ORIGINAL RESEARCH ARTICLE

CYP3A and CYP2C19 Activity Determined by Microdosed Probe Drugs Accurately Predict Voriconazole Clearance in Healthy Adults

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Abstract

Background and Objective Voriconazole is an important broad-spectrum anti-fungal drug with nonlinear pharmacokinetics. The aim of this single centre fixed-sequence open-label drug–drug interaction trial in healthy participants ($N = 17$) was to determine whether microdosed probe drugs for CYP3A and CYP2C19 reliably predict voriconazole clearance (CL_{VRZ}).
Methods At baseline, a single oral microdose of the paradigm substrates midazolam (CYP3A) and omeprazole (CYP2C19) were given to estimate their clearances (CL). Thereafter, a single oral dose of voriconazole was administered (50, 100, 200 or 400 mg), followed by the microdosed probe drugs.
Results The clearances of midazolam (CL_{MDZ} 790–2790 mL/min at baseline; 248–1316 mL/min during voriconazole) and omeprazole (CL_{OMZ} 66.4–2710 mL/min at baseline; 30.1–1420 mL/min during voriconazole) were highly variable. CL_{MDZ} [geometric mean ratio (GMR) 0.586 at 50 mg voriconazole decreasing to GMR 0.196 at 400 mg voriconazole] and CL_{OMZ} (GMR 0.590 at 50 mg decreasing to GMR 0.166 at 400 mg) were reduced with higher voriconazole doses. CL_{MDZ} was linearly correlated with CL_{VRZ} (slope 1.458; adjusted R^2 0.528) as was CL_{OMZ} (slope 0.807; adjusted R^2 0.898). Multiple linear regression resulted in an adjusted R^2 of 0.997 for the relationship $CL_{VRZ} \sim \log CL_{OMZ} + \log CL_{MDZ}$ using data during voriconazole treatment and an adjusted R^2 of 0.997 for the relationship $CL_{VRZ} \sim \log CL_{OMZ} + \log CL_{MDZ} +$ voriconazole dose, using baseline data for CL_{MDZ} and CL_{OMZ}.
Conclusion Microdosed midazolam and omeprazole accurately described and predicted total CL_{VRZ}
Trial Registration EudraCT No: 2020-001017-20, registered on March 5th, 2020. DRKS: DRKS00022547, registered on August 6th, 2020.

1 Introduction

Voriconazole is a second generation triazole antifungal agent that is on the World Health Organization (WHO) essential medicines list [1, 2]. Its broad spectrum of activity, high tissue penetration and particular efficacy against *Aspergillus* make voriconazole a cornerstone of prevention and treatment of invasive fungal infections in vulnerable patient populations, such as patients with neutropenia or immunosuppression after stem cell or solid organ transplantation [3–6].

However, due to the complex pharmacokinetics of voriconazole, it is a challenge in clinical practice to reliably and quickly achieve therapeutic antifungal exposures in individual cases [7]. Voriconazole has non-linear and highly variable pharmacokinetics; it is metabolised by cytochrome P450 (CYP) isozymes and is therefore susceptible to

numerous drug–drug interactions, some of which are also genotype dependent [8]. CYP2C19 and CYP3A appear to play a major role, but the extent of their involvement in vivo and possible contributions of CYP2C9 and the family of flavin-dependent monooxygenases (FMO3) are quantitatively uncertain [7]. At the same time, voriconazole and its major N-oxide metabolite are inhibitors of CYP3A and CYP2C19 and likely cause concomitant enzyme induction, because increasing doses of voriconazole are required over time to maintain stable plasma concentrations [9–11]. Furthermore, CYP2C19 polymorphisms substantially modulate the metabolism of voriconazole and its interaction potential, and co-morbidities such as systemic inflammation further modulate voriconazole clearance (CL_{VRZ}) [8, 12–15].

In this trial, we aimed to mechanistically investigate the relative contribution of CYP2C19 and CYP3A to the clearance of voriconazole using an in vivo approach with microdosed probe drugs to quantify CYP activities [16, 17].

Extended author information available on the last page of the article

Key Points

This in vivo study aimed to investigate the contribution of CYP2C19 and CYP3A activity to oral voriconazole clearance using microdosed probe drugs for CYP2C19 (omeprazole) and CYP3A (midazolam) and different voriconazole doses.

Multiple linear regression demonstrated that omeprazole and midazolam clearance accurately describe voriconazole clearance when given together, and that their clearances together with voriconazole dose can predict voriconazole clearance precisely.

After oral administration, voriconazole clearance exclusively depends on the activity of CYP2C19 and to a lesser extent on CYP3A, indicating that other enzymes have no or only a minor contribution to its metabolism in vivo in healthy volunteers.

Because the pharmacokinetics of microdosed midazolam and omeprazole are linear, they reflect isozyme activities at therapeutic doses well [16–18], without causing interactions and with minimal risk of adverse events (AE) [19]. In this trial, different doses of voriconazole were used and participants were enrolled regardless of their CYP2C19 genotype to cover a spectrum of CL_{VRZ} values as broad as possible and to investigate its association with CYP3A and CYP2C19 activity.

