



Time Course of the Interaction Between Oral Short-Term Ritonavir Therapy with Three Factor Xa Inhibitors and the Activity of CYP2D6, CYP2C19, and CYP3A4 in Healthy Volunteers

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Abstract

Background We investigated the effect of a 5-day low-dose ritonavir therapy, as it is used in the treatment of COVID-19 with nirmatrelvir/ritonavir, on the pharmacokinetics of three factor Xa inhibitors (FXaI). Concurrently, the time course of the activities of the cytochromes P450 (CYP) 3A4, 2C19, and 2D6 was assessed.

Methods In an open-label, fixed sequence clinical trial, the effect and duration of a 5-day oral ritonavir (100 mg twice daily) treatment on the pharmacokinetics of three oral microdosed FXaI (rivaroxaban 25 µg, apixaban 25 µg, and edoxaban 50 µg) and microdosed probe drugs (midazolam 25 µg, yohimbine 50 µg, and omeprazole 100 µg) was evaluated in eight healthy volunteers. The plasma concentrations of all drugs were quantified using validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods and pharmacokinetics were analysed using non-compartmental analyses.

Results Ritonavir increased the exposure of apixaban, edoxaban, and rivaroxaban, but to a different extent the observed area under the plasma concentration–time curve (geometric mean ratio 1.29, 1.46, and 1.87, respectively). A strong CYP3A4 inhibition (geometric mean ratio > 10), a moderate CYP2C19 induction 2 days after ritonavir (0.64), and no alteration of CYP2D6 were observed. A CYP3A4 recovery half-life of 2.3 days was determined.

Conclusion This trial with three microdosed FXaI suggests that at most the rivaroxaban dose should be reduced during short-term ritonavir, and only in patients receiving high maintenance doses. Thorough time series analyses demonstrated differential effects on three different drug-metabolising enzymes over time with immediate profound inhibition of CYP3A4 and only slow recovery after discontinuation.

Clinical Trial Registration EudraCT number: 2021-006643-39.

1 Introduction

The COVID-19 pandemic affects people worldwide and can lead to hospitalization and death. Nirmatrelvir-ritonavir (Paxlovid[®]) has been available through an Emergency Use Authorization (EUA) since early 2022 and was approved by the US Food and Drug Administration (FDA) in May

2023 for the treatment of mild to moderate COVID-19 in adults at high risk for progression to severe COVID-19 and within 5 days of symptom onset. This therapy consists of a low dose (100 mg) of ritonavir taken orally every 12 h for 5 days, which inhibits the metabolism of nirmatrelvir, reducing its clearance (CL) and increasing its bioavailability [1]. This short-term use for only 5 days is new for ritonavir, as ritonavir-boosted treatments for HIV patients are chronic and therefore drug–drug interaction (DDI) studies usually examine the effects of long-term use. Ritonavir is a potent mechanism-based inhibitor of cytochrome P450 (CYP) 3A with inhibitory and time-dependent inductive effects on numerous other CYPs and drug transporters such as p-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and organic anion-transporting polypeptides (OATP) [2–7]. Using a 300-mg twice-daily (bid) schedule, potent CYP3A

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Key Points

The effect of short-term low-dose ritonavir on the pharmacokinetics of factor Xa inhibitors (FXaI) is not well studied and the time course of interaction with major cytochrome P450 (CYP) pathways has not been described in detail.

We simultaneously quantified the effect of 5 days of ritonavir 100 mg twice daily on three microdosed oral FXaI and concurrently assessed the time course of CYP3A4, CYP2C19, and CYP2D6 activity during ritonavir and for 4 days after discontinuation.

Of the three FXaI, only rivaroxaban exposure increased relevantly during ritonavir treatment while apixaban and edoxaban exposures were only mildly increased.

Ritonavir inhibited CYP3A4 rapidly and profoundly and the recovery of its activity was delayed. CYP2C19 was mildly and transiently induced at the end of treatment, while CYP2D6 activity was unchanged throughout the observation period.

inhibition of ritonavir over time was already demonstrated during 9 days of treatment [8].

Nirmatrelvir/ritonavir therapy is indicated for patients at high-risk for severe COVID-19 courses, who frequently take other drugs such as factor Xa inhibitors (FXaI). Infection with SARS-CoV-2 seems additionally associated with an increased risk of thrombotic events [9]. All three available FXaI are substrates of P-gp and BCRP but their clearance pathways differ, with edoxaban being mostly excreted unchanged [10], apixaban being metabolized mainly by CYP3A with a minor contribution from other CYP isozymes [11], and rivaroxaban being metabolized mainly by CYP2J2 and CYP3A [12–14]. Therefore, DDIs are known and expected for strong CYP3A and P-gp inhibitors and are associated with increased haemorrhagic risk [15, 16]. However, there are limited data on coadministration with ritonavir showing a 2.5-fold exposure increase in rivaroxaban in healthy volunteers after 6 days of high-dose (600 mg bid) ritonavir therapy [17], as well as case reports of increased plasma concentrations and/or bleeding risk of FXaI with concomitant ritonavir-boosted therapy [18–23]. The manufacturer does not recommend the use of nirmatrelvir/ritonavir with rivaroxaban, while there is no statement for apixaban and edoxaban [24].

Therefore, we aimed to assess the influence of shorter and much lower standard boosting doses of ritonavir, as recommended for COVID-19 treatment (100 mg ritonavir bid for 5 days), on the pharmacokinetics of FXaI to quantify the magnitude of these DDI. We administered FXaI microdoses, which are predictive of their therapeutic doses [25, 26], and evaluated all three FXaI simultaneously.

In the absence of mechanistic studies on the time course of the interaction of short-term ritonavir with other major CYPs, we also examined the effects on CYP2D6 and CYP2C19 activities over time. Therefore, the pharmacokinetics of the established microdosed CYP probe drugs midazolam [8], yohimbine [27], and omeprazole [28] were studied daily during ritonavir intake and for another 4 days after stopping ritonavir.

2 Methods

2.1 Trial Design

This phase I trial was conducted as a single-centre, open-label, fixed sequence clinical trial. Ritonavir was administered orally as 100-mg tablets (Norvir[®] 100-mg film-coated tablets, Abbvie, Ludwigshafen, Germany) bid for 5 days. Before ritonavir administration (baseline day) and on the last day of ritonavir intake, the participants received a microdosed cocktail of FXaI (Fig. 1) consisting of apixaban 25 µg (Eliquis[®], Bristol-Myers Squibb, Munich, Germany), edoxaban 50 µg (Lixiana[®], Daiichi Sankyo Europe, Munich, Germany), and rivaroxaban 25 µg (Xarelto[®], Bayer, Leverkusen, Germany) [25]. The oral stock solutions of edoxaban (30 µg/mL), rivaroxaban (25 µg/mL), and apixaban (25 µg/mL) were prepared by the Pharmacy of Heidelberg University Hospital in compliance with GMP guidelines according to the pharmaceutical development protocols approved by the competent authority (BfArM, Bonn, Germany).

