PHARMACOKINETICS AND DRUG DISPOSITION

Potent cytochrome P450 2C19 genotype–related interaction between voriconazole and the cytochrome P450 3A4 inhibitor ritonavir

Objectives: **Cytochrome P450 (CYP) 2C19 and CYP3A4 are the major enzymes responsible for voriconazole elimination. Because the activity of CYP2C19 is under genetic control, the extent of inhibition with a CYP3A4 inhibitor was expected to be modulated by the CYP2C19 metabolizer status. This study thus assessed the effect of the potent CYP3A4 inhibitor ritonavir after short-term administration on voriconazole pharmacokinetics in extensive metabolizers (EMs) and poor metabolizers (PMs) of CYP2C19.**

Methods: **In a randomized, placebo-controlled crossover study, 20 healthy participants who were stratified according to CYP2C19 genotype received oral ritonavir (300 mg twice daily) or placebo for 2 days. Together with the first ritonavir or placebo dose, a single oral dose of 400 mg voriconazole was administered. Voriconazole was determined in plasma and urine by liquid chromatography–mass spectrometry, and pharmacokinetic parameters were estimated by noncompartmental analysis.**

Results: **When given alone, the apparent oral clearance of voriconazole after single oral dosing was 26% ± 16% ($P > .05$) lower in *CYP2C19*1/*2* individuals and 66% ± 14% ($P < .01$) lower in CYP2C19 PMs. The addition of ritonavir caused a major reduction in voriconazole apparent oral clearance (354 ± 173 mL/min versus 202 ± 139 mL/min, $P = .0001$). This reduction occurred in all CYP2C19 genotypes (463 ± 168 mL/min versus 305 ± 112 mL/min [$P = .023$] for **1/*1*, 343 ± 127 mL/min versus 190 ± 93 mL/min [$P = .008$] for **1/*2*, and 158 ± 54 mL/min versus 22 ± 11 mL/min for **2/*2*) and is probably caused by inhibition of CYP3A4-mediated voriconazole metabolism.**

Conclusions: **Coadministration of a potent CYP3A4 inhibitor leads to a higher and prolonged exposure with voriconazole that might increase the risk of the development of adverse drug reactions on a short-term basis, particularly in CYP2C19 PM patients. (Clin Pharmacol Ther 2006;80:126-35.)**

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The new triazole antifungal voriconazole is used in the treatment of candidiasis and invasive aspergillosis, as well as other mold infections. Voriconazole is primarily metabolized by CYP2C19 and, to a lesser extent, by CYP3A4 and CYP2C9, with only minimal involvement of the latter in human liver microsomes.[1]

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Consequently, other drugs affecting the activity of these enzymes are expected to interact with the antifungal in vivo. Indeed, inhibition of voriconazole metabolism[2] and induction by phenytoin[3] and St John's wort[4] have been reported. In addition, voriconazole also inhibits these 3 enzymes with similar potency (50% inhibitory concentration between 8 and 10 μmol/L),[5] pointing to the potential for drug interactions with voriconazole acting as an inhibitor of the metabolism of other drugs[6–8] whose metabolism is impaired by the triazole.

CYP2C19 is a polymorphically expressed enzyme,[9] with 2.2% of the white population being poor metabolizers (PMs)[10] with a genetically determined absence of active enzyme. It is known that voriconazole pharmacokinetics is substantially influenced by the CYP2C19 genotype.[4,11,12] A reduction in voriconazole metabolic clearance in PMs of CYP2C19 is expected; data published thus far indicate that voriconazole area under the plasma concentration–time curve (AUC) and maximum observed plasma concentration (C_{max}) values are approximately 3-fold higher in CYP2C19 PMs as compared with homozygous extensive metabolizers (EMs).[4,12,13]

In vitro data suggest that CYP3A4 does contribute to the metabolism of voriconazole, but the affinity of voriconazole to CYP3A4 is about 50-fold lower compared with the affinity to CYP2C19.[1] Hence CYP3A4 may not play a major role in overall voriconazole metabolism.[1] However, in CYP2C19 PMs, who lack this important pathway of voriconazole elimination, alternative metabolic pathways via CYP3A4 may become more important for the elimination of voriconazole. It was shown by in vitro inhibition experiments on voriconazole metabolism that ketoconazole as a specific CYP3A4 inhibitor was more potent as an inhibitor in CYP2C19 PM liver microsomes.[1] This may suggest that the extent of interaction may be genetically determined and that CYP2C19 PMs may indeed have a rather different interaction profile compared with the more prevalent EMs. Such a difference would likely be of clinical relevance because CYP3A4 is a frequent site of drug interactions and inhibition of this major drug-eliminating enzyme is a frequent phenomenon in clinical practice.

With this study, we aimed to determine the short-term effect of CYP3A4 inhibition on voriconazole pharmacokinetics and to assess to what extent this interaction is modulated by the CYP2C19 genotype. The most potent CYP3A4 inhibitor known thus far is ritonavir,[14] a human immunodeficiency virus protease inhibitor that also inhibits CYP2D6 and, to a lesser extent, CYP2C9.[15] There is increasing evidence that ritonavir exhibits mechanism-based inactivation of CYP3A4 with persistence of inhibition after discontinuation of therapy.[16,17] Indeed, clinically relevant drug interactions with short-term ritonavir administration have been reported to affect triazolam[18] and clarithromycin,[19] with long-term ritonavir administration (10 days) increasing rifabutin concentrations[20] and other human immunodeficiency virus protease inhibitors leading to systemic accumulation of these drugs.[21] We expected that inhibition of the CYP3A4 pathway would impair voriconazole clearance much more in the absence of functional CYP2C19, suggesting that the CYP2C19 genotype would critically determine the dose requirements in this situation.

