Scholz J, Oberwittler H, Riedel KD, Burhenne J, Weiss J, Haefeli WE, 
Mikus G. 2009. Pharmacokinetics, metabolism and bioavailability of the 
7. Walsh TJ, Driscoll T, Milligan PA, Wood ND, Schlamm H, Groll 
AH, Jafri H, Arrieta AC, Klein NJ, Lutsar I. 2010. Pharmacokinetics, safety, and tolerability of voriconazole in immunocompromised chil-
K, Annaert P, Spijts I. 2014. Impact of hypoalbuminemia on voricona-
10. Troke PF, Hockey HP, Hope WW. 2011. Observational study of the 
clinical efficacy of voriconazole and its relationship to plasma concentra-
EY, Park SH, Choi JH, Yoo JH. 2011. Voriconazole-related severe ad-
verse events: clinical application of therapeutic drug monitoring in Ko-
Pilewski JM, Crespo MM, Bermudez C, Bhma JK, Clancy CJ. 2012. Prospective, observational study of voriconazole therapeutic drug moni-
toring among lung transplant recipients receiving proflaxins: factors impact-
13. Pak WB, Kim NH, Kim KH, Lee SH, Nam WS, Yoon SH, Song KH, 
Choe PG, Kim NJ, Jang JJ, Oh MD, Yu KS. 2012. The effect of thera-
Steady-state pharmacokinetics and metabolism of voriconazole in pa-
monitoring: established and emerging indications. Antimicrob Agents 
Thakker DR. 2008. Role of flavin-containing monooxygenase in ox-
idation of voriconazole by human liver microsomes. Drug Metab Dispos 
17. Yanni SB, Anhett PP, Augustinis P, Bridges A, Gao Y, Benjamin DJ, 
Thakker DR. 2010. In vitro hepatic metabolism explains higher clearance 
of voriconazole in children versus adults: role of CYP2C19 and flavin-
doi.org/10.1128/AAC.00920-10.
18. Gautier-Veyer E, Fonrose X, Tonini J, Thiebaut-Bertrand A, Bartoli M, 
of voriconazole plasma concentrations after allogeneic hematopoietic 
stem cell transplantation: impact of cytochrome P450 polymorphisms and 
comedications on initial and subsequent trough levels. Antimicrob Agents 
E, Díaz de Heredia C, Figueras C. 2012. Voriconazole drug monitoring in 
20. Stevens DA. 2013. Reflections on the approach to treatment of a myco-
21. Lockhart SR, Pham CD, Gade L, Igbal N, Scheel C, Cleveland AA, 
Whitney AM, Noble-Wang J, Chiller TM, Park BJ, Litvintseva AP, 
Brandt ME. 2013. Preliminary laboratory report of fungal infections as-
ociated with contaminated methylprednisolone injections. J Clin Micro-
22. Kerkering TM, Grifasi ML, Baffoe-Bonnie AW, Bansal E, Garner DC, 
Smith JA, Demicco DD, Schleupner CJ, Alagothai RA, Savalia VA. 2013. Early clinical observations in prospectively followed patients with 
24. Wiedenhold NP, Pennick GJ, Dorsey SA, Furmaga W, Lewis JS, Patt-
tson TF, Sutton DA, Fothergill AW. 2014. A laboratory experience of 
clinical achievable voriconazole, posaconazole, and itraconazole 
AAC.01558-13.
Jiang Y, Chaftra A-M, Raad II. 2014. Invasive aspergillosis caused by 
Aspergillus terreus: an emerging opportunistic infection with poor out-


