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RESEARCH PAPER

Investigating Intestinal Transporter Involvement in Rivaroxaban Disposition through Examination of Changes in Absorption

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ABSTRACT

Purpose The involvement of the intestinally expressed xenobiotic transporters P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) have been implicated in rivaroxaban disposition based on *in vitro* studies, similar to what had previously been proposed for apixaban. We recently showed that these efflux transporters were not clinically relevant for apixaban disposition and examine here their relevance for this second Factor Xa inhibitor.

Methods Using recently published methodologies to discern metabolic- from transporter- mediated drug interactions, a critical evaluation was undertaken of 9 rivaroxaban studies reporting 12 DDIs, one study of food effects and one study of hepatic function.

Results Rationale examination of these clinical studies using basic pharmacokinetic theory finds little support for the clinical significance of intestinal efflux transporters in rivaroxaban disposition. Drug-drug interactions are most likely adequately predicted based on the level of CYP 3A metabolism.

Conclusion These analyses indicate that inhibition of efflux transporters appears to have negligible, clinically insignificant effects on the rivaroxaban absorption process, which is consistent with the concern that predictions based on *in vitro* measures may not translate to a clinically relevant interaction *in vivo*. We emphasize the need to evaluate gastric emptying, dissolution and other processes related to absorption when using *MAT* changes to indicate efflux transporter inhibition.

KEY WORDS Bioavailability · complex drug-drug interactions · mean absorption time · rivaroxaban

ABBREVIATIONS

AUC	Area under the curve
AUMC	Area under the moment time curve
BCRP	Breast cancer resistance protein
CL/F	Apparent clearance
C_{max}	Maximum concentration
CYP	Cytochrome P450
DDIs	Drug-drug interactions
I_{gut}	Maximum perpetrator concentration in gut
MAT	Mean absorption time
MRT	Mean residence time
P-gp	P-glycoprotein
SJW	St. John's Wort
t_{max}	Time of maximum concentration
$t_{1/2}$	Terminal half-life

INTRODUCTION

Rivaroxaban is a highly selective and direct Factor Xa inhibitor used to prevent thrombin generation that is increasingly used clinically for the prevention and treatment of thromboembolism due to its ease of use and improved patient compliance as compared to warfarin (1–4). Rivaroxaban has also been approved for reduction of stroke risk in patients with non-valvular atrial fibrillation, and for prevention and treatment of deep vein thrombosis (5). The most severe adverse effect of rivaroxaban is bleeding and evidence suggests that

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renal or hepatic dysfunction or drug-drug interactions (DDIs) that increase exposure to rivaroxaban have the potential to increase the risk of bleeding complications (6).

Recently we reported that the intestinal efflux transporters P-gp and BCRP were not clinically relevant for apixaban (7), another approved Factor Xa inhibitor. Here we evaluated the relevance of those intestinal efflux transporters on the disposition of rivaroxaban, suspecting that our analysis may not be as clear-cut since rivaroxaban is a Biopharmaceutics Drug Disposition Classification System (BDDCS) Class 2 drug *versus* apixaban being a BDDCS Class 1 drug. The BDDCS system suggests that transporter effects would generally be clinically irrelevant for highly soluble, extensively metabolized Class 1 drugs (8), but that intestinal efflux transporters may exhibit clinically relevant interactions for poorly soluble highly metabolized BDDCS Class 2 drugs (9).

Rivaroxaban is eliminated mainly by hepatic metabolism and renal excretion (10, 11). Approximately 51% of an orally administered [^{14}C]-rivaroxaban dose was recovered as inactive metabolites in urine (30%) and feces (21%). Oxidative degradation catalyzed by CYP3A4/5 and CYP2J2 and hydrolysis are the major routes of biotransformation (12).

Based on *in vitro* studies, rivaroxaban is also a substrate of the efflux transporters P-gp and BCRP (13–15). According to the BDDCS classification system, rivaroxaban belongs to Class 2 due to its low solubility and high permeability and thus intestinal apical efflux transporters might be expected to exert an effect on absorption (9). A 2013 clinical study conducted by Mueck *et al.* (16) investigated rivaroxaban and P-gp/CYP3A4 inhibitors in healthy volunteers, concluding that rivaroxaban can be co-administered with P-gp and/or CYP3A4 substrates/moderate inhibitors, such as clarithromycin, erythromycin and fluconazole, but not with strong P-gp/CYP3A4 inhibitors such as ritonavir and ketoconazole due to the substantially increased rivaroxaban exposure. The FDA approved label for rivaroxaban states that concomitant use with known combined P-gp and strong CYP3A inhibitors or inducers should be avoided (12). However, no analysis related to differentiating the contribution of efflux transporter and enzymes on disposition of rivaroxaban *in vivo* has been conducted. Due to the *in vitro* evidence that rivaroxaban is a substrate of efflux transporters, it was of interest to examine the potential of rivaroxaban to interact with perpetrator drugs that can inhibit efflux transporters. Inhibition of intestinal transporters has the potential to alter the absorption rate of a drug in addition to the amount absorbed (17), whereas enzyme inhibition can only influence the amount of drug absorbed, but not the rate of absorption.

