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ORIGINAL ARTICLE

Application of a microdosed cocktail of 3 oral factor Xa inhibitors to study drug–drug interactions with different perpetrator drugs

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Aims: Using 3 different perpetrators the impact of voriconazole, cobicistat and rifampicin (single dose), we evaluated the suitability of a microdose cocktail of factor Xa inhibitors (FXaI; rivaroxaban, apixaban and edoxaban; 100 μg in total) to study drug–drug interactions.

Methods: Three cohorts of 6 healthy volunteers received 2 treatments with microdoses of rivaroxaban, apixaban and edoxaban alone and with coadministration of 1 of the perpetrators. Plasma and urine concentrations of microdosed apixaban, edoxaban and rivaroxaban were quantified using a validated ultra-performance liquid chromatography–tandem mass spectrometry with a lower limit of quantification of 2.5 pg/mL.

Results: Voriconazole caused only a minor interaction with apixaban and rivaroxaban, none with edoxaban. Cobicistat significantly increased exposure of all 3 FXaI with area under the plasma concentration–time curve ratios of 1.67 (apixaban), 1.74 (edoxaban) and 2.0 (rivaroxaban). A single dose of rifampicin decreased the volume of distribution and elimination half-life of all 3 FXaI.

Conclusions: The microdosed FXaI cocktail approach is able to generate drug interaction data and can help elucidating the mechanism involved in the clearance of the different victim drugs. This is a safe approach to concurrently study drug–drug interactions with a drug class. (EudraCT 2016–003024-23).

KEYWORDS

apixaban, cobicistat, drug interaction, edoxaban, rifampicin, rivaroxaban, voriconazole

1 | INTRODUCTION

A new class of anticoagulants—the factor Xa inhibitors (FXaI)—has been introduced in the last decade; these are increasingly used for the prevention of thromboembolic events in patients with nonvalvular atrial fibrillation,[1] the prevention and treatment of pulmonary embolism, and the prevention and treatment deep vein thrombosis.[2] FXaI pharmacokinetics are linked to their pharmacodynamics, efficacy and safety.[3] It has been claimed that FXaI have fewer interactions with food and drugs in comparison to vitamin K antagonists.[4] Clearance mechanisms of FXaI vary substantially between representatives of this

The authors confirm that Gerd Mikus is the Principal Investigator of this clinical trial and that he had direct clinical responsibility for the participants.

class. **Apixaban**, **edoxaban** and **rivaroxaban** are all substrates of cytochrome P450 isozymes (predominantly **CYP3A4**) and efflux transporters, such as **P-glycoprotein** (P-gp) and the **Breast Cancer Resistance Protein** (BCRP), albeit to varying extents.[5–9] Therefore, these FXaI are prone to be victims of perpetrators drugs altering the activity of CYP isozymes and/or drug transporters.[5,10,11] However, due to differences in their clearance mechanisms, evidence obtained with 1 FXaI cannot be extrapolated to other class members and, thus, each FXaI has to be studied individually with a number of different perpetrators. A cocktail approach using microdoses of the 3 FXaI could reduce the number of studies and enable the possibility to compare the data within the same subjects for all 3 FXaI at once. Using this microdosed FXaI cocktail approach with 25 μg apixaban, 50 μg edoxaban and 25 μg rivaroxaban, we have recently shown that **ketoconazole's** drug interactions with therapeutic FXaI doses could precisely be predicted with this microdose cocktail.[12] The exposure changes, expressed as the ratio of the area under the plasma concentration–time curve (AUC) with and without ketoconazole, as a measure of the drug interaction effect were 1.90 for apixaban, 2.35 for edoxaban and 2.27 for rivaroxaban.[12] These results are well comparable to drug–drug interaction trials at therapeutic doses of apixaban (1.99[11]), edoxaban (1.87[10]) and rivaroxaban (1.82 single-dose, 200 mg ketoconazole; and 2.58 steady-state, 400 mg ketoconazole).[5]

To further evaluate the suitability of this FXaI microdosing approach, 3 different paradigm perpetrators (**cobicistat**, **rifampicin** and voriconazole) were studied in the same participants as an extension of the earlier ketoconazole drug interaction trial. The aim was to generate interaction data of the 3 victim FXaI simultaneously using a total dose of 100 μg. In addition, the effect of these paradigm perpetrators on CYP3A activity and, thus, the contribution of the CYP3A pathway to FXaI clearance pathways was measured by the established microdosed **midazolam** methodology.[13,14] Furthermore, coagulation parameters were monitored to assess the safety of this approach.

What is already known about this subject

- Using an approach where in total only 1% of a therapeutic dose of 3 FXa inhibitors is administered simultaneously, a drug interaction study with ketoconazole predicts precisely the known magnitude of inhibition for each of the 3 drugs when given as a therapeutic dose.
- A minimal risk for the study participants is associated with the use of FXa inhibitors when using the microdose approach.

What this study adds

- With a single study, valid drug interaction data for several victim drugs can be concurrently obtained in the same participants.
- Especially for a new drug class, knowledge on drug interactions is often limited. This microdose cocktail offers the opportunity to generate mechanistic insights into drug–drug interactions with the new drug class of FXaI and can help to elucidate FXaI clearance mechanisms completely.
- This approach might be a valuable tool because it identifies the impact of currently unknown drug–drug interactions with all European Medicines Agency-approved FXaI.