2 Materials and Methods

The trial protocol was approved by the competent authority (BfArM, Bonn) in Germany (EudraCT No: 2020-001017-20) and the responsible ethics committee of the Medical Faculty of Heidelberg University on 13 July 2020. The trial was conducted in the DIN EN ISO9001-certified Clinical Research Unit (KliPS) of the Department of Clinical Pharmacology and Pharmacoepidemiology at Heidelberg University Hospital according to the standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice) and in compliance with the Declaration of Helsinki and all specific legal requirements in Germany. Participants were only enrolled into the trial after they had given their written informed consent.

2.1 Study Population

All participants were physically and mentally healthy as confirmed by a thorough medical history, physical exam, blood pressure measurement, a 12-lead electrocardiogram

and standard laboratory analyses, including a urine drug screen and a pregnancy test (in women of childbearing potential). Participants were required to consent to use a highly effective method of contraception during the trial.

Participants were excluded if any of the following criteria were met: clinically relevant abnormalities in the medical history, physical examination or laboratory evaluation as assessed by the investigator, any medical disorder that may require significant treatment, or make the participant unlikely to fully complete the trial, or any condition that presents undue risk from the investigational medicinal products or trial interventions, clinically relevant ongoing or past history of physical or psychiatric illness as judged by the investigator, pregnancy or breast feeding, any acute or chronic illness or clinically relevant finding known or expected to modify the absorption, distribution, metabolism, or excretion of voriconazole, omeprazole, or midazolam, including the use of any co-medications or consumption of known inducers (including St. John's Wort) in the past 2 weeks or inhibitors of the CYP of interest such as grapefruit, and finally any known allergies to the specific trial medication, triazole derivatives in general, or additives.

2.2 Genotyping

Prior to assigning the voriconazole dose group, CYP2C19 genotyping was performed for *CYP2C19*2* (*rs4244285*), *CYP2C19*3* (*rs4986893*), and *CYP2C19*17* (*rs12248560*) as described previously [20]. The presence of two wild-type alleles was assumed if none of the tested polymorphisms was present.

2.3 Study Design

This was a single centre fixed sequence open-label four-arm phase I trial in healthy volunteers. The trial included a screening visit, two treatment visits 3–7 d apart, and an end-of-trial visit.

At baseline, participants were all administered oral microdoses of midazolam (10 μg) and omeprazole (100 μg). For the preparation of midazolam, 0.01 mL Dormicum® V 5 mg/5 mL (Cheplapharm Arzneimittel GmbH, Greifswald, Germany) was added to 100 mL of tap water [21]. Because uncoated omeprazole (OMEP® 40 mg HEXAL powder for solution for infusion, Hexal AG, Holzkirchen, Germany) is subject to degradation in gastric acid, the powder was dissolved in 100 mL of normal saline and 250 μL of the solution were further diluted in 100 mL of sodium bicarbonate buffer (4.2 %, w/v). In addition, 10 min prior to oral administration of the omeprazole microdose, participants drank 100 mL of sodium bicarbonate buffer [17]. Blood was collected in 4.9 mL lithium–heparin tubes before and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 h

after drug administration. Midazolam and omeprazole were given in a fasted state. Fluid intake was prohibited during the first 2 h after administration of the trial medication, and food for the first 4 h. Thereafter, participants were served a breakfast.

On the second trial day, participants were given a single dose of either 50, 100, 200, or 400 mg of voriconazole (Voriconazol Hexal® 50 mg film tablets, Hexal AG, Holzkirchen, Germany). Participants were sequentially assigned to voriconazole doses with four participants per dose, regardless of the CYP2C19 genotype, except for CYP2C19 poor metabolisers who were always assigned 400 mg voriconazole. One hour after voriconazole, participants were given midazolam 10 μg and omeprazole 100 μg as on the baseline day. Blood samples were collected in 7.5 mL lithium-heparin tubes before and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 9, 11 and 24 h after voriconazole administration. As on day 1, participants arrived fasted, were allowed to drink water 2 h after the microdoses, and were given a breakfast after 4 h. All samples were centrifuged within 30 min at 2500g and 4 °C for 10 min, distributed to 2 aliquots per substance and sample, and stored at − 20 °C until analysis.

2.4 Quantification of Midazolam, Omeprazole, and Voriconazole

Midazolam and omeprazole plasma concentrations were quantified by ultra-high performance liquid chromatography coupled to tandem mass spectrometry with a lower limit of quantification (LLOQ) of 1 pg/mL for midazolam [22] and 10 pg/mL for omeprazole [20]. Voriconazole concentrations were quantified using a validated high-performance liquid chromatography coupled to tandem mass spectrometry method, with a LLOQ of 1 ng/mL [23]. All methods were validated according to the current US Food and Drug Administration (FDA) and European Medical Agency (EMA) standards [24, 25].

2.5 Data Analysis

The primary endpoint was the correlation of CL_{MDZ} and CL_{OMZ} with CL_{VRZ} after a single oral voriconazole dose of 50, 100, 200 or 400 mg. No formal sample size calculation was performed. Secondary endpoints were the pharmacokinetics of midazolam, omeprazole and voriconazole, and the frequency, severity, seriousness, relatedness, expectedness and outcome of AE.