To monitor the different CYP isozyme activities, midazolam (Dormicum[®]; 5 mg/5 mL solutions for infusion; Roche, Grenzach-Wyhlen, Germany; 30 µg at baseline and 10 µg at all other administration times), yohimbine 50 µg (Yohimbinum hydrochloricum D4[®] tablets, DHU-Arzneimittel GmbH, Karlsruhe, Germany), and omeprazole 100 µg (OMEP[®] 40 mg power for solution for infusion; Hexal, Holzkirchen, Germany) were administered as oral solutions together with the FXaI cocktail before ritonavir was initiated, every day with the intake of ritonavir, and 2 and 4 days after stopping ritonavir. To buffer gastric acid and prevent degradation of the uncoated omeprazole, sodium hydrogen carbonate (4.2% w/v) was administered 10 min before (50 mL equivalent to 25 mmol) and simultaneously

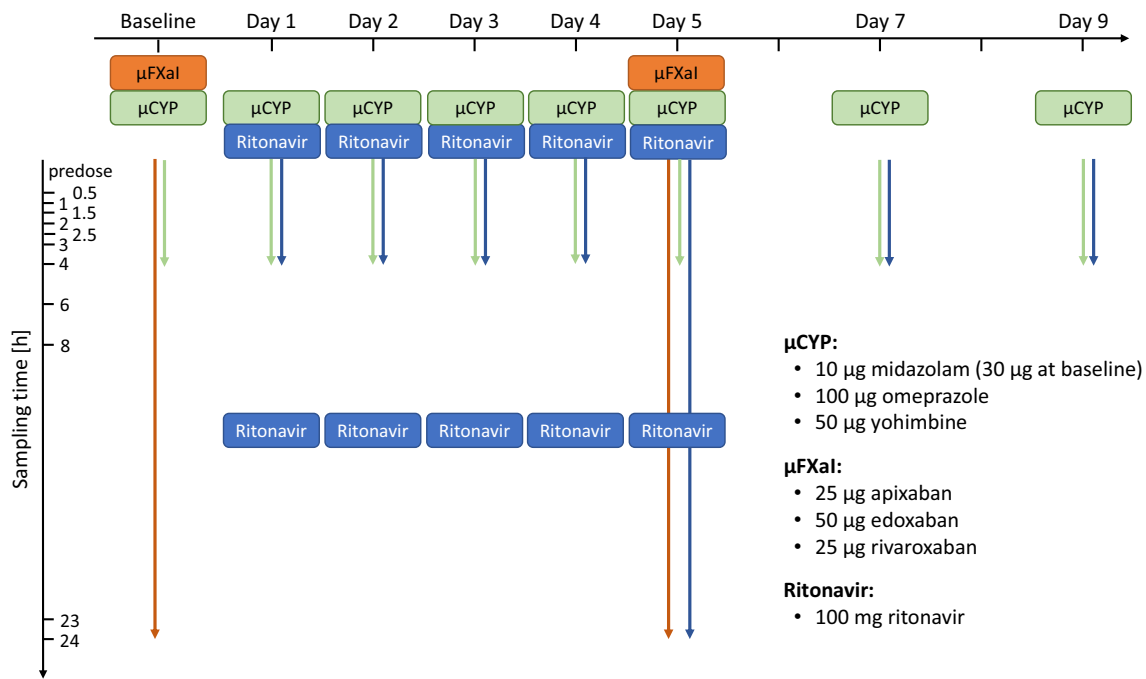


Fig. 1 Eight healthy volunteers received oral ritonavir 100 mg (blue boxes) twice daily from day 1 to 5. At baseline and on day 5, a microdosed cocktail consisting of apixaban 25 μg, edoxaban 50 μg, and rivaroxaban 25 μg was administered (μFXaI, orange boxes). Additionally, at each visit, a cocktail of CYP probe drugs (green boxes) consisting of midazolam 10 μg (at baseline 30 μg), yohimbine 50 μg, and omeprazole 100 μg together with sodium hydrogen carbonate (50 mL

100 mL equivalent to 50 mmol) was administered. Blood samples (red arrows) for the analyses of FXaI concentration were taken before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 23, and 24 h after drug administration at baseline and on day 5. On other study visits, blood samplings were taken over a period of 4 h (before, 0.5, 1, 1.5, 2, 2.5, 3, and 4 h post dose). Urine was collected for 24 h at baseline and on day 5. CYP cytochrome P450, FXaI factor Xa inhibitor

(100 mL equivalent to 50 mmol) with the 100-μg uncoated omeprazole dose as used previously [28]. Microdoses were always prepared immediately before administration and were administered with ritonavir always in the same sequence (first the FXaI, followed by yohimbine, midazolam, and omeprazole) within 1 min.

2.2 Trial Population

We enrolled volunteers who were in good physical and mental health and had no clinically relevant findings in medical history, physical examination, electrocardiogram, and routine laboratory analyses including renal and liver function. Not enrolled were pregnant or lactating women, participants with known intolerance or contraindication to any component of the trial medication, a history of anaphylactic shock, or participants of an interventional clinical trial within 30 days before inclusion. The use of medicines (except iodine and levothyroxine), cannabis, or citrus fruits (including grapefruit) was not allowed during the trial, and enrolment in the study was allowed at the earliest 5 half-lives (for inhibitors) or 2 weeks (for inductors) after discontinuation of the previously taken medicines. Alcohol and smoking

were not allowed on study days. All participants used highly effective contraceptive methods (intrauterine devices, intrauterine hormone-releasing systems, vasectomized partner, or sexual abstinence for female volunteers and condom or sexual abstinence for men) during the trial and for a further 2 weeks after the last medication intake.