2 | METHODS

The study protocol (EudraCT 2016–003024-23) was approved by the responsible Ethics Committee of Heidelberg University Hospital, Germany and the competent authority (BfArM, Bonn, Germany). The study was performed at the Department of Clinical Pharmacology and Pharmacoepidemiology at Heidelberg University Hospital (Heidelberg, Germany) in accordance with the actual declaration of Helsinki. Before inclusion in the clinical trial, each participant signed a written declaration of informed consent.

2.1 | Study population

Eighteen healthy Caucasian women (n = 11) and men (n = 7; 18–56 years; body mass index of 19.8–29.7 kg/m^2 and haemoglobin concentrations >11 g/dL) participated and completely finished the trial. No regular drug intake within the last 2 weeks except for oral contraceptives was permitted and a participation in other clinical trials within 6 weeks before inclusion was not allowed. Additional details have previously been reported.[12]

2.2 | Study design

After the randomised cross-over part using ketoconazole and the FXaI cocktail alone,[12] an additional trial part was amended in which the same 18 participants (7 male, 11 female) were randomised to 1 of 3 treatments (6 participants each; Figure 1):

i Voriconazole: (Voriconazol-ratiopharm, ratiopharm GmbH, Ulm, Germany; 3 male, 3 female). Two doses of voriconazole (400 mg orally) were administered starting 24.5 and 12.5 hours before FXaI administration and were followed by 4 doses of voriconazole (200 mg orally) 30 minutes before and 11.5, 23.5, and 35.5 h after FXaI administration.

ii Rifampicin: (Eremfat, RIEMSER Pharma GmbH, Greifswald, Germany); 1 male, 5 female). A single oral dose of 600 mg rifampicin was administered 30 minutes before FXaI administration.

iii Cobicistat (administered as the combination product Genvoya [cobicistat/elvitegravir/emtricitabine/tenofovir alafenamide

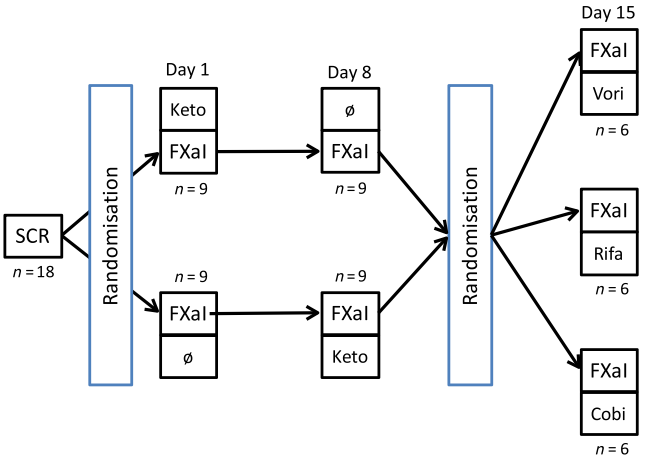

FIGURE 1 Study flow diagram of the complete study with n = 18 study participants. SCR: screening; FXaI: Microdosed cocktail of 3 factor Xa inhibitors (apixaban, edoxaban, rivaroxaban) plus 10 µg midazolam; Keto: ketoconazole; Vori: voriconazole; Rifa: rifampicin; Cobi: cobicistat

fumarate, Gilead Sciences International Ltd., Cambridge, UK]; 3 male, 3 female). In total, 3 doses of Genvoya containing 150 mg of cobicistat, 150 mg of elvitegravir, 200 mg of emtricitabine and 10 mg of tenofovir alafenamide fumarate were administered 24.5 and 0.5 hours before and 23.5 hours after FXaI administration.

Immediately after each FXaI administration, an oral solution of 10 µg midazolam (Dormicum V 5 mg/5 mL, Roche Pharma AG, Grenzach-Wyhlen, Germany) was administered. Oral stock solutions of each FXaI were prepared and provided by the hospital pharmacy according to a pharmaceutical development protocol approved by the competent authority (BfArM, Bonn, Germany). Each FXaI solution was prepared in a separate bottle containing 2.5 µg/mL apixaban, 30 µg/mL edoxaban and 2.5 µg/mL rivaroxaban. Oral solutions were freshly prepared by dilution of the stock solutions in 1 step 30 minutes before administration. For rivaroxaban and apixaban, 10 mL stock solution, and for edoxaban, 1.66 mL stock solution were transferred into ~100 mL tap water in a plastic cup yielding final doses of 25 µg rivaroxaban, 25 µg apixaban, and 50 µg edoxaban.

2.3 | Study conduct

The study was conducted in the department's clinical trial unit KliPS, which is certified according to DIN EN ISO 9001. On the FXaI pharmacokinetic study days, blood samples (LiHep tubes) were collected before and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 32 and 48 hours after administration. To assess CYP3A activity, blood samples (LiHep tubes) were taken before and 2, 2.5, 3 and 4 hours after the midazolam microdose.[15] Urine was collected for 48 hours in 2 24-hour periods. Blood samples were immediately centrifuged for 10 minutes at 4°C and 2500 g. The separated plasma and 10 mL urine aliquots were stored at −20°C until analysis.