Standard non-compartmental pharmacokinetic parameters for all substances were determined using Phoenix WinNonlin™ version 8.3 (Certara Inc., Princeton, NJ, USA). This included maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), terminal elimination half-life ($t_{1/2}$), area under the plasma concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$) and apparent oral clearance (CL/F). Descriptive statistics of pharmacokinetic parameters were calculated. Paired t tests of the geometric mean ratios on log-transformed data were performed to assess pharmacokinetic differences at baseline and under different doses of voriconazole.

Linear regression analysis was used to individually assess the relationships between CL_{VRZ} and clearance of the probe drugs at baseline and during voriconazole treatment. To analyse skewed non-normally distributed data, the analysis was carried out using a log–log linear equation.

Multiple linear regression analysis was used to assess whether a better fit could be achieved when the variables were combined. CL_{VRZ} was defined as the dependent variable and CL_{MDZ}, CL_{OMZ} and voriconazole dose as independent variables as described in Eq. (1), where β_1, β_2 and β_3 are the regression coefficient estimates resulting from the analysis.

$$CL_{VRZ} \sim \beta_1 CL_{OMZ} + \beta_2 CL_{MDZ} + \beta_3 \text{voriconazole dose.} \quad (1)$$

The analysis was performed first with CL_{OMZ} alone, then after systematically including the other variables. The regression was also performed after log transformation of CL_{VRZ}, CL_{OMZ} and CL_{MDZ}. The most suitable models were selected by comparing the adjusted R^2 and Akaike's information criterion corrected for sample size (AICc). To assess predictive performance, predictive R^2 (i.e., the correlation coefficient between predicted and observed values) was calculated in-sample and out-of-sample via enhanced non-parametric bootstrap from 1000 bootstrap samples. All statistical analyses were performed using Prism Version 9.0.1 (GraphPad Software Inc., La Jolla, CA, USA), R Version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria), and a p value < 0.05 was considered statistically significant.

3 Results

Seventeen participants [8 females; all Caucasians; 1 ultra-rapid (*17/*17), 7 rapid (*1/*17), 5 normal (*1/*1), 3 intermediate (*1/*2, *2/*17), and 1 poor metaboliser (*2/*2)] were enrolled and included in all analyses; each dose group was assigned four participants irrespective of the genotype, one poor metaboliser was assigned to the 400 mg group, which therefore had five participants. Their mean age was 31 years (range: 22–50) and body mass index was 24.6 kg/m^2 (range 19.3–29.2). All participants completed the trial.

3.1 Midazolam

At baseline, CL_{MDZ} was highly variable ranging from 790 to 2790 mL/min. Coadministration of voriconazole

dose-dependently increased midazolam plasma concentrations over time and prolonged its terminal half-life in all participants (Fig. 1, Table 1). Midazolam $AUC_{0-\infty}$ showed an increase by a factor of 1.58, 2.42, 3.0 and 5.24 compared with baseline. Terminal elimination half-life was dose dependently prolonged by voriconazole with a 2.1-fold increase at 400 mg. During voriconazole, CYP3A was inhibited and CL_{MDZ} was reduced in all participants ranging from 248 to 1316 mL/min. Concurrently, CL_{MDZ} were reduced to 58.6% of the baseline value (50 mg), 42.6% (100 mg), 34.1% (200 mg) and 19.6% (400 mg) (Fig. 2; Table 2).

3.2 Omeprazole

The pharmacokinetics of microdosed omeprazole was highly variable with CL_{OMZ} ranging from 66.4 to 2710 mL/min at baseline. Dependent on the voriconazole dose CL_{OMZ} decreased in every participant (Fig. 3; Table 1). With the 50 mg voriconazole dose omeprazole $AUC_{0-\infty}$ was increased by a factor of 1.3 compared with baseline and by a factor of 12.1 with the 400 mg dose (Table 1). Correspondingly, CL_{OMZ} during 400 mg voriconazole was reduced to only 8.3 % compared with baseline (Fig. 2; Table 2). Interestingly, the CYP2C19 poor metaboliser had a an $AUC_{0-\infty}$ of 25.1 h ng/mL and a CL_{OMZ} of 66.4 mL/min at baseline, which was altered by 400 mg voriconazole to 55.4 h ng/mL and 30.1 mL/min. The ultra-rapid metaboliser showed an $AUC_{0-\infty}$ of 0.62 h ng/mL and a CL_{OMZ} of 2712 mL/min at baseline, which was altered by 100 mg voriconazole to 2.22 h ng/mL and 711 mL/min, respectively.