2.3 Trial Conduct

The trial was conducted at the Clinical Research Centre of the Department of Clinical Pharmacology and Pharmacoepidemiology (KliPS), Heidelberg University Hospital, which is certified according to DIN EN ISO 9001:2015. Participants arrived fasting (for at least 8 h) and remained fasting for a further 4 h after taking all microdoses simultaneously. Blood samples for the analyses of FXaI concentration were drawn before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 23, and 24 h after drug administration on the first day (baseline) and at the fifth day of ritonavir intake (Fig. 1). On days with FXaI intake, the entire 24-h urine was collected. Blood samples for analyses of yohimbine, omeprazole, and ritonavir were taken before and 0.5, 1, 1.5, 2, 2.5, 3, and 4 h on every day of ritonavir intake and on days 2 and 4 after discontinuation of ritonavir. Additional ritonavir plasma samples were taken

6, 8, 23, and 24 h after dosing on baseline and on day 5. Midazolam plasma samples were taken before and 2, 2.5, 3, and 4 h after drug administration on each study day.

All samples were collected in lithium-heparin tubes and centrifuged for 10 min at 20 °C and 2500g within 30 min. Urine aliquots and separated plasma aliquots were stored at – 20 °C until analysis.

2.4 Quantification of Factor Xa Inhibitors and Probe Drugs

The concentrations of FXaI, midazolam, yohimbine, and omeprazole with 5'-hydroxy omeprazole were quantified by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS) according to published assays [29–32] with a lower limit of quantification (LLOQ) of 1 pg/mL for midazolam and yohimbine and 5 pg/mL for omeprazole, 5'-hydroxy omeprazole, and the FXaI. Ritonavir plasma concentrations were quantified by high-performance LC–MS/MS with a LLOQ of 10 ng/mL [2]. Urine samples were diluted 1:10 with blank human plasma for analyses of FXaI concentrations [33]. Accuracy and precision values were always $\leq \pm 15\%$ and all assays complied with the relevant guidelines for the validation of bioanalytical methods of the European Medicines Agency and the US Food and Drug Administration [34, 35].

2.5 Calculations and Statistical Analysis

The sample size was determined using the interaction data from a previous trial (EudraCT 2017-004453) [36] investigating the effect of ketoconazole on FXaI, which was assumed to be comparable to ritonavir. A sample size of 3 (apixaban and rivaroxaban) and 5 (edoxaban) was calculated to detect the observed area under the plasma concentration–time curve (AUC) extrapolated to infinity (AUC_{inf}) ratio of 1.68 ± 0.36 for apixaban, 2.28 ± 1.10 for edoxaban, and 2.37 ± 0.53 for rivaroxaban with a power of 0.8 and an α error of 5%. Using these data, a two-sided paired t-test with a sample size of $N = 8$ (log-transformed AUC_{inf}) would allow to detect a 20% change of exposure (i.e., an AUC_{inf} ratio of 1.20) for apixaban, rivaroxaban, and edoxaban with a power of 80% and an α error of 5%.

The standard non-compartmental pharmacokinetic parameters of each FXaI were calculated using Phoenix™ WinNonlin Software 8.3.5 (Certara, Princeton, NJ, USA). The calculated parameters included maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), AUC_{inf} , AUC from 0 to 24 h (AUC_{0-24}), 0 to 4 h (AUC_{0-4} , omeprazole, yohimbine), and 2 to 4 h (AUC_{2-4} , midazolam), apparent volume of distribution at steady-state (V_z/F), apparent oral clearance

(CL/F), terminal elimination half-life ($t_{1/2}$), renal clearance (CL_{renal}), expressed as the amount excreted unchanged in urine over 24 h divided by AUC_{0-24} , and non-renal clearance ($CL_{nonrenal}$) as the difference between CL/F and renal clearance. The AUC was calculated using a mixed log-linear trapezoidal model.

To determine the effect of ritonavir on CYP3A activity, the estimated midazolam partial metabolic clearance (eCL_{met}) was calculated using the data of a limited sampling methodology [8, 37, 38]. Due to the prolonged midazolam elimination half-life with coadministration of ritonavir, midazolam concentrations above the LLOQ were observed in predose samples from the second day of ritonavir intake until 4 days after ritonavir discontinuation, which was taken into account as follows: the residual AUC was calculated using the predose concentration and the current half-life in the interval 2–4 h of the individual visit and then subtracted accordingly. The CYP3A4 recovery half-life was calculated as described previously [39].

The effect of ritonavir on CYP2C19 and CYP2D6 activity was determined using AUC_{0-4} and C_{max} of yohimbine and omeprazole. The exposure of ritonavir was determined using AUC_{0-4} after dose 1, 3, 5, 7, and 9, and after discontinuation.

Data are reported as geometric means with 95% confidence intervals (CIs) except t_{max} , which is reported as median with range. To test for differences of each FXaI separately between baseline and ritonavir, the geometric mean ratios (GMRs) and their 90% CIs were calculated. The default no-effect boundary of point estimates is 0.8–1.25 and when 90% CIs for systemic exposure (AUC) ratios are entirely within the equivalence range of 0.85–1.25, according to the standard practice of the FDA, it is concluded that no clinically relevant interaction is present. For other FXaI PK parameters, a ratio paired t-test (two-tailed) was performed to compare day 5 of ritonavir intake with baseline.

To account for the multiple occasions of assessing midazolam, yohimbine, and omeprazole exposure, repeated-measure one-way ANOVA of logarithmic transformed data with correction for multiple testing (Dunnett's multiple comparisons test) was performed to test for differences to baseline.

All statistics were performed with Prism 9.0.0 (GraphPad Software, La Jolla, CA, USA). A p value < 0.05 was considered significant.

3 Results

Nine healthy volunteers were included in the study. One participant dropped out on the last day of ritonavir intake due to an unrelated COVID-19 infection. Therefore, eight

participants (7 females) with a median age of 25.5 years (range 21–58) and a body mass index of 24.2 kg/m² (range 18.0–28.1) completed the study.

3.1 Factor Xa Inhibitors

Over the observation period, all plasma concentrations were above the LLOQ, except for the 23- and 24-h edoxaban concentrations in two and the 24-h edoxaban concentration in three participants. The 24-h urine collection from one participant was excluded from analyses due to suspected incomplete urine collection on day 5.

Coadministration of ritonavir 100 mg bid for 5 days increased plasma concentrations of all three FXaI (Fig. 2; Tables 1 and 2). The individual increase in AUC_{0–24} for apixaban and edoxaban was heterogeneous: three participants had an AUC_{0–24} ratio of 0.8–1.2 versus two and three participants, respectively, with AUC_{0–24} increases of 2–2.3 (Fig. 3). The largest and consistent change was observed for rivaroxaban. A slightly but significantly prolonged *t*_{1/2} (from 5.14 h to 6.57 h) and a significantly reduced CL_{renal} (from 49.4 to 34.8 mL/min) were observed for rivaroxaban but not for the other two FXaI. The volume of distribution was significantly reduced for edoxaban and rivaroxaban but not for apixaban.