METHODS

The study protocol was approved by the Ethics Committee of the University Hospital, Heidelberg, Germany, and was conducted at the Department of Internal Medicine VI, Clinical Pharmacology and Pharmacoepidemiology, Heidelberg, Germany, in accordance with the Declaration of Helsinki and subsequent amendments. Written informed consent was obtained from each participant.

Study population. Overall, 21 participants were included in the trial. One heterozygous EM dropped out after the first study part because of a protocol violation. This volunteer was replaced by a carrier of the same CYP2C19 genotype. Finally, 20 healthy, nonsmoking white men (n = 13) and women (n = 7) (age, 19-37 years; body mass index, 17.7-27.2 kg/m^2) were enrolled and completed the trial. All participants were drug-free for at least 6 weeks before entry into the study. They were ascertained to be healthy by clinical examination, electrocardiography, and routine laboratory tests. Women were required to undergo pregnancy testing and were only enrolled if the result was negative and they were using a barrier contraceptive.

Study design. A randomized, double-blind, 2-way crossover study was carried out with single-dose administration of voriconazole and 4 doses of ritonavir or placebo. Polymorphisms of CYP2C19 were determined before the study, and participants were randomized within each genetic group as follows: group 1, *CYP2C19*1/*1* (n = 8, homozygous EMs); group 2, *CYP2C19*1/*2* or **1/*3* (n = 8, heterozygous EMs); and group 3, *CYP2C19*2/*2*, **2/*3*, or **3/*3* (n = 4, PMs).

The presence of the *CYP2C19*2* or **3* allele in the genomic deoxyribonucleic acid derived from leukocytes of the participants was determined by use of the

hybridization probe format (LightCycler CYP2C19 Mutation Detection Kit with specific primers; Roche Applied Science, Mannheim, Germany) on a LightCycler (Roche Applied Science). The presence of the wild-type allele *CYP2C19*1* was inferred from the absence of the **2* and **3* alleles.

After an overnight fast, participants received a standard breakfast, consisting of white bread, cheese, butter, and caffeine-free tea, 30 minutes before oral administration of the study drugs. Alcoholic and caffeinated beverages were not allowed from 12 hours before drug administration until the last blood sample was taken on the kinetic study day. Lunch and dinner consisted of regular hospital meals. During the whole study (days 1 to 10), beverages containing grapefruit juice were forbidden because of the known influence on CYP3A4 enzyme activity. Participants stayed at the Clinical Research Unit of the Department of Internal Medicine VI for 10 hours after drug administration. For safety reasons, electrocardiographic monitoring was carried out over a 4-hour period after voriconazole administration by use of a Surveyor II monitoring system (Mortara Instruments, Essen, Germany). Up to 48 hours after voriconazole administration, the participants returned to the Clinical Research Unit for blood sampling at the required times. During that period, participants maintained their usual daily activities.

After cannulation of a forearm vein, the participants received 300 mg ritonavir (3 capsules of Norvir, 100 mg; Abbott, Wiesbaden, Germany) or placebo (3 capsules). Immediately thereafter, they were administered 400 mg voriconazole orally (2 capsules of Vfend, 200 mg; Pfizer, Karlsruhe, Germany), together with 200 mL of mineral water. Ritonavir and placebo were administered 4 times with a 12-hour dosing interval to ensure effective ritonavir concentrations over the whole 48-hour observation period. Placebo capsules were prepared at the university hospital pharmacy. After a washout period of 7 days, the identical procedure was repeated with ritonavir and placebo interchanged. The washout period of 7 days for short-term ritonavir administration was used previously,[22] and recovery from CYP3A4 inhibition by ritonavir has recently been reported to be nearly complete at 3 days after discontinuation.[23]

Blood sampling and urine collection. On study days 1 and 8, blood samples (7.5 mL each) were taken at the following times: immediately before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 24, 25, 33, 36, and 48 hours after administration of voriconazole. Blood samples were immediately centrifuged at 4°C, and separated plasma was stored at −20°C until analysis. Urine was collected in two 24-hour intervals, and a 10-mL aliquot of each was kept frozen at −20°C until analysis.

Quantification of voriconazole. Voriconazole concentrations in plasma and urine were determined after solid-phase extraction on the basis of an HPLC assay[24] and optimized by use of mass-spectrometric detection (liquid chromatography–mass spectrometry), as described previously in detail.[4] The limit of quantification was 0.05 μg/mL, and the calibration ranged between 0.05 and 10.0 μg/mL, with coefficients of variation always being below 10%.

Calculations and statistic analysis. The sample size calculation was performed by use of PS Power and Sample Size Calculations for a 2-sided paired *t* test.[25] With an SD of 30% for the difference in voriconazole AUC with and without ritonavir assumed, a sample size of 6 was estimated to detect a 50% increase in voriconazole AUC with a significance level of 5% and a statistical power of 90%; for unpaired comparisons, the power was 74%. Data are presented as median values and range. Noncompartmental analysis by use of WinNonlin software (version 4.1; Pharsight, Mountain View, Calif) was performed to determine the following pharmacokinetic parameters of voriconazole: C_{max}, time to reach C_{max}, AUC from time 0 to the last measurable concentration ($AUC_{0\text{-}48}$), and AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), calculated by use of the linear trapezoidal rule, as well as terminal elimination half-life ($t_{½}$). The apparent oral clearance (Cl_{oral}) was calculated as the dose of voriconazole divided by $AUC_{0\text{-}\infty}$, and the volume of distribution based on the terminal phase was calculated as the dose of voriconazole divided by the elimination rate constant (λ_z) times $AUC_{0\text{-}\infty}$. Renal clearance (Cl_{renal}) of voriconazole was determined as the amount excreted in urine from 0 to 48 hours divided by the corresponding $AUC_{0\text{-}48}$ values, and nonrenal clearance ($Cl_{nonrenal}$) was determined as the difference between Cl_{oral} and Cl_{renal}. Differences in these pharmacokinetic parameters between placebo and ritonavir treatment were assessed by use of the nonparametric Wilcoxon matched pairs signed rank test. $P < .05$ was considered to be significant. The Kruskal-Wallis test with Dunn multiple comparisons was used to assess the differences between the CYP2C19 genotypes.