3.3 Voriconazole

The plasma concentration–time curves of the four voriconazole dose groups and the corresponding pharmacokinetics are shown in Fig. 4, Table 1 and Supplementary Table S1. Voriconazole was rapidly absorbed (t_{max} 0.66 h). C_{max} and $AUC_{0-\infty}$ showed a disproportionate increase after the three stepwise dose doublings, with C_{max} increasing twofold, 6.7-fold and 1.6-fold for each doubling of the dose from 50 to 400 mg, while $AUC_{0-\infty}$ showed an increase by a factor of 2.2, 6.2 and 4.3. Compared with the next lower dose group, CL_{VRZ} decreased by 9.4%, 67.8% and 54%, respectively, and CL_{VRZ} at 400 mg of voriconazole was reduced to 13.3% of CL_{VRZ} at 50 mg. Furthermore, $t_{1/2}$ increased by 15.4%, 23.4%, and 27.4% with each dose step. CL_{VRZ} values varied 44-fold across the different dose groups with the lowest clearance observed in the CYP2C19 PM with 200 mL/min and the highest in the 50 mg dose group (8860 mL/min). The ultra-rapid metaboliser had a clearance of 3602 mL/min after 100 mg voriconazole.

3.4 Bivariate Clearance Relationships

Linear regression analyses with the clearance data collected during voriconazole coadministration resulted in a slope of 1.458 ($p < 0.001$) with an adjusted R^2 of 0.528 for the relationship of CL_{VRZ} and CL_{MDZ}, and a slope of 0.807 ($p < 0.0001$) with an adjusted R^2 of 0.898 for CL_{VRZ} and CL_{OMZ}. Using the clearance data for midazolam and omeprazole at baseline revealed slopes of 0.612 ($p = 0.488$) (CL_{MDZ}) and 0.846 ($p < 0.001$) (CL_{OMZ}) with adjusted R^2 values of -0.032 (CL_{MDZ}) or 0.617 (CL_{OMZ}).

Fig. 1 Semilogarithmic plot of mean ($\pm$ standard deviation) plasma concentration–time curves of midazolam (10 µg) at baseline (grey) and after co-administration of a single dose of either 50, 100, 200 or 400 mg voriconazole (VRZ)
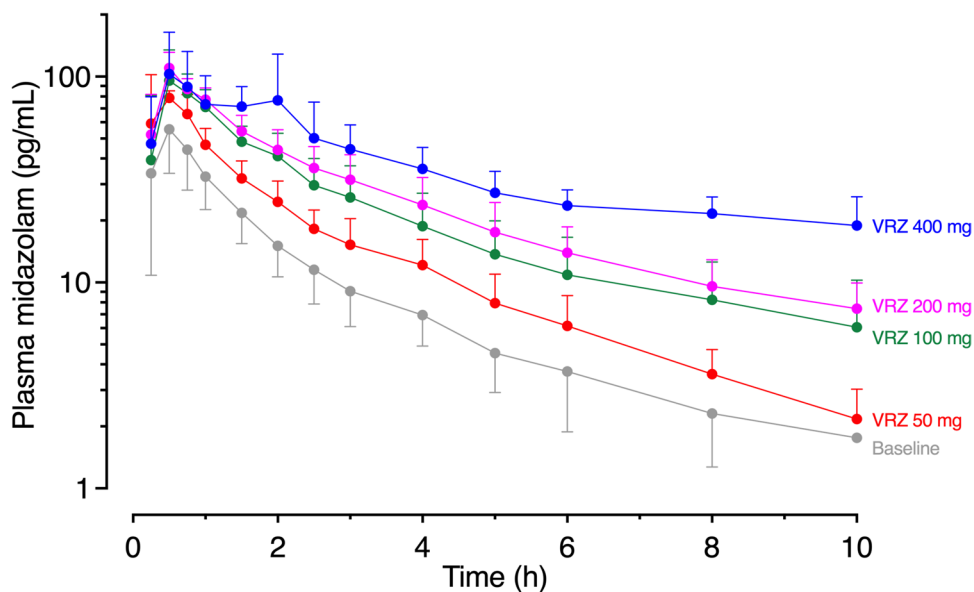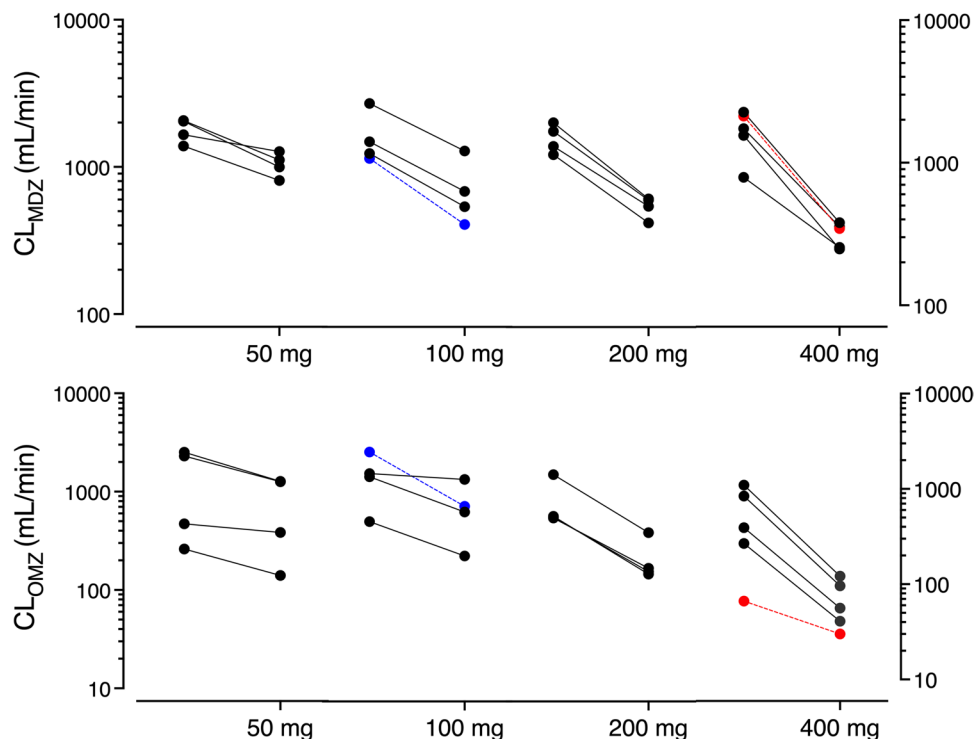
Fig. 2 Individual clearances of midazolam 10 μg (CL_{MDZ}) and omeprazole 100 μg (CL_{OMZ}) at baseline and after administration of four different single doses of voriconazole. The CYP2C19 poor metaboliser is marked in red, the ultra-rapid metaboliser in blue