3.2 CYP Probe Drugs

3.2.1 Midazolam

Ritonavir profoundly reduced the CL_{met} of midazolam (Table 3) and already after the first intake of ritonavir 100 mg, midazolam AUC_{2–4} increased 5.1-fold. From day 2 to day 5 of ritonavir treatment, the AUC_{2–4} of midazolam continued to increase to 11.1–15.5 times baseline values and recovered only slowly after ritonavir discontinuation, with AUC_{2–4} values still 10.2-fold and 2.9-fold higher 2 and 4 days after ritonavir discontinuation, respectively (Fig. 4). The estimated CYP3A4 recovery half-life was 2.30 ± 0.89 days.

3.2.2 Omeprazole and Yohimbine

The baseline pharmacokinetics of yohimbine and omeprazole are shown in Table 3. During ritonavir, omeprazole pharmacokinetics were unchanged but after ritonavir discontinuation, the AUC_{0–4} was decreased by approximately 35% and the AUC_{0–4} ratio also decreased (Fig. 5). The molar AUC_{0–4} ratio of omeprazole and 5-OH-omeprazole decreased from baseline during ritonavir treatment and was significantly reduced on days 4 and 5 (32%), and remained significantly reduced afterwards (Table 3).

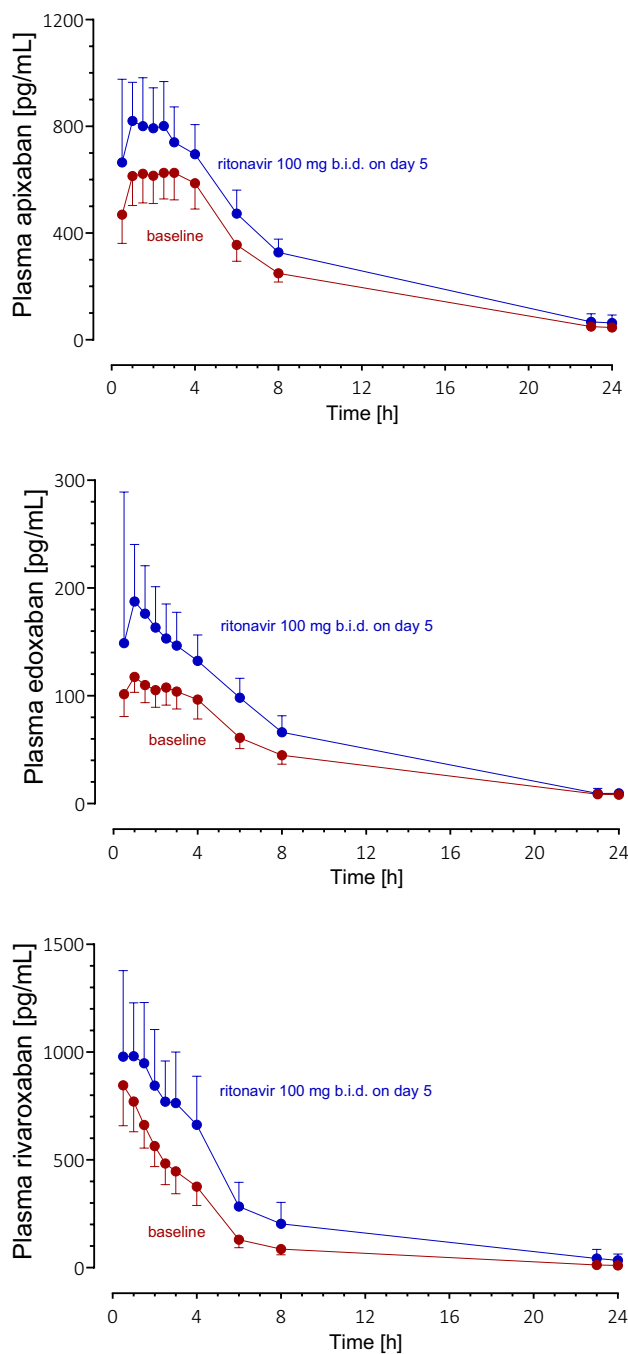


Fig. 2 Geometric mean plasma concentration–time curves (± 95% confidence intervals) after oral administration of apixaban 25 µg, edoxaban 50 µg, and rivaroxaban 25 µg alone (baseline; red symbols and line) and at day 5 of ritonavir therapy (blue symbols and line) in 8 healthy volunteers. *bid* twice daily

Ritonavir gradually, but not significantly, increased yohimbine AUC_{0–4} to a GMR of approximately 1.5; this effect was short-lived and 2 days after ritonavir

Table 1 Pharmacokinetic parameters of apixaban, rivaroxaban (both 25 µg), and edoxaban (50 µg) after a single oral dose before ritonavir and on the fifth day of ritonavir (100 mg bid) in 8 healthy volunteers

	Apixaban		Edoxaban		Rivaroxaban	
	Baseline	Ritonavir day 5	Baseline	Ritonavir day 5	Baseline	Ritonavir day 5
C_{max} [pg/mL]	671	906	125	212*	891	1130*
	556–810	747–1099	111–140	167–269	727–1093	916–1395
t_{max}^a [min]	90	60	75	30	30	60
	60–240	30–150	30–240	30–150	30–60	30–120
AUC_{0-24} [pg/mL·h]	6230	8074	1028	1522*	3574	6700*
	5360–7241	6592–9889	809.3–1305	1269–1826	2835–4506	4848–9258
CL/F [mL/min]	66.9	51.6	811	548*	117	62.2*
	57.5–77.7	42.13–63.1	639–1030	456–657	92.5–147	45.1–85.9
CL_{renal}^b [mL/min]	13.1	11.6	165	143	49.4	34.8*
	10.3–16.7	9.27–14.4	141–193	116–177	36.2–67.4	23.7–51.3
$CL_{nonrenal}^b$ [mL/min]	53.9	40.7	629	407*	70.4	30.9*
	42.7–68.1	30.4–54.6	453–873	453–873	54.5–90.9	21.4–44.0
Ae^b [% of dose]	17.7	16.5	19.6	24.8	40.0	48.8
	12.7–24.6	10.52–26.0	14.9–25.7	19.8–31.1	35.3–45.2	40.3–59.2
V_z/F [L]	38.5	30.2	368	248*	51.9	35.4*
	30.6–48.4	27.6–33.0	300–450	205–299	39.2–68.6	27.2–46.0
$t_{1/2}$ [h]	6.65	6.76	5.24	5.23	5.14	6.57*
	5.33–8.30	5.64–8.10	3.76–7.30	4.44–6.15	4.47–5.91	5.02–8.61