To predict steady-state concentrations of voriconazole (C_{ss}), the dose rate (400 mg/24 h) was divided by Cl_{oral}. Compartmental and noncompartmental analysis (WinNonlin software, version 4.1) results regarding clearance were used.

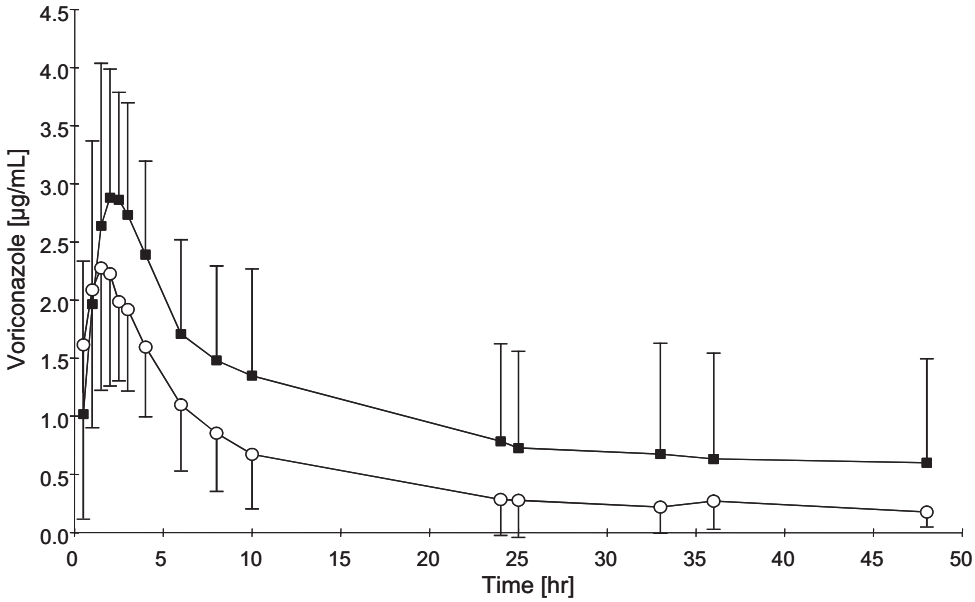

Fig 1. Mean (±SD) voriconazole plasma concentration–time profile after single oral administration of 400 mg voriconazole in combination with placebo (*circles*) or 300 mg ritonavir twice daily (*squares*) to 20 healthy subjects.

Table I. Pharmacokinetic parameters after noncompartmental analysis of voriconazole during placebo and ritonavir administration in 20 healthy individuals

	Placebo	*Ritonavir*	*P value*
t_{max} (h)	1.95 ± 1.31	2.03 ± 0.60	.762
C_{max} (µg/mL)	2.97 ± 1.07	3.49 ± 1.12	.015
$AUC_{0\text{-}48}$ (h · µg/mL)	23.50 ± 17.78	48.16 ± 38.81	.0001
$AUC_{0\text{-}\infty}$ (h · µg/mL)	25.26 ± 17.09	114.80 ± 221.17	.0001
V_z (L/kg)	3.56 ± 1.52	2.95 ± 1.78	.012
Cl_{oral} (mL/min)	354 ± 173	202 ± 139	.0001
Cl_{oral} ($mL \cdot min^{-1} \cdot kg^{-1}$)	4.85 ± 2.45	2.75 ± 1.89	.0001
$t_{½}$ (h)	9.5 ± 3.5	28.4 ± 42.6	.0001
MRT (h)	11.5 ± 5.8	39.2 ± 62.4	.0001
$Ae_{0\text{-}48}$ (µg)	1851 ± 1843	3020 ± 1551	.0003
Cl_{renal} (mL/min)	1.41 ± 0.66	1.39 ± 0.76	.475
$Cl_{nonrenal}$ (mL/min)	353 ± 172	201 ± 138	.0001

Data are given as mean ± SD. Difference between placebo and ritonavir treatment were assessed by use of the Wilcoxon matched pairs signed rank test.

t_{max}, Time to reach maximum observed plasma concentration; C_{max}, maximum observed plasma concentration; $AUC_{0\text{-}48}$, AUC from time 0 to last measurable concentration; $AUC_{0\text{-}\infty}$, AUC extrapolated to infinity; V_z, volume of distribution; Cl_{oral}, oral clearance; $t_{½}$, terminal elimination half-life; MRT, mean residence time; $Ae_{0\text{-}48}$, amount excreted in urine from 0 to 48 hours; Cl_{renal}, renal clearance; $Cl_{nonrenal}$, nonrenal clearance.