Multiple linear regression All evaluated equations provided good fits to the data as shown by very high adjusted R^2 values exceeding 0.99 (Supplementary Table S2). Using the data collected during voriconazole administration, CL_{VRZ} was best predicted (adjusted R^2 0.997; AICc − 53.2) by including the predictors CL_{MDZ} and CL_{OMZ} during voriconazole administration only (log $CL_{VRZ} \sim \beta_1$ log CL_{OMZ} + β_2 log CL_{MDZ}) (Fig. 5). Adding voriconazole dose did not improve the model, likely because dose is reflected in the clearance values. With the final equation, an in-sample predictive R^2 of 0.861 and an out-of-sample predictive R^2 of 0.849 was calculated.

Using the baseline clearance data of midazolam and omeprazole, CL_{VRZ} was best predicted (adjusted R^2 0.997; AICc −54.53) with all predictors included (log $CL_{VRZ} \sim \beta_1$ log CL_{OMZ} + β_2 log CL_{MDZ} + β_3 voriconazole dose) (Fig. 5) yielding a predictive R^2 of 0.891 (in-sample) and 0.852 (out-of-sample).

3.5 Safety and Tolerability

All trial medications were well tolerated, and no serious AE occurred. A total of 22 AE in 11 participants occurred. Most AE were mild [common terminology criteria for AE (CTCAE) grade 1], while two cases of headache were treated with ibuprofen (CTCAE grade 2). Two cases of visual disturbances (photopsia, xanthopsia) were deemed probably related to the investigational medicinal products (voriconazole) and eight AE (headache, bloating,

diarrhoea) possibly related to the study. The remaining AE were considered unrelated to the trial procedures and interventions or their relationship was not assessable.

4 Discussion

This trial demonstrated that combining microdose pharmacokinetics of midazolam and omeprazole as surrogates of current CYP3A and CYP2C19 activity can reliably predict CL_{VRZ} in healthy volunteers. Recently, contributions of CYP2C19 and CYP3A to voriconazole N-oxide formation in vitro were estimated to be 63% and 30%, respectively [26], which is consistent with our in vivo observations that these enzymes are responsible for almost all metabolic clearance. Interestingly, a reduction of CL_{VRZ} by ritonavir (which is only a weak CYP2C19 inhibitor [27]) by only approximately 150 mL/min was observed, corresponding to about one-third of the CL_{VRZ} in CYP2C19 normal metabolisers [8], confirming the important but not dominant role of CYP3A in voriconazole metabolism. Major factors known to affect CL_{VRZ} include the dose and metaboliser status for CYP2C19 [7, 14, 28, 29].

To evaluate a possible relationship between CL_{VRZ} and the clearances of midazolam and omeprazole, a large range of voriconazole doses (and thus CYP activities) was tested by administering four different doses of voriconazole to a variety of CYP2C19 ultra-rapid, rapid, normal and

Table 1 Pharmacokinetic parameters of voriconazole, midazolam [10 µg orally (p.o.)], and omeprazole (100 µg p.o.) at baseline and during co-administration of different voriconazole doses to 16 ultra-rapid, rapid, normal and intermediate metaboliser and one poor metaboliser of CYP2C19