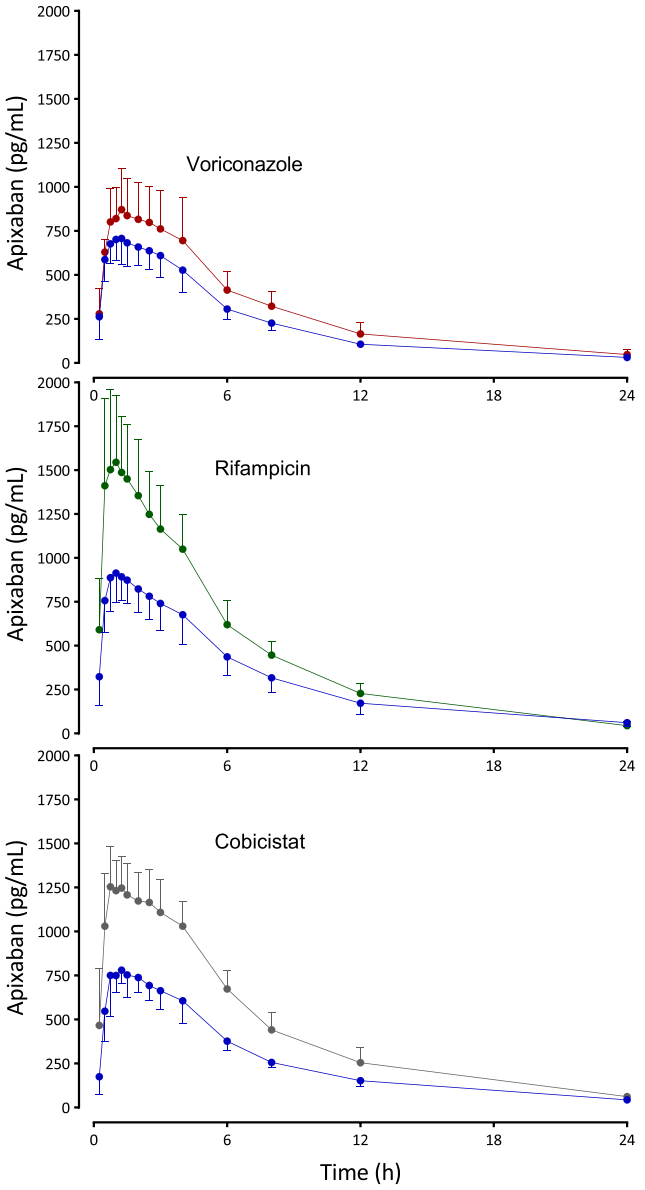

FIGURE 2 Mean (±standard deviation) plasma concentration-time profiles of apixaban (blue closed circles) after simultaneous oral administration of 25 mg apixaban, 50 µg edoxaban and 25 µg rivaroxaban alone and during voriconazole (red closed circles), rifampicin (green closed circles) and cobicistat (grey closed circles) in 6 healthy volunteers each

Additional blood samples (citrate 3.2%) to quantify international normalised ratio (INR) and activated partial thromboplastin time (aPTT) were taken at the screening visit, 2 hours after FXaI administration (in each study part, expected peak concentration [C_{max}]), and at the end of the study.

2.4 | Quantification of FXaI and midazolam

A previously published ultra-performance liquid chromatography–tandem mass spectrometry method was used to quantify midazolam

concentrations in plasma.[15] Plasma concentrations of apixaban, edoxaban and rivaroxaban were analysed using an ultra-sensitive ultra-performance liquid chromatography–tandem mass spectrometry assay.[12,16] All assays fulfilled the pertinent guidelines on bioanalytical method validation of the US Food and Drug Administration[17] and the European Medicines Agency[18] with accuracy and precision values of ≤ ± 15%. The lower limits of quantification were 0.093 pg/mL for midazolam and 2.5 pg/mL for each FXaI.

2.5 | Calculations and statistical analysis

Standard pharmacokinetic parameters of each FXaI were determined using Kinetica 5.0 (Thermo Fisher Scientific, Waltham, MA, USA). The following pharmacokinetic parameters were calculated by a noncompartmental analysis using plasma concentrations of apixaban, edoxaban and rivaroxaban: C_{max}, time to C_{max} (t_{max}), terminal elimination half-life ($t_{1/2}$), $AUC_{0-\infty}$, volume of distribution (V_{ss}/F) and apparent oral clearance (Cl/F). AUCs were calculated by a mixed log-linear model. Renal clearance was calculated as amount excreted unchanged in urine divided by AUC. Nonrenal clearance is the difference between Cl/F and renal clearance. AUC ratio (AUCR) is the quotient of AUC of FXaI during the perpetrator divided by AUC of the FXaI alone; the C_{max} ratio was calculated correspondingly.

Descriptive statistics were calculated for each treatment and each pharmacokinetic parameter with geometric mean and the respective 95% confidence interval listed in the result tables. The statistical model used for the analysis of $AUC_{0-\infty}$ and C_{max} of each perpetrator interaction (n = 6) with each FXaI is a repeated measures ANOVA (analysis of variance) for a cross-over design after logarithmic transformation. The 90% confidence intervals for the ratios were calculated by re-transformation of the logarithmic results. Graphical and statistical analysis was done with Prism 7.0 (GraphPad Software Inc., La Jolla, CA, USA).