RESULTS

All subjects. Compared with placebo, mean voriconazole plasma concentrations were higher during ritonavir treatment, which was a persistent effect over the whole 48-hour observation period (Fig 1). Pharmacokinetic analysis showed significant increases in C_{max}, $AUC_{0\text{-}\infty}$ (25.26 ± 17.09 h · ng/mL versus 114.8 ± 221.2 h · ng/mL, $P < .0001$), and $t_{½}$ and decreases in Cl_{oral} (43%) and $Cl_{nonrenal}$, whereas Cl_{renal} was low and unchanged (Table I). The volume of distribution of voriconazole was slightly reduced (17.3%) by ritonavir (Table I).

CYP2C19 genotypes. Treatment with ritonavir increased voriconazole plasma concentrations in each CYP2C19 genotype group (Fig 2). The detailed pharmacokinetic parameters are given in Table II. The impact of ritonavir on voriconazole kinetics was related

Table II. Noncompartmental analysis of voriconazole pharmacokinetics during placebo and ritonavir according to CYP2C19 genotype

	**1/*1 (n = 8)*		
	Placebo	*Ritonavir*	*P value*
t_{max} (h)	1.38 ± 0.64	1.94 ± 0.42	.109
C_{max} (μg/mL)	2.97 ± 1.14	2.99 ± 1.17	.742
$t_{½}$ (h)	8.11 ± 1.35	11.15 ± 3.82	.055
$AUC_{0\text{-}48}$ (h · μg/mL)	15.86 ± 7.07	24.28 ± 12.12	.023
$AUC_{0\text{-}\infty}$ (h · μg/mL)	16.52 ± 7.21	25.50 ± 12.57	.016
V_z (L/kg)	4.38 ± 1.73	4.02 ± 2.35	.461
Cl_{oral} (mL/min)	463 ± 168	305 ± 112	.023
Cl_{oral} ($mL \cdot min^{-1} \cdot kg^{-1}$)	6.34 ± 2.47	4.15 ± 1.54	.023
MRT (h)	8.50 ± 2.10	12.61 ± 3.81	.008
$Ae_{0\text{-}48}$ (μg)	1186 ± 510	2282 ± 1112	.023
Cl_{renal} (mL/min)	1.36 ± 0.62	1.76 ± 0.84	.383
$Cl_{nonrenal}$ (mL/min)	461 ± 167	303 ± 112	.023

Data are given as mean ± SD. Difference between placebo and ritonavir treatment were assessed by use of the Wilcoxon matched pairs signed rank test, and differences between the genotype groups were calculated by use of the Kruskal-Wallis test with Dunn multiple comparisons.
†$P < .01$ for **1/*1* versus **2/*2* calculated by Kruskal-Wallis test with Dunn multiple comparisons.
‡$P < .01$ for **1/*2* versus **2/*2*.

to the number of inactive CYP2C19 alleles. In homozygous EMs some parameters such as $AUC_{0\text{-}\infty}$ and Cl_{oral} showed statistically significant differences, whereas in heterozygous EMs additional parameters ($t_{½}$, C_{max}, and volume of distribution) were also different (Table II). Voriconazole pharmacokinetics was profoundly affected by ritonavir in CYP2C19 PMs (Fig 2). This was best reflected by $t_{½}$, apparent Cl_{oral}, and $Cl_{nonrenal}$, whereas Cl_{renal} of voriconazole was very low and was not changed by ritonavir. Cl_{oral} in PMs decreased from 158 ± 54 to 22 ± 11 mL/min during ritonavir treatment. Fig 3 shows the genotype-dependent effect of ritonavir on the apparent Cl_{oral} of voriconazole with 34% and 45% reductions in homozygous and heterozygous EMs, respectively, and a blunted metabolism (86% reduction) in CYP2C19 PMs.

During placebo and ritonavir treatment, significant differences in voriconazole pharmacokinetics were observed between homozygous EM and PM individuals for $AUC_{0\text{-}48}$, $AUC_{0\text{-}\infty}$, Cl_{oral}, $Cl_{nonrenal}$, and mean residence time (MRT), respectively. Only MRT was different between heterozygous EM and PM individuals (Table II).

Within the genotype groups, no obvious influence of sex was observed. Three of the 8 homozygous EMs, 2 heterozygous EMs, and 2 PMs were women. The pharmacokinetic parameters of the women were well within the range of the corresponding CYP2C19 group.

Safety and tolerability. Overall, the study drugs were well tolerated; no serious adverse drug events (ADEs) occurred, and all ADEs were mild and transient. Thirteen participants reported ADEs, 4 of whom (1 homozygous EM, 1 heterozygous EM, and 2 PMs) had ADEs in both trial phases. ADEs were observed during voriconazole and placebo administration in 10 participants and during voriconazole and ritonavir administration in 7 individuals. Visual disturbances occurred on 8 occasions, with each PM being affected during at least 1 study part. They started 1 to 2 hours after voriconazole dosing and lasted, on average, 30 minutes. Other ADEs were headaches and gastrointestinal disturbances.

DISCUSSION

The results of this study have several important implications. First, there is a reduction in voriconazole clearance in relation to the CYP2C19 genotype (25% in heterozygous EMs and 65% in PMs) after single oral dosing, confirming the recently published data of Rengelshausen et al.[4] Second, short-term administration of ritonavir caused a major reduction in voriconazole apparent Cl_{oral}, probably as a result of CYP3A4 inhibition of voriconazole metabolism. Third, this reduction occurred in all CYP2C19 genotype groups with a similar absolute magnitude (reduction in Cl_{oral} of 158 mL/min in EMs, 152 mL/min in heterozygous EMs, and 136 mL/min in PMs).