		T_{max} (h)	C_{max} (ng/mL)		$AUC_{0-\infty}$ (h ng/mL)		CL/F (mL/min)		$t_{1/2}$ (h)	
Baseline										
	Midazolam	0.504	0.056	[0.048–0.065]	0.102	[0.088–0.120]	1630	[1400–1910]	2.92	[2.46–3.48]
	Omeprazole	0.266	2.70	[2.00–3.62]	2.28	[1.38–3.76]	732	[444–1210]	0.772	[0.642–0.928]
Voriconazole dose (number of participants)	Voriconazole									
50 mg (N = 4)	Voriconazole	0.706	125	[27.3–574]	274	[71.0–1060]	3036	[786–11730]	4.30	[2.53–7.31]
	Midazolam	0.429	0.091	[0.066–0.125]	0.161	[0.119–0.218]	1040	[764–1400]	2.73	[1.79–4.18]
	Omeprazole	0.333	3.57	[1.40–9.09]	3.07	[0.566–16.7]	542	[100–2940]	0.786	[0.558–1.11]
100 mg (N = 4)	Voriconazole	0.632	258	[96.6–690]	606	[256–1436]	2750	[1160–6510]	5.08	[2.37–10.9]
	Midazolam	0.600	0.094	[0.053–0.167]	0.247	[0.113–0.544]	673	[306–1480]	3.58	[1.89–6.80]
	Omeprazole	0.333	3.15	[1.48–6.70]	2.63	[0.796–8.68]	634	[192–2100]	0.613	[0.391–0.962]
200 mg (N = 4)	Voriconazole	0.600	1730	[1120–2660]	3760	[342–4130]	886	[808–973]	6.63	[3.52–12.5]
	Midazolam	0.545	0.112	[0.087–0.144]	0.306	[0.232–0.404]	544	[413–718]	3.70	[2.85–4.80]
	Omeprazole	0.333	5.79	[3.40–0.86]	8.23	[3.95–17.1]	203	[97.3–422]	1.10	[0.818–1.49]
400 mg (N = 5)	Voriconazole	0.714	2789	[1880–4150]	16490	[7830–34740]	404	[192–851]	9.14	[5.89–14.2]
Including 1 CYP2C19 PM	Midazolam	0.638	0.129	[0.085–0.195]	0.534	[0.417–0.684]	312	[244–400]	6.19	[4.79–8.00]
	Omeprazole	0.45	9.24	[6.04–14.1]	27.6	[13.4–56.7]	60.5	[29.4–124]	1.58	[0.729–3.41]

$AUC_{0-\infty}$ area under the concentration-time curve, CL/F apparent oral clearance, C_{max} maximum plasma concentration, geometric mean [95 % confidence interval], t_{max} time to reach C_{max} (harmonic mean), $t_{1/2}$ terminal elimination half-life

Table 2 Geometric mean ratios [90 % confidence interval] of midazolam and omeprazole clearances at baseline (reference) and during different voriconazole doses (test) in healthy volunteers

Oral voriconazole dose (number of participants)	Oral midazolam		Oral omeprazole	
50 mg (N = 4)	0.586	[0.466; 0.737]	0.590	[0.457; 0.762]
100 mg (N = 4)	0.426	[0.365; 0.497]	0.464	[0.266; 0.807]
200 mg (N = 4)	0.341	[0.302; 0.386]	0.271	[0.244; 0.302]
400 mg (N = 5)	0.196	[0.148; 0.260]	0.166	[0.096; 0.288]

intermediate metaboliser, and one poor metaboliser treated with 400 mg voriconazole to decrease CL_{VRZ} to a (potential) minimum.

The clearances were highly variable for all substances and spanned a range of about 1.5–2 orders of magnitude. CYP3A activity, as indicated by CL_{MDZ}, was increasingly suppressed with each increase in voriconazole exposure. This was very similar for CPY2C19 activity with the strongest inhibition with 400 mg voriconazole. With this voriconazole dose, a clearance of less than 20% of baseline was observed for both midazolam and omeprazole. Since all drugs were given orally, it is not possible to distinguish the contribution of the small intestine and the liver to the resulting overall