In addition, the effect of the paradigm perpetrators on the CYP3A marker substance midazolam was tested equally. Point estimates (geometric mean ratios) and the 90% confidence intervals for the pairwise ratios of AUC_{2-4} and estimated metabolic clearance (eCl_{met}) were calculated.[19]

2.6 | Nomenclature of target and ligands

Drug/molecular targets in this article were hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY and are permanently archived in the Concise Guide to PHARMACOLOGY.[20,21]

3 | RESULTS

3.1 | Apixaban

Apixaban plasma concentrations were significantly increased by coadministration of voriconazole, rifampicin and cobicistat (Figure 2). C_{max} showed significant increases for rifampicin (C_{max} ratio 1.69; 95% confidence interval [CI]: 1.17–2.45) and cobicistat (C_{max} ratio 1.60; 95% CI: 1.29–1.99), but not for voriconazole (C_{max} ratio 1.15; 95% CI: 0.94–1.41; Table 1; Figure 2). Apixaban AUC increased significantly for each perpetrator with AUCRs significantly different from 1 for voriconazole (1.33; 95% CI: 1.01–1.75), rifampicin (1.33; 95% CI: 1.06–1.68) and cobicistat (1.67; 95% CI: 1.33–2.09). The terminal elimination half-life was unchanged except for rifampicin where an almost 40% shortening was observed (8.37 *vs* 5.16 h). Apixaban Cl/F was consequently reduced to 75% by voriconazole and rifampicin, and to 60% by cobicistat. The oral cobicistat and rifampicin reduced V_{ss}/F to almost 50%. Apixaban renal clearance was reduced to 70% during cobicistat.

3.2 | Edoxaban

Plasma concentrations of edoxaban were significantly increased by coadministration of rifampicin and cobicistat (Figure 3). No significant change of any edoxaban pharmacokinetic parameter was observed during voriconazole (Table 2). C_{max} and AUC of edoxaban significantly increased during rifampicin and cobicistat (Table 2). Both rifampicin and cobicistat showed significant differences from 1 for edoxaban AUCR (1.90; 95% CI: 1.43–2.52/1.74; 95% CI: 1.37–2.21) and C_{max} ratio (2.76; 95% CI: 1.76–4.32/2.40; 95% CI: 1.45–4.00). Edoxaban terminal elimination half-life was shorter for rifampicin (30%) and cobicistat (25%). Consequently, edoxaban apparent oral clearance was reduced to 53% by rifampicin and to 57% by cobicistat. Rifampicin reduced the oral V_{ss}/F to 37% and cobicistat to 42%.

3.3 | Rivaroxaban

Average plasma concentrations of rivaroxaban were higher after coadministration of voriconazole, rifampicin and cobicistat (Figure 4). However, C_{max} of rivaroxaban significantly increased for cobicistat only (Table 3). Voriconazole resulted in 33% changes of AUC and apparent oral clearance (Table 3). Cobicistat showed significant differences from 1 for rivaroxaban AUCR (2.00; 95% CI: 1.57–2.55) and C_{max} ratio (1.39; 95% CI: 1.20–1.62). Rivaroxaban terminal elimination half-life was shorter for rifampicin (31%), but prolonged by cobicistat (40%). The oral V_{ss}/F was reduced by both rifampicin to 64% and cobicistat to 72%.

3.4 | Sex differences

No sex differences were observed for apixaban, edoxaban and rivaroxaban pharmacokinetics (7 male, 11 female), in addition no obvious sex differences for each of the DOACs during voriconazole and cobicistat with 3 male and 3 female participants each. For the rifampicin part, no valid statement can be made due to the imbalanced sex distribution (1 male, 5 female).

TABLE 1 Pharmacokinetics of 25 μg apixaban alone and during voriconazole, single dose rifampicin, and cobicistat treatment in 6 healthy participants each

	Apixaban	Apixaban + voriconazole		Apixaban	Apixaban + rifampicin		Apixaban	Apixaban + cobicistat	
	Geom. Mean (95% CI)	Geom. Mean (95% CI)	*P*-value	Geom. Mean (95% CI)	Geom. Mean (95% CI)	*P*-value	Geom. Mean (95% CI)	Geom. Mean (95% CI)	*P*-value
C_{max} (pg/mL)	741 (619–886)	853 (652–1115)	n.s.	914 (751–1114)	1547 (1220–1961)	<.05	836 (685–1021)	1341 (1192–1509)	<.001
t_{max} (h)	1.0 (0.75–2.0)	1.25 (1.0–2.0)	n.s.	1.0 (0.75–1.25)	1.0 (0.5–1.25)	n.s.	1.25 (0.75–1.5)	1.0 (0.75–2.5)	n.s.
AUC_{∞} (h pg/mL)	5341 (4714–6052)	7086 (5121–9807)	<.05	7773 (6447–9372)	10352 (8422–12724)	<.05	6273 (5667–6943)	10436 (8836–12389)	<.001
$t_{1/2}$ (h)	6.32 (4.31–9.60)	7.23 (4.43–11.8)	n.s.	8.37 (4.90–14.3)	5.16 (3.88–6.86)	<.05	5.85 (4.74–7.21)	5.91 (4.61–7.58)	n.s.
V_{ss}/F (l)	36.4 (27.7–47.9)	30.5 (24.2–38.4)	n.s.	31.9 (20.7–49.2)	16.1 (12.9–20.1)	<.05	31.9 (26.0–39.3)	18.5 (16.7–20.4)	<.005
Cl/F (mL/min)	78.0 (69.0–88.4)	58.8 (42.5–81.4)	<.05	53.6 (44.5–64.6)	40.3 (32.8–49.5)	<.05	66.4 (60.0–73.5)	39.8 (33.6–47.2)	<.005
Ae (% of dose)	22.2 (17.5–28.0)	25.5 (22.2–29.3)	n.s.	26.2 (16.1–42.6)	30.8 (21.9–43.5)	n.s.	24.6 (17.3–35.1)	27.3 (20.9–35.7)	n.s.
Cl_{ren} (mL/min)	17.3 (13.9–21.5)	15.0 (11.3–19.8)	n.s.	14.1 (9.3–21.2)	12.4 (8.4–18.3)	n.s.	16.4 (12.4–21.6)	10.9 (7.7–15.3)	<.05
Cl_{nonren} (mL/min)	60.3 (51.2–71.0)	43.7 (30.7–62.1)	n.s.	38.2 (28.6–50.8)	27.0 (20.7–35.3)	<.05	49.1 (40.0–60.2)	28.6 (23.9–34.1)	<.005