Data are expressed as geometric mean and 95% confidence interval, except ^amedian and range; ^b $n = 7$

Ae amount excreted in urine as parent drug, AUC_{0-24} area under the concentration–time curve from 0 to 24 h, CL/F apparent oral clearance, $CL_{nonrenal}$ non-renal clearance, CL_{renal} renal clearance, C_{max} peak concentration, t_{max} time to C_{max} , $t_{1/2}$ terminal elimination half-life, V_z/F apparent volume of distribution

* $p < 0.05$ vs baseline

Table 2 Geometric mean ratios (GMR) of apixaban, rivaroxaban (both 25 µg), and edoxaban (50 µg) as single oral doses before ritonavir and on the fifth day of ritonavir (100 mg bid) in 8 healthy volunteers

	Apixaban		Edoxaban		Rivaroxaban	
	GMR	90% confidence interval	GMR	90% confidence interval	GMR	90% confidence interval
AUC_{0-24}	1.29	1.04–1.61	1.46*	1.23–1.73	1.87*	1.54–2.28
C_{max}	1.35	1.05–1.74	1.69*	1.34–2.14	1.27*	1.11–1.45

AUC_{0-24h} area under the concentration–time curve from 0 to 24 h, C_{max} peak concentration

discontinuation, CYP2D6 activity had returned to baseline activity (Table 3; Fig. 6).

3.3 Ritonavir

The AUC_{0-4} of ritonavir was 906 ng/mL·h (95% CI 543–1512) and C_{max} was 373 ng/mL (95% CI 244–572) after intake of the first dose. Exposure and C_{max} were similar on the remaining 4 days, ranging between 4593–5331 ng/mL·h and 1776–2107 ng/mL, respectively (Fig. 7). Two days after the last ritonavir dose, concentrations were close to or below the LLOQ.

3.4 Safety Data

Overall, the study medication was well tolerated. Twenty adverse events (5 considered related to study drug) were observed in six participants with most adverse events occurring on baseline day, that is, before ritonavir administration (Supplementary Table S1, see electronic supplementary material [ESM]), and all were transient. One adverse event (presyncope) was moderate and one (syncope) was severe and both occurred as an immediate consequence of unsuccessful venepunctures; all other adverse

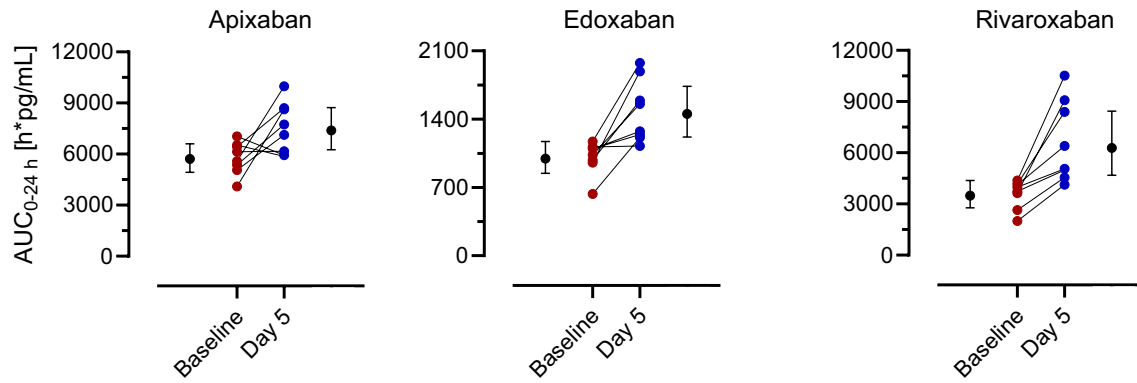


Fig. 3 Individual AUC_{0-24} changes of apixaban, edoxaban, and rivaroxaban and geometric population mean (with 95% confidence interval) of 8 healthy volunteers after oral administration of apixaban

25 μ g, edoxaban 50 μ g, and rivaroxaban 25 μ g alone and during low-dose ritonavir for 5 days. AUC_{0-24} area under the plasma concentration–time curve from 0 to 24 h

Table 3 Pharmacokinetics of midazolam, yohimbine, and omeprazole before, during, and after treatment with ritonavir 100 mg bid in 8 healthy volunteers

	Before ritonavir	Oral ritonavir 100 mg bid					After ritonavir	
	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 9
Midazolam								
AUC_{2-4} [pg/mL·h]	14.3 10.2–20.0	72.9* 46.9–114	222* 192–256	211* 175–256	187* 159–221	158* 130–192	147* 121–178	41.7* 28.3–61.3
GMR AUC_{2-4}	–	5.10 3.76–6.92	15.5 11.2–21.4	14.8 10.5–20.8	13.1 9.30–18.4	11.1 7.72–15.8	10.2 7.60–13.8	2.91 1.99–4.27
eCL_{met} [mL/min]	1291 923–1805	253* 163–394	83.3* 72.3–96.0	87.4* 72.2–106	98.5* 83.4–116	117* 96.1–142	126* 104–153	443* 301–653
Omeprazole								
AUC_{0-4} [pg/mL·h]	1942 1242–3037	2053 1302–3236	2389 1456–3921	1909 1116–3266	1603 864–2973	1583 951–2636	1234* 753–2021	1287 748–2214
GMR AUC_{0-4}	–	1.06 0.87–1.29	1.23 1.02–1.49	0.98 0.76–1.28	0.83 0.60–1.13	0.82 0.65–1.02	0.64 0.51–0.79	0.66 0.51–0.86
Molar AUC ratio [% of 5-OH-omeprazole]	121 82.2–179	122 78.2–189	120 76.7–187	101 61.2–166	90.3* 55.7–147	82.3* 52.6–129	72.0* 45.3–115	83.6* 53.0–132
Yohimbine								
AUC_{0-4} [pg/mL·h]	135 61.4–295	162 71.9–366	165 66.3–412	212 92.8–484	200 83.8–478	201 83.4–485	165 74.1–367	175 74.5–413
GMR AUC_{0-4}	–	1.20 0.88–1.66	1.23 0.90–1.68	1.57 1.20–2.07	1.49 1.13–1.96	1.49 1.12–1.99	1.22 0.94–1.59	1.3 0.90–1.89

Data are expressed as geometric mean values with 95% confidence intervals and, in case of GMR, with 90% confidence intervals. Molar AUC ratio: the molar AUC ratio of omeprazole is expressed as a percentage of 5-OH-omeprazole; it is calculated as the AUC_{0-4} of omeprazole (nmol/L·h) divided by the AUC_{0-4} of 5-hydroxy omeprazole (nmol/L·h) and multiplied by 100

AUC_{0-4} area under the concentration–time curve from 0 to 4 h, AUC_{2-4} area under the concentration–time curve from 2 to 4 h, eCL_{met} estimated midazolam partial metabolic clearance, GMR geometric mean ratio.