This study therefore highlights the very large interindividual variability of voriconazole pharmacokinetics, whose clearance is critically determined by genetic

*1/*2 (n = 8)			*2/*2 (n = 4)	
Placebo	*Ritonavir*	*P value*	*Placebo*	*Ritonavir*
2.13 ± 1.06	2.00 ± 0.76	.844	2.75 ± 2.33	2.25 ± 0.65
2.85 ± 0.73	3.65 ± 0.65	.008	3.24 ± 1.70	4.20 ± 1.56
8.07 ± 2.22	11.51 ± 3.52	.008	15.21 ± 3.06†	96.8 ± 60.1†
20.68 ± 10.27	40.55 ± 18.65	.008	42.40 ± 19.51†	111.1 ± 37.7†
22.65 ± 10.88	44.05 ± 23.01	.008	47.96 ± 23.33†	434.9 ± 370.1†
3.13 ± 1.41	2.33 ± 0.93	.023	2.80 ± 0.11	2.05 ± 0.23
343 ± 127	190 ± 93	.008	158 ± 54†	22 ± 11†
4.69 ± 1.88	2.58 ± 1.28	.008	2.21 ± 0.53†	0.30 ± 0.13†
9.67 ± 3.26	14.79 ± 6.46	.008	21.20 ± 4.65†	141.3 ± 84.7†‡
2242 ± 1654	3180 ± 1644	.195	2398 ± 1357	4173 ± 1660
1.71 ± 0.73	1.41 ± 0.62	.195	0.92 ± 0.19	0.63 ± 0.19
341 ± 127	189 ± 93	.008	157 ± 54†	21 ± 11†

factors and also substantially influenced by exogenous factors such as coadministered drugs. Short-term administration of the protease inhibitor ritonavir had a major impact on voriconazole elimination. On average, a 42% reduction in the apparent Cl_{oral} was observed. On the one hand, this effect was not surprising because ritonavir is a very potent CYP3A4 inhibitor (inhibition constant [K_i] = 0.017 μmol/L).[15] On the other hand, it was proposed from in vitro data that CYP3A4 is not the main pathway of voriconazole elimination.[1] The Michaelis-Menten constant (K_m) of voriconazole oxidase activity in expressed human CYP enzymes was 4 μmol/L for CYP2C19 and 235 μmol/L for CYP3A4,[1] which indicates a very low affinity to CYP3A4. When the K_m and K_i values of these substances (K_m for CYP3A4 metabolism of voriconazole, 236 μmol/L; K_i for CYP3A4 inhibition by ritonavir, 0.078 μmol/L[26]; and K_i for CYP3A4 inhibition by erythromycin, 46.6 μmol/L[27]) were taken into account, some interaction with ritonavir was expected, but its exact extent was not predictable. Interestingly, in vivo no interaction was observed between voriconazole and erythromycin, another potent CYP3A4 inhibitor.[13] Therefore it was somewhat unexpected to have such a profound interaction with ritonavir.

In line with 2 recent reports[4,12] the presence of deficient *CYP2C19*2* alleles resulted in higher AUC and lower apparent Cl_{oral} values of voriconazole compared with the *CYP2C19* wild-type group. To further elucidate the mechanism of this clearance reduction, separate analysis of ritonavir's effects according to the different CYP2C19 genotypes can be helpful. Because ritonavir is not known to be a substrate or inhibitor of CYP2C19, there should be a similar effect of ritonavir in each of the 3 CYP2C19 genotype groups, which was indeed the case because the absolute reduction in the apparent Cl_{oral} of voriconazole by ritonavir was rather similar across all 3 genotypes. In view of these data it seems likely that ritonavir does inhibit voriconazole metabolism not via CYP2C19 but rather via CYP3A4. This is in accordance with recent in vitro findings which revealed that ketoconazole was most potent as an inhibitor of voriconazole metabolism in human liver microsomes of CYP2C19 PMs, suggesting a dominant role of CYP3A4 in these individuals.[1] Because in PMs the Cl_{oral} of voriconazole alone was already low, coadministration of ritonavir almost abolished voriconazole elimination to 22 ± 11 mL/min. This is only 4.75% of the voriconazole clearance observed in EMs without ritonavir and an 86% reduction compared with the change of only 34% in EMs. Hence the effect of CYP3A4 inhibition is of major consequence in CYP2C19 PMs.

Despite a slightly smaller volume of distribution, the reduction in Cl_{oral} in PMs by ritonavir caused a prolongation of the terminal elimination half-life from approximately 8 hours in EMs without ritonavir to approximately 100 hours in PMs during ritonavir administration. Voriconazole pharmacokinetics was assessed during continuous short-term exposure with