Data are expressed as geometric mean (95% CI) for all parameters, with the exception of t_{max}, which is given as median (range).

Ae, amount excreted in urine as parent drug; AUC_{∞}, area under the concentration–time curve extrapolated to infinity; CI, confidence interval; Cl/F, apparent oral clearance; Cl_{ren}, renal clearance; Cl_{nonren}, non-renal clearance; C_{max}, peak concentration; n.s., not significant $P > .05$; t_{max}, time to peak concentration; $t_{1/2}$, terminal elimination half-life; V_{ss}/F, oral steady-state volume of distribution.

FIGURE 3 Mean (±standard deviation) plasma concentration–time profiles of edoxaban (blue closed diamonds) after simultaneous oral administration of 25 mg apixaban, 50 μg edoxaban and 25 μg rivaroxaban alone and during voriconazole (red closed diamonds), rifampicin (green closed diamonds) and cobicistat (grey closed diamonds) in 6 healthy volunteers each

3.5 | CYP3A activity

To assess the effect of the 3 perpetrators on CYP3A activity, midazolam was orally coadministered as an ineffective microgram dose.[17] All perpetrators significantly increased midazolam AUC_{2-4} and accordingly decreased the calculated partial metabolic midazolam clearance (Table 4). Midazolam AUCR accounted for 8.16 during voriconazole, 1.30 during rifampicin, and 8.77 during cobicistat coadministration.

TABLE 2 Pharmacokinetics of 50 µg edoxaban alone and during voriconazole, rifampicin, and cobicistat treatment in 6 healthy participants each

	Edoxaban	Edoxaban + voriconazole		Edoxaban	Edoxaban + rifampicin		Edoxaban	Edoxaban + cobicistat	
	Geom. Mean (95% CI)	Geom. Mean (95% CI)	*P*-value	Geom. Mean (95% CI)	Geom. Mean (95% CI)	*P*-value	Geom. Mean (95% CI)	Geom. Mean (95% CI)	*P*-value
C_{max} (pg/mL)	141 (108–184)	179 (127–251)	n.s.	171 (127–228)	471 (344–644)	<.005	176 (115–267)	422 (320–557)	<.01
t_{max} (h)	0.75 (0.5–3.0)	1.0 (0.25–2.5)	n.s.	0.75 (0.5–1.25)	0.75 (0.5–1.5)	n.s.	1.25 (0.75–1.5)	0.625 (0.5–2.5)	n.s.
AUC_{∞} (h pg/mL)	945 (664–1345)	1191 (758–1871)	n.s.	1296 (1006–1670)	2461 (2126–2849)	<.005	1223 (1036–1443)	2131 (1859–2443)	<.005
$t_{1/2}$ (h)	5.47 (4.24–7.06)	5.81 (3.92–8.59)	n.s.	5.60 (3.43–9.14)	3.90 (3.25–4.68)	<.05	5.19 (4.03–6.67)	3.88 (2.79–5.39)	<.05
V_{ss}/F (l)	373 (259–537)	302 (219–415)	n.s.	297 (196–452)	107 (83–136)	<.005	302 (212–431)	127 (101–161)	<.005
Cl/F (mL/min)	882 (619–1255)	700 (445–1100)	n.s.	643 (499–829)	339 (293–392)	<.005	682 (578–804)	391 (341–448)	<.01
Ae (% of dose)	24.4 (17.7–33.6)	29.4 (21.2–40.8)	n.s.	27.2 (19.1–38.8)	48.7 (35.9–66.2)	<.05	32.3 (23.6–44.3)	42.5 (33.1–54.6)	n.s.
Cl_{ren} (mL/min)	215 (167–277)	206 (144–295)	n.s.	175 (146–210)	165 (119–229)	n.s.	220 (151–321)	166 (120–230)	n.s.
Cl_{nonren} (mL/min)	655 (420–1021)	482 (280–832)	n.s.	458 (320–656)	163 (120–222)	<.005	448 (362–556)	218 (181–262)	<.01

Data are expressed as geometric mean (95% CI) for all parameters, with the exception of t_{max}, which is given as median (range).

Ae, amount excreted in urine as parent drug; AUC_{∞}, area under the concentration–time curve extrapolated to infinity; CI, confidence interval; Cl/F, apparent oral clearance; Cl_{ren}, renal clearance; Cl_{nonren}, non-renal clearance; C_{max}, peak concentration; n.s., not significant $P > .05$; t_{max}, time to peak concentration; $t_{1/2}$, terminal elimination half-life; V_{ss}/F, oral steady-state volume of distribution.