The present study evaluates changes in rivaroxaban absorption time when rivaroxaban is co-administered with other drugs or differing conditions to understand if the efflux transporter inhibition potential observed *in vitro* has any clinical significance.

MATERIALS AND METHODS

A systematic literature search was conducted to identify published pharmacokinetic drug interaction studies performed in humans of rivaroxaban in combination with perpetrator drug. PubMed, Medline and Google Scholar were searched from inception through December 2020. The search was restricted to studies with published pharmacokinetic curves with at least 2 time points (except for those only depicting time zero and time to maximal concentration (t_{max})) in the absorption phase. Pharmacokinetic profiles were then digitized to generate parameters including area under the curve (*AUC*), area under the moment time curve (*AUMC*), maximum concentration (C_{max}), t_{max}, terminal half-life ($t_{1/2}$) and apparent clearance (*CL/F*) via non-compartmental analysis with WinNonlin® Professional Edition Version 2.1 (Pharsight, Mountain View, CA). The values of *AUC* from the digitized curves were compared to published values, and only included in this analysis if these values were within 20% of one another, indicating that the published average pharmacokinetic profiles adequately represented the data. As previously described (17), the concentration-time data were then fit to a two-compartment model with first order absorption using WinNonlin®, and mean absorption time (*MAT*) was calculated as reciprocal of the first order absorption rate constant. Mean residence time (*MRT*) was calculated as the ratio of $AUMC_{0-\infty}$ divided by $AUC_{0-\infty}$ minus *MAT*. The ratios of these pharmacokinetic parameters, treated group to control group, were also calculated. Ratios calculated from published parameters were reported in priority, with digitized results supplementing values that were not specifically reported in the investigated studies. The results are shown in Table I for drug-drug interactions and Table II for food effects and hepatic dysfunction, with values derived from publications specifically noted with the superscript "R". Pharmacokinetic parameters that displayed a decrease more than 30% and an increase more than 43% (i.e. ratios outside of the range of 0.70 and 1.43) were considered as potential evidence of a clinically significant interaction. These limits are equivalent on a logarithmic scale, similar to ranges utilized to compare bioequivalence.

According to the FDA draft guidance on predicting drug-drug interactions (49), an orally dosed drug has the potential to inhibit intestinal enzymes and transporters *in vivo* if the total concentration in gut (I_{gut}) is larger than ten-fold of IC_{50}, where I_{gut} = dose of inhibitor/250 mL. Therefore, IC_{50} values of perpetrator drugs against BCRP/P-gp were identified from the literature and used to calculate I_{gut}/IC_{50} for P-gp and BCRP for each perpetrator drug. These values are reported in Table I as an indication of potential to inhibit intestinal efflux transporters.

Table I Ratios of Oral Rivaroxaban Pharmacokinetic Parameters Reported as Drug-Drug Interaction (DDI)/Control (Con) Results

Perpetrator drugs	$\frac{I_{gut}}{IC_{50}}$ (P-gp)	$\frac{I_{gut}}{IC_{50}}$ (BCRP)	MAT^{Con} (h)	$\frac{MAT^{DDI}}{MAT^{Con}}$	$\frac{t_{max}{}^{DDI}}{t_{max}{}^{Con}}$	CL/F^{Con} ($L\ h^{-1}$)	$\frac{CL/F^{DDI}}{CL/F^{Con}}$	$\frac{AUC^{DDI}}{AUC^{Con}}$	$\frac{t_{1/2}{}^{DDI}}{t_{1/2}{}^{Con}}$
Aspirin (18)	NR	NR	1.01	2.42	2.00[R]	12.4	1.13	0.91[R]	0.88[R]
Clarithromycin (16)	40.5–652.1 (19, 20)	NI (21)	2.74	0.71	1.00[R]	10.4[R]	0.65[R]	1.54[R]	0.85[R]
Cyclosporine (22)	53.8–415.6 (23, 24)	42.6–665.0 (25, 26)	1.87	1.10	1.50[R]	9.6[R]	0.68[R]	1.47[R]	0.70[R]
Erythromycin (16)	22.9–272.5 (27, 28)	NI (29)	0.74	0.84	1.00[R]	9.4[R]	0.75[R]	1.34[R]	0.86[R]
Fluconazole (16)	NI (30)	NI (31)	2.0	0.83	1.00[R]	11.3[R]	0.71[R]	1.42[R]	1.25[R]
Ketoconazole (16)	538.0–2248.8 (32, 33)	196.9–251.0 (34, 35)	0.79	0.96	1.33[R]	11.2[R]	0.39[R]	2.58[R]	1.35[R]
Macitentan (36)	1.06–15.4 (37, 38)	0.91–5.15 (37, 38)	1.91	0.73	0.69[R]	10.3[R]	1.02[R]	0.98[R]	0.86[R]
Macitentan + SJW[*22]	–	–	1.91	0.67	0.95[R]	10.3[R]	1.35[R]	0.74[R]	0.74[R]
Naproxen (39)	NI (40)	NI (40)	1.09	1.07	2.00[R]	13.2	0.86	1.12[R]	0.91[R]
Omeprazole (41)	4.8–24.4 (19, 42)	12.8 (43)	3.0	1.05	1.40[R]	8.45[R]	0.99[R]	1.01[R]	1.00[R]
Ritonavir (16)	118.0–875.7 (19, 32)	504.4 (34)	1.60	0.48	1.33[R]	10.0[R]	0.40[R]	2.53[R]	1.21[R]
Verapamil (Healthy) (44)	94.5–791.2 (19, 24)	NI (45, 46)	0.86	1.33	NR	7.9[R]	0.72[R]	1.39[R]	1.18[R]
Verapamil (44) (mild renal function)	94.5–791.2 (19, 24)	NI (45, 46)	0.44	1.05	NR	7.38[R]	0.68[R]	1.42[R]	1.43[R]