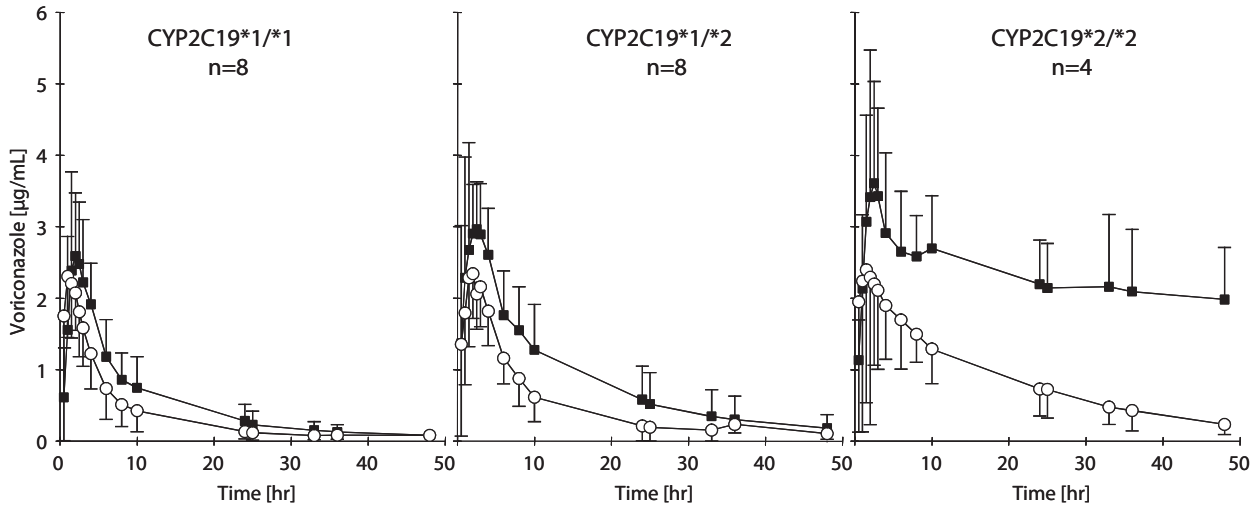

Fig 2. Mean (±SD) voriconazole plasma concentrations after single oral administration of 400 mg voriconazole in combination with placebo (*circles*) and 300 mg ritonavir twice daily (*squares*) to *CYP2C19*1/*1* (n = 8), *CYP2C19*1/*2* (n = 8), and *CYP2C19*2/*2* (n = 4) subjects.

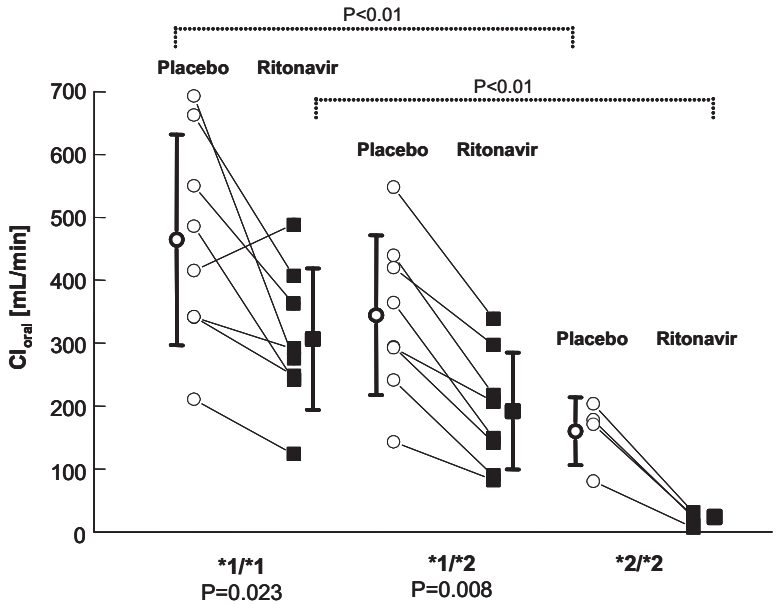

Fig 3. Mean (±SD) and individual apparent oral voriconazole clearance (Cl_{oral}) after single oral administration of 400 mg voriconazole in combination with placebo (*circles*) or 300 mg ritonavir twice daily (*squares*) to *CYP2C19*1/*1* (n = 8), *CYP2C19*1/*2* (n = 8), and *CYP2C19*2/*2* (n = 4) participants. *P* values between placebo and ritonavir were obtained by use of the Wilcoxon matched pairs signed rank test. *P* values among the 3 CYP2C19 genotypes were calculated by use of the Kruskal-Wallis test with Dunn multiple comparisons.

ritonavir to ensure maximum enzyme inhibition throughout the trial. However, we administered voriconazole as a single dose because we expected quite a substantial interaction to occur at least in CYP2C19 PMs and attempted to avoid the risk for adverse effects. Indeed, extrapolation of the clearance data of the

CYP2C19 PMs to voriconazole steady state suggests that in these volunteers the combination of a potent CYP3A4 inhibitor with voriconazole will result in a C_{ss} value of 18.3 ± 15.7 μg/mL when regular daily doses of 400 mg are administered and linear kinetics is assumed. Compared with CYP2C19 EM individuals without a concomitant CYP3A4 inhibitor (C_{ss} <1 μg/mL), this will be at least a 20-fold increase. With a frequency of CYP2C19 PMs of 2.2% in white persons and 15.8% in Asian persons,[10] this becomes even more relevant in the Asian population. Whether, under steady-state conditions with ritonavir, such a substantial voriconazole accumulation will indeed occur is, however, still undetermined. Prolonged ritonavir exposure inhibited CYP3A4-mediated rifabutin metabolism,[20] leading to increased concentrations; on the other hand, ritonavir may cause induction of CYP1A2 and hepatic glucuronidation,[28] as well as autoinduction with higher doses,[29] thereby possibly counteracting the inhibitory effect of the compound.