Fig. 3 Semilogarithmic plot of mean (± standard deviation) plasma concentration–time curves of omeprazole 100 μg at baseline (grey) and after co-administration of a single oral dose of 50, 100, 200 or 400 mg voriconazole (VRZ). The concentration–time curves were capped at 5 h because thereafter most concentrations (especially in the control group and the low-dose voriconazole groups) were below the limit of quantification
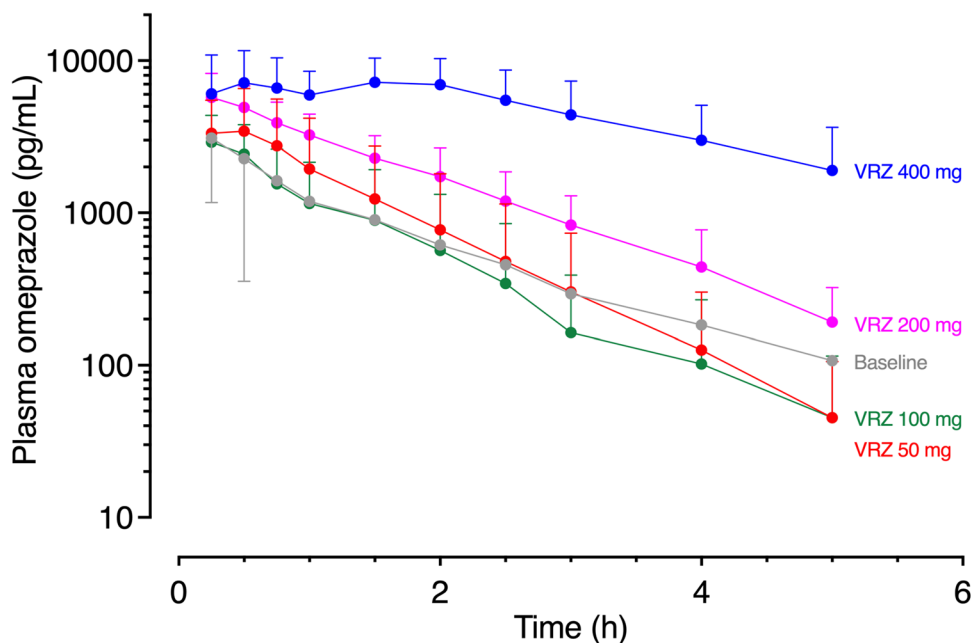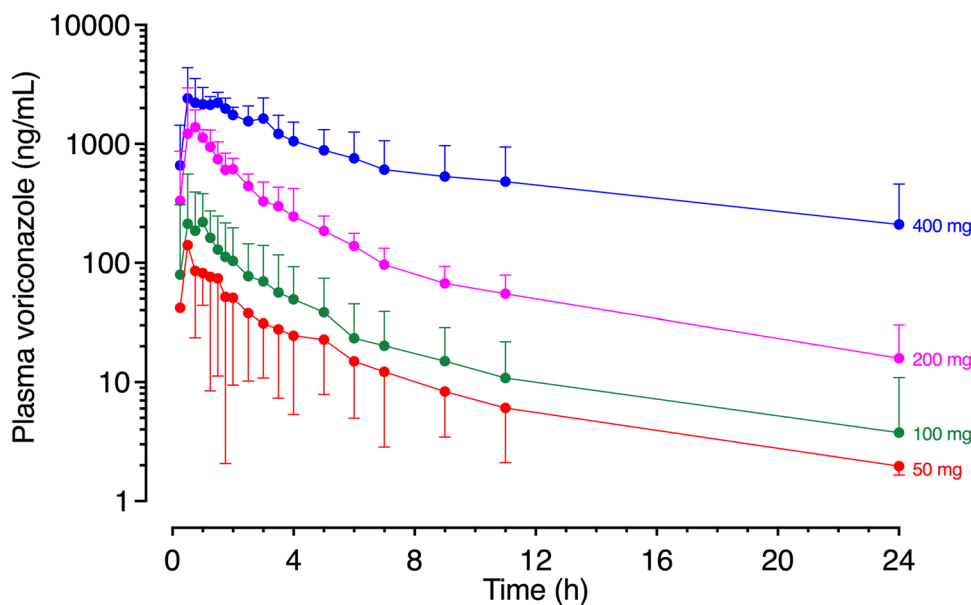

Fig. 4 Semilogarithmic plot of mean (± standard deviation) plasma concentration–time curves of voriconazole after administration of a single dose of 50 mg (N = 4), 100 mg (N = 4), 200 mg (N = 4) or 400 mg (N = 5) to healthy volunteers
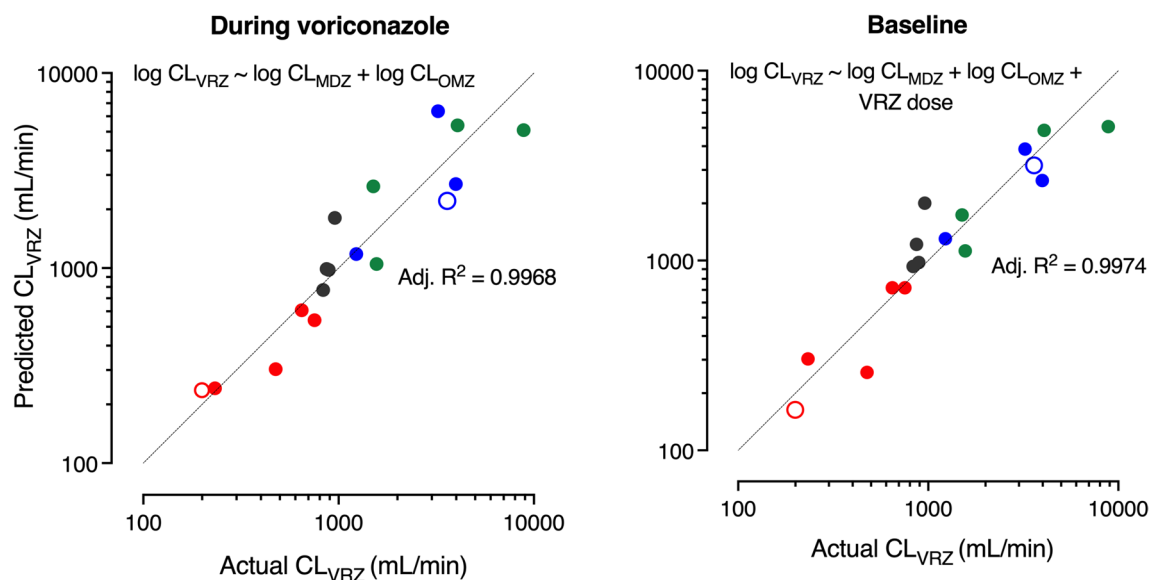
Fig. 5 Best-fit multiple regression models: plot of actual voriconazole clearance (CL_{VRZ}) versus predicted CL_{VRZ} during voriconazole (using CL_{MDZ} and CL_{OMZ} during VRZ) and at baseline (using CL_{MDZ} and CL_{OMZ} at baseline without VRZ) with different single doses of voriconazole (red 400 mg; grey 200 mg; blue 100 mg; green 50 mg). Open circles depict the CYP2C19 poor metaboliser (red) and ultrarapid metaboliser (blue). Both plots include the line of identity

inhibition. However, because C_{max} of omeprazole and midazolam increased dose-dependently, it can be assumed that at the intestinal level, both CYP isozymes are inhibited with increasing voriconazole doses.