* $p < 0.05$ vs baseline

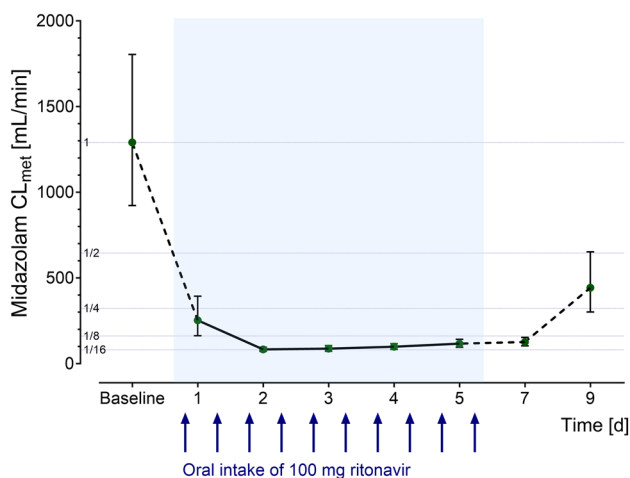


Fig. 4 Time course of the estimated geometric mean metabolic clearance (CL_{met} , with 95% confidence interval) of midazolam in 8 healthy volunteers before (baseline) and during ritonavir therapy (100 mg twice daily, days 1–5; light blue box) as well as 2 and 4 days after ritonavir discontinuation (day 7 and 9, respectively)

events were mild. The most common adverse event was headache on the first study day without ritonavir therapy (5 out of 8 participants affected). A relationship was considered unlikely, as only microdosed substances were administered on this day and headaches only occurred in one participant after repeated administration on the fifth day of ritonavir intake.

4 Discussion

4.1 Interaction of Low-Dose Ritonavir With Factor Xa Inhibitors

Treatment with ritonavir 100 mg bid for 5 days increased the AUC_{0-24} of all currently marketed FXaI but with different magnitude and likely little clinical relevance in the absence of other clearance impairment (e.g., renal dysfunction) [40–42]. The largest increase was observed with rivaroxaban but this change was below the order of magnitude that usually prompts dose reductions (factor 2). Unless very high doses are administered, as required in the initial treatment of major embolic events [43], dose reductions should be individually planned and it should also be considered that COVID-19 can trigger a prothrombotic state and at least prophylactic anticoagulation is generally recommended [44].

The differences in the magnitude of interaction between the different FXaI are not fully elucidated. They are certainly influenced by the different effects of ritonavir on the diverse clearance mechanisms involved, and similar differences have also been observed in a series of patients receiving these FXaI together with protease inhibitors boosted with ritonavir

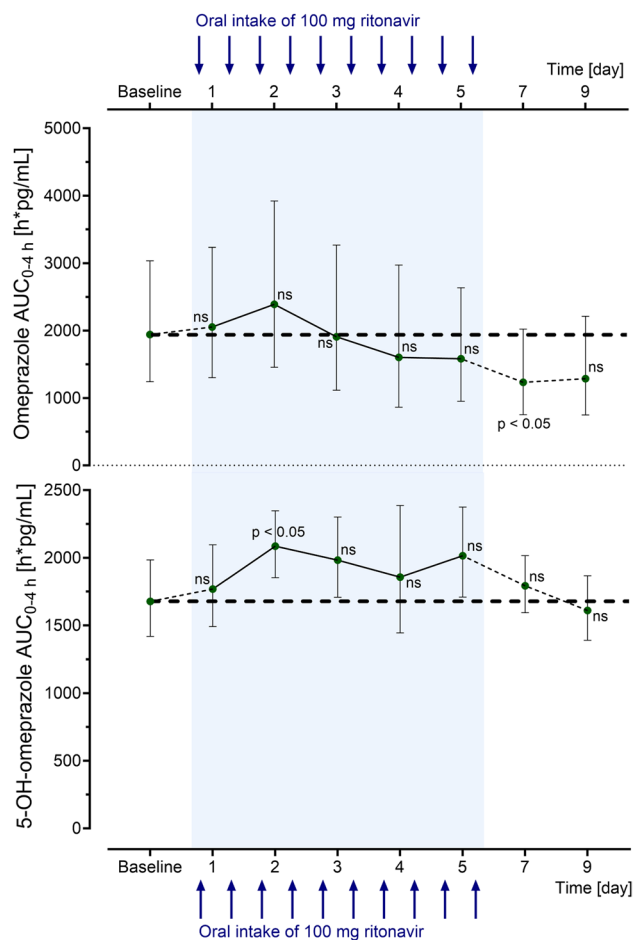


Fig. 5 Geometric means of the omeprazole AUC_{0-4} and 5-OH-omeprazole AUC_{0-4} of a 100- μ g omeprazole dose (with 95% confidence intervals) before (baseline) and during ritonavir (100 mg bid) for 5 days (light blue box), as well as 2 and 4 days after ritonavir discontinuation (day 7 and 9, respectively). ns: $p > 0.05$. AUC_{0-4} area under the concentration–time curve from 0 to 4 h, bid twice daily

100 mg bid [18–23]. In these earlier case series and in our study, the interaction was most pronounced with rivaroxaban, the FXaI with the largest bioavailability (and complete bioavailability at low doses [45]), indicating that the exposure change was not caused by a change in bioavailability but rather a reduction of systemic drug clearance, which is also reflected in a prolongation of the elimination $t_{1/2}$. Other than with other FXaI, these clearance changes were partly due to reduced renal excretion, likely reflecting combined inhibition of efflux transporters (P-gp and BCRP) and of the organic anion transporter 3 (OAT3, SLC22A8), which plays a major role in the renal elimination of rivaroxaban [46, 47]. In our study with low ritonavir doses, the effect was smaller than the interaction observed with very high doses of ritonavir (600 mg bid), where a 2.5-fold increase in AUC and an even more extensive reduction of CL_{renal} were observed [17, 25, 36]. These data therefore suggest