Hepatotoxicity is the dose-limiting adverse event observed in patients taking voriconazole.[30] Clinically significant hepatic dysfunction occurs in more than 10% of patients receiving regular doses and is a major cause for discontinuation of the drug. Hepatotoxicity is concentration-dependent,[31] and it has been estimated that for every 1-mg/L increase in plasma concentration the incidence of hepatic dysfunction increases by 7% to 17%.[32] These data therefore suggest that the high concentrations observed in our CYP2C19 PMs will substantially increase the risk for adverse events in this subgroup of patients. It has been suggested that voriconazole plasma concentrations grater than 10 μg/mL should be avoided.[33] In addition, a retrospective study with voriconazole concentrations from 1053 patients showed an association of higher voriconazole concentrations with the risk of visual adverse events and abnormalities of AST, alkaline phosphatase, and bilirubin levels.[34] These findings, together with the results of this study, therefore suggest that in CYP2C19 PM individuals taking voriconazole, ritonavir and other potent CYP3A4 inhibitors should be used with caution. Concomitant therapy of voriconazole with other efficient CYP3A4 inhibitors likely requires a dose reduction in voriconazole and careful monitoring of adverse drug reactions. Indeed, a case of fatal liver failure in a child receiving voriconazole in combination with highly active antiretroviral therapy (including lopinavir and ritonavir) has recently been reported.[35] Unfortunately, the CYP2C19 genotype was not determined and voriconazole concentrations were not quantified, leaving us uncertain as to whether this patient was a PM and whether voriconazole concentrations were elevated.

Because CYP2C19 genetic analysis for this study included only the alleles *2 and *3, which account for more than 85% of defective CYP2C19 alleles in white persons,[36] the occurrence of other defective alleles in the study population and thus misclassification of individuals cannot be ruled out.

Another limitation of this study is that ritonavir was only dosed to steady state but not for a prolonged period of time. This would be critical only if a longer duration of exposure would induce substantial changes in either bioavailability or the capacity of clearance processes (or both). In an earlier study whose results are available in abstract form, voriconazole (200 mg twice daily for 10 days) was added to ritonavir treatment on day 10 (400 mg twice daily for 20 days).[37] It was reported that ritonavir decreased the exposure with voriconazole substantially. There is, however, too little information given to elucidate the mechanisms of interaction and to differentiate between impaired absorption and increased elimination (eg, enzyme induction), whereas our results can be followed by the known CYP inhibition of ritonavir. In contrast, it is well known that even after prolonged exposure of combinations of ritonavir with other protease inhibitors, CYP3A4 inhibition persists, indicating that even if enzyme induction occurs the dominant effect is still CYP3A4 inhibition.[38]

In conclusion, CYP3A4 inhibition by short-term ritonavir treatment increases voriconazole concentrations after single oral administration. It appears likely that other strong inhibitors (eg, other protease inhibitors) will have a similar effect. Whereas ketoconazole coadministration is not a clinically relevant combination, it might now be more interesting to assess the impact of grapefruit juice on voriconazole pharmacokinetics. This, however, is expected to modulate only bioavailability and not elimination. Erythromycin has only been studied in 1 PM thus far, but no details are available.[13] Individuals with CYP2C19 deficiency might be at risk for the development of elevated voriconazole concentrations when undergoing concomitant short-term therapy with ritonavir, together with more frequent and severe side effects.