The clearances of the probe drugs midazolam and omeprazole closely reflect actual CYP isozyme activities and, when administered in microdoses, do not exert any perpetrator effects. Voriconazole, however, as an inhibitor of both CYP2C19 and CYP3A, affects the clearances of both probe drugs, which were therefore expected to be related to CL_{VRZ} and to its exposure. It is therefore not surprising that CL_{VRZ} correlated with CL_{MDZ} or CL_{OMZ} during voriconazole treatment but also with baseline clearances before inhibitor administration. While baseline CL_{OMZ} was correlated with CL_{VRZ}, there was no significant relationship between baseline CL_{MDZ} and CL_{VRZ}, confirming that the contribution of CYP2C19 to voriconazole metabolism is more substantial. This is also in line with the considerable impact of the CYP2C19 genotype on voriconazole clearance and thus exposure [15].

According to the relative contributions of the two isozymes to the total CL_{VRZ}, CL_{OMZ} achieved a better correlation than CL_{MDZ}, but an almost perfect correlation with CL_{VRZ} was only achieved when both clearances were considered together. Accounting for voriconazole doses did not improve the regression, probably because voriconazole exposure (and thus dose) is already accounted for by CL_{MDZ} or CL_{OMZ} via voriconazole's perpetrator characteristics. This is consistent with the assumption that CYP3A and CYP2C19 almost exclusively determine CL_{VRZ} in healthy volunteers.

In vitro, the involvement of CYP2C9 and FMO3 in the metabolism of voriconazole has also been observed [30, 31], but our findings clearly suggest that the metabolic contribution of other enzymes is minor and likely not clinically relevant in adult patient populations. This might be different in a paediatric population [32]. Moreover, the activities of FMO3 cannot be induced by xenobiotics [33], and almost all inducers of CYP2C19 are also and often stronger inducers of CYP3A, suggesting that even in the presence of CYP inducers other enzymes are unlikely to participate in voriconazole metabolism. Finally, in addition to genetic variants of CYP2C19 as the strongest modulators of CL_{VRZ}, an indirect modulation of voriconazole concentrations in inflammatory states has also been reported [30, 31] with exposure increases caused by inflammatory states that down-regulate CYP3A and possibly also CYP2C19 [34].

5 Limitations

This trial was conducted in healthy participants and it remains to be shown whether the suggested exclusive dependence of CL_{VRZ} from CYP3A and CYP2C19 activities can be generalised to patient populations. This appears likely because the major known alterations of voriconazole pharmacokinetics beyond genetics in patients (inflammatory states, drug–drug interactions) could all be mediated through modulation of the activities of the same isozymes. Furthermore, the low prevalence of CYP2C19

poor metabolisers in the local population resulted in the recruitment of only one poor metaboliser, making the results of the lowest evaluated clearance range less certain. However, its results fit well into the regression of the whole group, well supporting the concept. Finally, this trial used a single oral dose of voriconazole, for the moment limiting interpretation to acute voriconazole effects. However, it appears likely that neither intravenous administration nor multiple dosing of voriconazole will change these findings, except in rare occasions in which autoinduction of the metabolism has been suggested [9].

6 Conclusions

In healthy volunteers, the CL_{VRZ} exclusively depends on the activity of CYP2C19 and CYP3A, indicating that other enzymes metabolising voriconazole in vitro (CYP2C9, FMO3) do not contribute to its metabolism in vivo. Whether chronic treatment, enzyme inducing comedication or comorbidities can recruit additional enzymes remains to be studied, but appears unlikely.

Declarations

Conflict of interest No conflicts of interest have been declared by any author.

Ethics approval The trial protocol was approved by the responsible ethics committee of the Faculty of Medicine of Heidelberg University (Afmo-431/2020) and the German competent authority (BfArM) on 13 July 2020 (EudraCT No: 2020-001017-20, registered on March 5th, 2020).

Availability of data and material The collected and analysed data can be made available upon reasonable request.

Code availability Not applicable.

Consent for publication Not applicable.

Informed consent Informed consent was obtained from all participants included in this trial prior to carrying out any trial procedures.

Author contributions G.M. proposed the research topic. A.M., A.B., F.S., G.M. and W.E.H. developed the trial protocol. A.M. and A.B. executed the trial. J.B. and K.I.F. analysed the samples. A.M., G.M. and A.D.M. performed the statistical analyses. A.M. wrote the first draft of the manuscript. All authors discussed and interpreted the results and contributed to the manuscript.

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