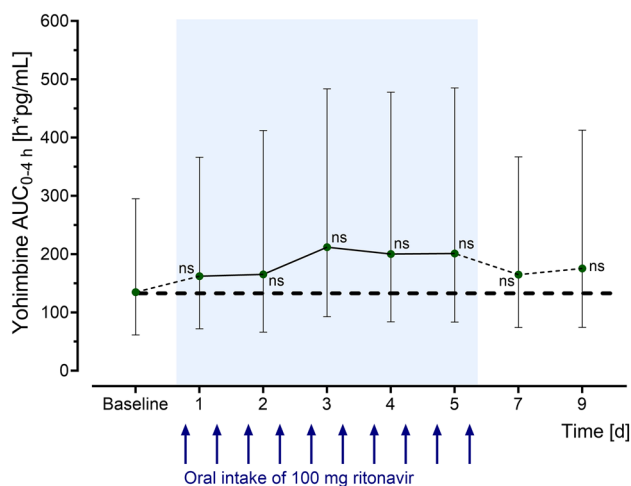


Fig. 6 Geometric means of AUC_{0–4} of a 50-µg yohimbine dose (with 95% confidence intervals) before (baseline) and during ritonavir (100 mg bid) for 5 days (light blue box), as well as 2 and 4 days after ritonavir discontinuation (day 7 and 9, respectively). ns: $p > 0.05$. AUC_{0–4} area under the concentration–time curve from 0 to 4 h, bid twice daily

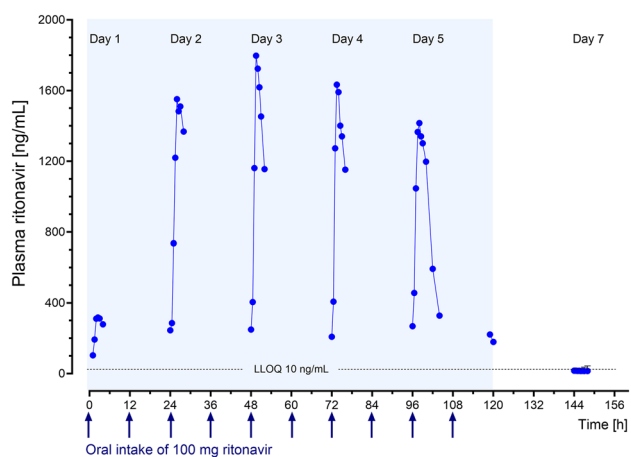


Fig. 7 Ritonavir plasma concentration–time curves during 5 days of ritonavir therapy (100 mg twice daily; light blue box) and 2 days after discontinuation (day 7) in 8 healthy volunteers. LLOQ lower limit of quantification

that boosting doses of ritonavir do not completely inhibit renal efflux transporters while they are likely high enough to inhibit CYP3A4 (and possibly CYP2J2 [7, 17, 48]) as efficiently as higher doses [2].

In contrast, there was only a minor DDI with apixaban, which is in agreement with the minimal interaction with potent CYP3A inhibitors such as voriconazole [25, 36] and the limited suspected contribution of efflux transporters [49] or OAT3 [47].

Ritonavir also increased edoxaban exposure but, in contrast to rivaroxaban, CL_{renal} and $t_{1/2}$ were not altered,

suggesting an increase in bioavailability as a result of intestinal inhibition of efflux transporters. This is consistent with other DDI studies and physiology-based pharmacokinetic modelling, which suggested a role of intestinal P-gp (and possibly BCRP) in edoxaban bioavailability [50–52]. It also agrees well with the effect size observed with the P-gp substrate dabigatran etexilate when it was administered with the same dose of ritonavir [53]. In addition, ritonavir also inhibits carboxylesterase 1 (CES1) in vitro [54], suggesting that concurrent inhibition of this minor pathway [10] could additionally be involved. The different influence of ritonavir on the urinary excretion of FXaI could be due to the fact that edoxaban and apixaban are not substrates of OAT3 and therefore the activity of the downstream renal P-gp is less important for their urinary excretion [47, 51].