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References

1. Hyland R, Jones BC, Smith DA. Identification of the cytochrome P450 enzymes involved in the N-oxidation of voriconazole. Drug Metab Dispos 2003;31:540-7.
2. Purkins L, Wood N, Kleinermans D, Nichols D. Histamine H2-receptor antagonists have no clinically significant effect on the steady-state pharmacokinetics of voriconazole. Br J Clin Pharmacol 2003;56(Suppl 1):51-5.
3. Purkins L, Wood N, Ghahramani P, Love ER, Eve MD, Fielding A. Coadministration of voriconazole and phenytoin: pharmacokinetic interaction, safety, and toleration. Br J Clin Pharmacol 2003;56(Suppl 1):37-44.
4. Rengelshausen J, Banfield M, Riedel K-D, Burhenne J, Weiss J, Thomsen T, et al. Opposite effects of short-term and long-term St John's wort intake on voriconazole pharmacokinetics. Clin Pharmacol Ther 2005; 78:25-33.
5. Niwa T, Shiraga T, Takagi A. Effect of antifungal drugs on cytochrome P450 (CYP) 2C9, CYP2C19, and CYP3A4 activities in human liver microsomes. Biol Pharm Bull 2005;28:1805-8.
6. Pai MP, Allen S. Voriconazole inhibition of tacrolimus metabolism. Clin Infect Dis 2003;36:1089-91.
7. Venkataramanan R, Zang S, Gayowski T, Singh N. Voriconazole inhibition of the metabolism of tacrolimus in a liver transplant recipient and in human liver microsomes. Antimicrob Agents Chemother 2002;46:3091-3.
8. Groll AH, Kolve H, Ehlert K, Paulussen M, Vormoor J. Pharmacokinetic interaction between voriconazole and ciclosporin A following allogeneic bone marrow transplantation. J Antimicrob Chemother 2004;53:113-4.
9. Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol 2001;52:349-55.
10. Shimizu T, Ochiai H, Asell F, Shimizu H, Saitoh R, Hama Y, et al. Bioinformatics research on inter-racial difference in drug metabolism I. Analysis on frequencies of mutant alleles and poor metabolizers on CYP2D6 and CYP2C19. Drug Metab Pharmacokinet 2003;18:48-70.
11. Wood N, Tan K, Purkins L, Layton G, Hamlin J, Kleinermans D, et al. Effect of omeprazole on the steady-state pharmacokinetics of voriconazole. Br J Clin Pharmacol 2003;56(Suppl 1):56-61.
12. Ikeda Y, Umemura K, Kondo K, Sekiguchi K, Miyoshi S, Nakashima M. Pharmacokinetics of voriconazole and cytochrome P450 2C19 genetic status. Clin Pharmacol Ther 2004;75:587-8.
13. Purkins L, Wood N, Ghahramani P, Kleinermans D, Layton G, Nichols D. No clinically significant effect of erythromycin or azithromycin on the pharmacokinetics of voriconazole in healthy male volunteers. Br J Clin Pharmacol 2003;56(Suppl 1):30-6.
14. Eagling VA, Back DJ, Barry MG. Differential inhibition of cytochrome P450 isoforms by the protease inhibitors, ritonavir, saquinavir and indinavir. Br J Clin Pharmacol 1997;44:190-4.
15. Kumar GN, Rodrigues AD, Buko AM, Denissen JF. Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. J Pharmacol Exp Ther 1996;277:423-31.
16. Koudriakova T, Iatsimirskaia E, Utkin I, Gangl E, Vouros P, Storozhuk E, et al. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: mechanism-based inactivation of cytochrome P4503A by ritonavir. Drug Metab Dispos 1998;26:552-61.
17. Ernest CS, Hall SD, Jones DR. Mechanism-based inactivation of CYP3A by HIV protease inhibitors. J Pharmacol Exp Ther 2005;312:583-91.
18. Greenblatt DJ, von Moltke LL, Harmatz JS, Durol AL, Daily JP, Graf JA, et al. Differential impairment of triazolam and zolpidem clearance by ritonavir. J Acquir Immune Defic Syndr 2000;24:129-36.
19. Ouellet D, Hsu A, Granneman GR, Carlson G, Cavanaugh J, Guenther H, et al. Pharmacokinetic interaction between ritonavir and clarithromycin. Clin Pharmacol Ther 1998;64:355-62.
20. Cato A III, Cavanaugh J, Shi H, Hsu A, Leonard J, Granneman R. The effect of multiple doses of ritonavir on the pharmacokinetics of rifabutin. Clin Pharmacol Ther 1998;63:414-21.
21. Malaty LI, Kuper JJ. Drug interactions of HIV protease inhibitors. Drug Saf 1999;20:147-69.
22. Greenblatt DJ, von Moltke LL, Harmatz JS, Fogelman SM, Chen G, Graf JA, et al. Short-term exposure to low-dose ritonavir impairs clearance and enhances adverse effects of trazodone. J Clin Pharmacol 2003;43: 414-22.
23. Culm-Merdek KE, von Moltke LL, Gan L, Horan KA, Reynolds R, Harmatz JS, et al. Effect of extended exposure to grapefruit juice on cytochrome P450 3A activity in humans: comparison with ritonavir. Clin Pharmacol Ther 2006;79:243-54.
24. Pennick GJ, Clark M, Sutton DA, Rinaldi MG. Development and validation of a high-performance liquid chromatography assay for voriconazole. Antimicrob Agents Chemother 2003;47:2348-50.
25. Dupont WD, Plummer WD. *PS* power and sample size program available for free on the internet [letter]. Control Clin Trials 1997;18:274.
26. von Moltke LL, Durol AL, Duan SX, Greenblatt DJ. Potent mechanism-based inhibition of human CYP3A in vitro by amprenavir and ritonavir: comparison with ketoconazole. Eur J Clin Pharmacol 2000;56:259-61.
27. Zhou S, Yung CS, Cher Goh B, Chan E, Duan W, Huang M, et al. Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs. Clin Pharmacokinet 2005;44:279-304.

28. Kakuda TN, Struble KA, Piscitelli SC. Protease inhibitors for the treatment of human immunodeficiency virus infection. Am J Health Syst Pharm 1998;55:233-54.
29. Hsu A, Granneman GR, Witt G, Locke C, Denissen J, Molla A, et al. Multiple-dose pharmacokinetics of ritonavir in human immunodeficiency virus-infected subjects. Antimicrob Agents Chemother 1997;41:898-905.
30. FDA Antiviral Drugs Advisory Committee. Briefing document for voriconazole (oral and intravenous formulations). October 4, 2001. Available at: http://www.fda.gov/ohrms/dockets/ac/01/briefing/3792b2_01_Pfizer.pdf. Accessed March 20, 2006.
31. Boucher HW, Groll AH, Chiou CC, Walsh TJ. Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. Drugs 2004;64:1997-2020.
32. Lutsar I, Hodges MR, Tomaszewski K, Troke PF, Wood ND. Safety of voriconazole and dose individualization. Clin Infect Dis 2003;36:1087-8.
33. Denning DW, Ribaud P, Milpied N, Caillot D, Herbrecht R, Thiel E, et al. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. Clin Infect Dis 2002;34:563-71.
34. Tan K, Brayshaw N, Tomaszewski K, Troke P, Wood N. Investigation of the potential relationships between plasma voriconazole concentrations and visual adverse events or liver function test abnormalities. J Clin Pharmacol 2006;46:235-43.
35. Scherpbier HJ, Hilhorst MI, Kuijpers TW. Liver failure in a child receiving highly active antiretroviral therapy and voriconazole. Clin Infect Dis 2003;37:828-30.
36. Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmacokinet 2002;41:913-58.
37. Liu P, Foster G, Labadie R, Allison MJ, Sharma A. Pharmacokinetic interaction between voriconazole and ritonavir at steady state in healthy subjects [abstract]. Clin Pharmacol Ther 2005;77:P40.
38. Ribera E, Lopez RM, Diaz M, Pou L, Ruiz L, Falco V, et al. Steady-state pharmacokinetics of a double-boosting regimen of saquinavir soft gel plus lopinavir plus minidose ritonavir in human immunodeficiency virus-infected adults. Antimicrob Agents Chemother 2004;48:4256-62.