FIGURE 4 Mean (±standard deviation) plasma concentration-time profiles of rivaroxaban (blue closed triangles) after simultaneous oral administration of 25 mg apixaban, 50 µg edoxaban and 25 µg rivaroxaban alone and during voriconazole (red closed triangles), rifampicin (green closed triangles) and cobicistat (grey closed triangles) in 6 healthy volunteers each

3.6 | Safety

Overall, 13 adverse events (AEs) occurred in 9 of 18 participants, of which all were transient, none was serious or resulted in a dropout. Five out of 6 participants reported in total 7 AEs during voriconazole treatment, mainly visual disturbances (n = 4). During cobicistat treatment 3 out of 6 participants showed a single AE (n = 3); none was observed during rifampicin. When the microdosed FXaI and midazolam cocktail was given alone, only in 3 out of 18 participants was an AE observed.

TABLE 3 Pharmacokinetics of 25 μg rivaroxaban alone and during voriconazole, rifampicin, and cobicistat treatment in 6 healthy participants each

	Rivaroxaban	Rivaroxaban + voriconazole		Rivaroxaban	Rivaroxaban + rifampicin		Rivaroxaban	Rivaroxaban + cobicistat	
	Geom. Mean (95% CI)	Geom. Mean (95% CI)	P-value	Geom. Mean (95% CI)	Geom. Mean (95% CI)	P-value	Geom. Mean (95% CI)	Geom. Mean (95% CI)	P-value
C_{max} (pg/mL)	822 (508–1330)	966 (553–1688)	n.s.	885 (750–1044)	1244 (830–1867)	n.s.	992 (736–1338)	1382 (1053–1813)	<.005
t_{max} (h)	0.5 (0.5–1.0)	0.75 (0.25–1.25)	n.s.	0.63 (0.5–1.25)	0.63 (0.5–1.5)	n.s.	0.75 (0.5–1.5)	0.5 (0.25–0.75)	<.05
AUC_{∞} (h pg/mL)	3449 (2424–4908)	4525 (3041–6734)	<.05	3686 (2750–4941)	4854 (3710–6350)	n.s.	3993 (3222–4949)	7984 (7092–8987)	<.001
$t_{1/2}$ (h)	5.00 (4.38–5.67)	6.28 (4.49–8.79)	n.s.	5.02 (3.86–6.52)	3.47 (2.37–5.09)	<.05	4.70 (4.36–5.07)	6.60 (5.31–8.22)	<.05
V_{ss}/F (l)	35 (22–59)	32 (21–49)	n.s.	33 (27–41)	21 (14–32)	<.05	29 (19–43)	21 (17–27)	<.05
Cl/F (mL/min)	121 (85–172)	92 (62–137)	<.05	113 (84–152)	86 (66–112)	n.s.	104 (84–129)	52 (46–59)	<.001
Ae (% of dose)	53.7 (46.2–60.1)	55.5 (47.8–64.4)	n.s.	54.8 (48.7–61.6)	55.0 (42.7–70.7)	n.s.	44.5 (32.5–61.1)	49.7 (39.1–63.2)	n.s.
Cl_{ren} (mL/min)	63.6 (42.0–96.5)	51.1 (31.6–82.5)	n.s.	61.9 (44.8–85.6)	47.2 (30.7–72.5)	n.s.	46.5 (31.0–69.8)	25.9 (18.5–36.4)	<.05
Cl_{nonren} (mL/min)	56.3 (39.8–79.5)	40.0 (27.9–57.5)	<.05	50.3 (36.4–69.4)	35.1 (23.4–52.6)	n.s.	54.1 (37.3–78.3)	24.9 (20.2–30.9)	<.001

Data are expressed as geometric mean (95% CI) for all parameters, with the exception of t_{max}, which is given as median (range). Ae, amount excreted in urine as parent drug; AUC_{∞}, area under the concentration–time curve extrapolated to infinity; CI, confidence interval; Cl/F, apparent oral clearance; Cl_{ren}, renal clearance; Cl_{nonren}, nonrenal clearance; C_{max}, peak concentration; n.s., not significant $P > .05$; t_{max}, time to peak concentration; $t_{1/2}$, terminal elimination half-life; V_{ss}/F, oral steady-state volume of distribution.

INR and aPTT were not significantly different between screening visit and end of study visit. INR was significantly increased by the microdosed FXaI cocktail (3.9%) and by the cocktail and the 3 perpetrators (3.3%); aPTT was also significantly increased by the microdosed FXaI cocktail (3.1%); no significant alteration was observed for the cocktail or the 3 perpetrators (1.5%).