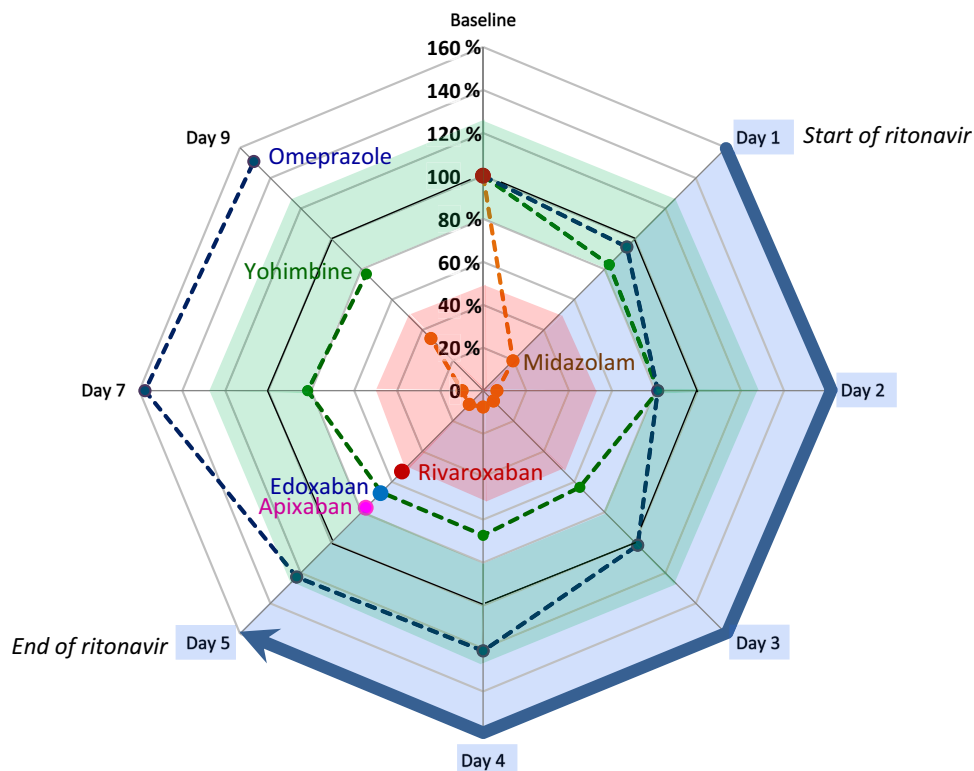
4.2 CYP Probe Drugs

Our trial generated activity data of relevant drug metabolizing CYPs over the time course of ritonavir treatment (5 days) and after its discontinuation. As expected [2], already after the first ritonavir dose, the CYP3A4 clearance profoundly decreased, indicating strong inhibition. This is in line with the dose–CYP3A4 inhibition relationship established in vivo, which estimated that the ritonavir dose inhibiting CYP3A4 by 50% (ED_{50}) was very low (3.4 mg) [2]. After ritonavir discontinuation, the CYP3A activity recovered slowly with a half-life of 2.3 days. On day 4 after ritonavir discontinuation, CYP3A activity was still reduced while no ritonavir was detectable in plasma, which suggests prolonged mechanism-based inactivation of CYP3A4. Based on these data, the full recovery of CYP3A4 activity is estimated to take 10–12 days after a 5-day treatment course with ritonavir 100 mg bid.

Regarding the effect of ritonavir on CYP2D6 activity, no significant changes of the probe drug yohimbine were observed. In contrast, using desipramine as an index substrate for CYP2D6, ritonavir (100 mg bid) caused a modest inhibitory effect on CYP2D6 activity in extensive metabolizers [55]. However, the dextromethorphan/dextrorphan metabolic ratio as another indicator for CYP2D6 activity was not altered. Thus, these findings with microdosed yohimbine as a sensitive CYP2D6 probe drug [27] confirm that no clinically relevant alteration of CYP2D6 enzyme activity during the course of a 5-day treatment with ritonavir 100 mg bid occurs.

Ritonavir had a small induction effect on CYP2C19 activity leading to slightly decreased omeprazole exposure on day 5 of ritonavir treatment, which was also reflected in corresponding changes in the molar AUC ratio of omeprazole and 5-hydroxy omeprazole. This inducing effect is time-dependent as it is more pronounced after a 10-day ritonavir (100 mg bid) treatment [56]. CYP2C19 induction is also

Fig. 8 Radar chart of oral clearance changes induced by 5 days of low-dose ritonavir therapy (100 mg twice daily) in 8 healthy volunteers. The percentage clearance compared with baseline, which is set at 100%, is shown for the different study days. The area of bioequivalence is highlighted in green, the risk area where dose adjustments may be required is highlighted in red



dose-dependent as reported with other CYP2C19 substrates; as an example, after 20 days of ritonavir (100 mg bid) treatment, voriconazole exposure was decreased by 33% [57] or 39% [58], which is only a small effect, probably attenuated by the autoinhibition of voriconazole [59, 60]. However, higher doses of ritonavir 400 mg bid for 20 days decreased voriconazole AUC by 83% [58]. Although short-term ritonavir treatment with low doses induces CYP2C19 activity, this effect is short lasting and likely does not require any dose adaptation of CYP2C19 substrates.

4.3 Limitations

The following limitations merit discussion. First, we only assessed the effect on the last day of the 5-day ritonavir treatment, which was expected to be the day with the maximum possible change in short-course low-dose therapies with ritonavir. Due to its short duration, this trial was not suitable to assess the potential inducing properties of ritonavir and its results should only be extrapolated to longer treatment regimens with caution. In addition, no transporter probe drugs were tested, so the time course of transporter inhibition is still not well characterized. Second, patients may differ from healthy volunteers in many ways. In patients, comorbidities such as renal impairment can potentiate apparently minor DDI [41, 42] and it will always be critical to consider all relevant clearance pathways when determining appropriate

individualized treatment plans for FXaI. Third, coadministration of the probe drugs with sodium hydrogen carbonate to buffer gastric acid could potentially influence their pharmacokinetics. However, the baseline pharmacokinetics agree well with prior studies using microdosed FXaI [25, 36], suggesting that buffering did not alter the pharmacokinetics of the three microdosed FXaI. This is in line with the absence of clinically relevant DDI of therapeutic FXaI doses with acid lowering drugs such as famotidine, ranitidine, or omeprazole [61–64]. In addition, the clearance values and relative contribution of renal excretion compare well with those reported for regular doses of FXaI in healthy volunteers [17, 18, 65], suggesting that microdoses adequately reflect the pharmacokinetics of regular doses and are therefore suitable for DDI studies.

5 Conclusion

We have successfully evaluated the effect of a 5-day treatment regimen with low doses of ritonavir (100 mg bid) on the pharmacokinetics of three FXaI in healthy volunteers (Fig. 8) and found that the clearance of all FXaI decreased with potential clinical relevance only for rivaroxaban, where a dose reduction by a factor of 0.5 might be considered if therapeutic rivaroxaban doses are administered.

Concurrently, we also monitored the daily activity changes of three important CYPs over the 5-day treatment and 2 days and 4 days after discontinuation of ritonavir and found that CYP2D6 activity was not significantly altered, while CYP2C19 was mildly induced and CYP3A was profoundly inhibited.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40262-024-01350-x>.

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Declarations

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Conflict of interest Walter E. Haefeli received grants for clinical trials, travel support, and lecture fees from Daiichi Sankyo, the manufacturer of edoxaban, not related to this work. All other authors have no conflicts of interest to declare.

Availability of data and material The collected and analysed data can be made available upon reasonable request. Part of the data was presented at the 19th World Congress of Clinical Pharmacology, Glasgow, and therefore published as an abstract [66].

Ethical approval and informed consent The trial protocol (EudraCT 2021-006634-39) was approved by the competent authority (BfArM, Bonn, Germany) and the responsible Ethics Committee of the Medical Faculty of Heidelberg University. All procedures performed in studies involving human participants were in accordance with Good Clinical Practice (ICH-GCP) guidelines, all pertinent German laws, and the ethical standards specified in the applicable version of the declaration of Helsinki. All volunteers gave their written informed consent prior to any study procedures.

Trial registration The trial was registered at the European Union Drug Regulating Authorities for Clinical Trials (EudraCT-No. 2021-006643-39) and at German Clinical Trial Register with ID DRKS00027838.

Author contributions WEH and GM proposed the research topic. BSR, AB, EK, WEH, and GM developed the trial protocol. BSR was the responsible study physician and executed the trial. KIF and JB analysed the samples. BSR and GM performed the trial analysis and the statistics. BSR wrote the first draft of the manuscript. All authors discussed and interpreted the results and contributed to the manuscript.

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