4 | DISCUSSION

This study successfully investigated the currently unknown impact of 3 different perpetrators on FXaI pharmacokinetics. Voriconazole, cobicistat and rifampicin (single dose) were chosen because the effect of these paradigm perpetrators is of value for many anticoagulated patients requiring long-term anti-infective therapy. The use of a FXaI microdose cocktail (total dose of 100 μg) allows the investigation of drug–drug interaction within the same drug class without generating pharmacologicals effects and reduces the risk for AE to the study participants. This approach might be a future direction to expand knowledge on drug–drug interaction with FXaI by reducing the number of drug–drug interaction trials but not the number of perpetrators. The approach to use low substrate concentrations and therapeutic perpetrator concentrations elicits the same degree of perpetrator effects in comparison to therapeutic substrate concentrations.[12,22,23] If the perpetrator dose is lowered, this results in dose and concentration dependent inhibition as shown for **ritonavir**.[24]

4.1 | Apixaban

Given orally, approximately 15% of apixaban is metabolised via CYP3A and about 6% by **CYP1A2** and CYP2J2.[6,7] The remainder (≥50%) is excreted unchanged into faeces and urine or has not been identified or recovered so far (about 22%).[6] Voriconazole decreased apixaban Cl/F by 25% most probably due to its potent inhibition of CYP3A. The AUCR of the coadministered CYP3A marker midazolam increased significantly, proving that the activity of CYP3A was attenuated by voriconazole. It is currently unknown whether CYP2J2 or CYP1A2 are also inhibited by voriconazole. However, taking into account that voriconazole markedly decreased apixaban Cl/F it might be possible that voriconazole also inhibited CYP2J2 and CYP1A2. In contrast to voriconazole, a single dose of rifampicin also reduced apixaban clearance by 25% but affected apixaban C_{max} stronger than voriconazole (69% *vs* 15%). These data suggest that rifampicin primarily affected the absorption (and/or distribution) of apixaban, which might be caused by an inhibition of intestinal P-gp[25] (and hepatic OATP1B1 and 1B3[26]), a transporter relevant during apixaban absorption. Interestingly, rifampicin significantly reduced apixaban $t_{1/2}$ but to a less pronounced extent as it has been observed with other substrates of P-gp, such as doravirine whose elimination half-life of 18.6 hours was reduced by a single dose of rifampicin to 5.5 hours.[27] An emerging early enzyme induction by rifampicin, which has been observed 12 hours after dosing of a single intravenous dose of

TABLE 4 Midazolam exposure and partial metabolic clearance after 10 μg midazolam alone and during voriconazole, rifampicin, and cobicistat treatment in 6 healthy participants each

	Midazolam		Midazolam + perpetrator		
Voriconazole	**Geom. Mean**	**95% CI**	**Geom. Mean**	**95% CI**	***P*-value**
AUC_{2-4} (h pg/mL)	34.9	20.4–59.8	285	242–334	<.0001
eCl_{met} (mL/min)	529	309–907	87	74–103	.0009
Rifampicin					
AUC_{2-4} (h pg/mL)	24.9	18.4–338	32.5	21.4–49.5	<.05
eCl_{met} (mL/min)	740	547–1002	568	373–865	<.05
Cobicistat					
AUC_{2-4} (h pg/mL)	30.7	19.4–48.7	270	222–328	<.0001
eCl_{met} (mL/min)	601	380–951	68	56–83	<.0001

Data are expressed as geometric mean (95% CI) for all parameters. AUC_{2-4}, area under the concentration–time curve from 2 to 4 hours after administration; CI, confidence interval; eCl_{met}, calculated partial metabolic clearance of midazolam to 1-OH-midazolam.

rifampicin with **warfarin**, might have caused the small impact on apixaban $t_{1/2}$.[28] The interaction observed after a single oral dose of rifampicin does not predict the interaction when rifampicin is administered repeatedly since the time-dependent CYP inducing effect will add to the transporter inhibiting effect. Recently, a modelling and simulation approach has been conducted for rifampicin to predict successfully the complex drug–drug interaction with **glibenclamide**.[29] Cobicistat, as the third perpetrator used, demonstrated the largest effects on apixaban pharmacokinetics with a 67% increase of AUC and a clearance reduction by 40%. Cobicistat acts as a strong CYP3A[30] and in addition as P-gp and BCRP inhibitor.[31] The inhibition of drug transporters by cobicistat might be an important mechanism for the increased AUC and C_{max} of apixaban. In combination with the CYP3A inhibition (Table 4), it can be anticipated that cobicistat increased apixaban absorption and reduced its first-pass metabolism.

4.2 | Edoxaban

The overall clearance mechanisms of an oral dose of edoxaban are very similar to those for apixaban. About 24% of an oral dose of edoxaban is excreted unchanged into urine and almost 50% of the dose can be found in faeces as parent drug.[9] However, cytochrome-mediated metabolism is less relevant for edoxaban clearance.[9] Consequently, the CYP3A inhibitor voriconazole did not significantly alter edoxaban pharmacokinetics. In contrast, a single oral dose of rifampicin resulted in a pronounced increase of edoxaban C_{max} (2.76-fold), which can probably be attributed to P-gp inhibition. P-gp in the gut wall probably contributes to the low oral bioavailability of edoxaban (~50%)[8] and high intestinal concentrations of rifampicin will most probably inhibit P-gp, thereby increasing edoxaban absorption and, thus, C_{max}. This is supported by an increased fraction of edoxaban excreted unchanged into urine (+48.7%) with unchanged renal clearance. P-gp actively secretes edoxaban into urine.[9] The intestinal concentration of rifampicin is assumed to be much higher than that in plasma following oral administration. Hence, inhibition of P-gp in the kidney will not be as prominent as in the intestine. Therefore, the intestine can be considered to be a primary tissue for the P-gp-mediated drug interaction for edoxaban. Almost equal results have been observed with cobicistat (P-gp and CYP3A inhibitor). Cobicistat did not alter renal clearance of edoxaban but increased C_{max} by almost the same magnitude as observed with rifampicin. The increased absorbed fraction of edoxaban again resulted in an increased renal excretion of unchanged edoxaban, which emphasises that drugs mainly affect edoxaban pharmacokinetics by altering the activity of intestinal drug transporters. CYP3A inhibition does not seem to be relevant for drug interactions with edoxaban as indicated by the absence of significant changes of edoxaban pharmacokinetics by voriconazole.

4.3 | Rivaroxaban

In addition to unspecific hydrolysis, rivaroxaban is metabolised via CYP3A and CYP2J2.[5] Each of these pathways contributes between 14 and 18% of rivaroxaban's overall clearance. Voriconazole reduced rivaroxaban clearance by 33%, which suggests that voriconazole inhibited both CYP3A and CYP2J2. It seems obvious that voriconazole inhibits CYP2J2, since CYP3A and CYP2J2 are responsible for ~32% of the total rivaroxaban clearance[5] and a 33% clearance reduction by voriconazole was observed (assuming complete rivaroxaban bioavailability), but there is no proof of this assumption. Rivaroxaban amount excreted unchanged in urine and renal clearance were not altered by voriconazole. In contrast, a single oral dose of rifampicin inhibiting drug transporters did not significantly change rivaroxaban exposure and clearance. Renal clearance was also unaffected which is not consistent with the known P-gp and BCRP mediated active renal secretion of rivaroxaban.[5,12,32] After 5 days of 400 mg ketoconazole or 5 days of 600 mg ritonavir twice daily, a strong inhibition of renal drug transporters was observed with significantly reduced renal clearances of rivaroxaban.[5] Systemic steady-state concentrations of both P-gp and BCRP inhibitors resulted in a diminished renal secretion of rivaroxaban. Rifampicin was not able to

demonstrate a similar effect, which might be due to the single dose administration to inhibit mainly intestinal drug transporters and its short half-life of 3 hours.[33] Therefore, the systemic concentration of rifampicin might not be high enough for the 48 hours observation period. Interestingly, the steady-state volume of distribution was reduced by the single rifampicin dose resulting in a shorter rivaroxaban terminal elimination half-life. Rivaroxaban distributes from the vessels into peripheral tissues,[34] which might be impaired by rifampicin altering uptake transporters. A similar observation has been reported for **atorvastatin**, where it was suggested that the uptake of atorvastatin was decreased by rifampicin.[35] Repeated administration of rifampicin will result in CYP induction, which then might cause a similar reduction in rivaroxaban exposure, like for St. John's wort where an almost 50% reduced AUC was observed.[36] Finally, cobicistat significantly affected all pharmacokinetic parameter of rivaroxaban except the amount excreted unchanged into urine. Cobicistat appears to affect rivaroxaban pharmacokinetics via inhibition of CYP3A and CYP2J2 (similar to voriconazole) and via inhibition of P-gp and BCRP (similar to rifampicin). In addition, the renal rivaroxaban clearance was reduced by almost 50%, which resembles the data obtained with ketoconazole and ritonavir.[5]

4.4 | CYP3A activity

Microdosed midazolam was used to monitor CYP3A activity during the different perpetrators used in this study. As expected, both voriconazole and cobicistat acted as strong CYP3A inhibitors and caused AUCR >5. Interestingly, the single oral dose of rifampicin also had a marginal inhibitory effect on midazolam exposure (30% increase). Rifampicin is known to induce its own elimination pathways after several days and thus reduce its terminal half-life by 50%.[33] However, it is not known which enzymes contribute to rifampicin clearance pathways. If CYP3A metabolises rifampicin to a certain extent, there is a possibility that rifampicin might also act as CYP3A inhibitor, especially when sensitive victim drug such as midazolam are investigated. However, only a weak inhibition was observed.

4.5 | Limitations

A limitation of this exploratory study is that the effect of each perpetrator on FXaI pharmacokinetics was investigated in a low number of participants (n = 6). However, with the cross-over design this was already sufficient to obtain valid results, which are statistically secured. To avoid enzyme induction by rifampicin, a single dose of rifampicin was administered, assuming that rifampicin inhibits drug transporters within the first hours after administration. However, changes in FXaI AUC_{∞} suggested that rifampicin already induced enzyme transcription during the 2-day observation period of this study and thus the effect of drug transporters on FXaI pharmacokinetics might be underestimated.

5 | CONCLUSION

In conclusion, a microdosed FXaI cocktail containing 25 μg apixaban, 25 μg rivaroxaban and 50 μg edoxaban was successfully applied to generate valid drug interaction data with voriconazole, cobicistat and a single oral dose of rifampicin on a single occasion for all 3 FXaI drugs. At least for cobicistat containing treatments a dose reduction by 30–50% should be considered for each of the 3 FXaI since AUCR ranged between 1.67 and 2.0. For future studies this approach of several perpetrators in 1 study might even be used with a smaller number of participants (e.g. 3 per perpetrator) to test for multiple perpetrators (e.g. 8 perpetrators in 24 participants). After data analysis, the most interesting perpetrators could then be studied with a proper sample size. This can be done in 1 study protocol.

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COMPETING INTERESTS

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

CONTRIBUTORS

G.M., K.I.F., M.S. and W.E.H. analysed and interpreted the data, and wrote the manuscript; G.M., M.L.L., M.S. and W.E.H. designed the study; M.S., M.L.L. and G.M. performed the trial; J.B. and K.I.F. developed the analytical methods and analysed the samples.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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