*SJW St John's wort; NI no inhibition observed; NR not reported

[R]: Ratios are calculated using reported values

RESULTS

The literature search identified 9 studies with published rivaroxaban concentration-time profiles that included a total of 12 perpetrator drugs, one food-rivaroxaban interaction and one study investigating rivaroxaban disposition with respect to hepatic dysfunction. Among the 12 perpetrator drugs, 7 had the potential to inhibit intestinal P-gp and/or BCRP (clarithromycin (16), erythromycin (16), ketoconazole (16), ritonavir (16), omeprazole (41), cyclosporine (22) and verapamil (44)), one was a P-gp inducer (St John's Wort (36) (SJW)), but it was co-administered with macitentan (not a BCRP but potentially a P-gp inhibitor), and the remaining 3 perpetrators are neither inhibitors or inducers of intestinal efflux transporters (aspirin (18), fluconazole (16), and naproxen (39)). Table I shows the ratios of rivaroxaban pharmacokinetic parameters with and without addition of different perpetrator drugs. Table II shows the influence of food or hepatic function on rivaroxaban pharmacokinetic parameters (47, 48). Of the 8 P-gp/BCRP inhibitors, 4 exhibited changes in AUC greater than 43% (clarithromycin, cyclosporine, ketoconazole and ritonavir), with 2 others quite close (fluconazole, 42%; verapamil 39% in healthy volunteers and 42% in mild renal failure patients). However, only one of these perpetrators (ritonavir) showed a decrease in *MAT* greater than 30%, (accompanied by a t_{max} change in the opposite direction, an increase of 33%). Although cyclosporine caused a greater than 43% increase in rivaroxaban *AUC*, slower absorption could be implied by the 50% increase in t_{max}, although only a 10% increase in *MAT* was observed. Fluconazole (a clinically recommended index inhibitor of CYP3A4) (50) exhibited a 42% increase in *AUC* and no changes in MAT nor t_{max}, consistent with its lack of inhibitory effects on P-gp/BCRP. Aspirin showed significant increases in *MAT* by 2.42-fold and macitentan plus SJW resulted in a 33% decrease in *MAT*. As for t_{max}, aspirin and naproxen exhibited a marked increase (2-fold for each) but macitentan (without SJW) decreased t_{max} by 31%. Food also increased *MAT* and t_{max}, with modest, clinically insignificant effects on *AUC* and *CL/F* (Table II). In hepatic dysfunctional patients, only moderate hepatic dysfunction subjects exhibited decreased *CL/F* (ratio of 0.43), but *MAT* showed no significant change in these patients. In patients with mild hepatic dysfunction, *MAT* decreased by 40% but *CL/F* showed no change (Table II).

DISCUSSION

The results of this analysis indicate that the intestinal efflux transporters are not strongly involved in the absorption phase of rivaroxaban contrary to what had been previously hypothesized by the field and suggested in the package insert (12). To verify this, *MAT* and t_{max} changes were examined in DDIs studies involving rivaroxaban with P-gp/BCRP inhibitors *versus* non-inhibitors. Rivaroxaban is suggested to be a substrate of P-gp and BCRP, therefore inhibition of P-gp or BCRP is expected to result in decreases in *MAT* and t_{max} as the efflux transporters suggested to be involved in rivaroxaban disposition can no longer cycle rivaroxaban between the enterocytes and intestinal lumen, thereby decreasing absorption rate. When rivaroxaban was co-administered with inhibitors of P-gp or BCRP, the *MAT* and t_{max} values for

Table II Ratios of Oral Rivaroxaban Pharmacokinetic Parameters Under Fed Condition or Different Hepatic Function Reported as Treated (Trt)/Control (Con) Results.

	MAT^{Con} (h)	$\frac{MAT^{Trt}}{MAT^{Con}}$	$\frac{t_{max}{}^{Trt}}{t_{max}{}^{Con}}$	CL/F^{Con} (L h^{-1})	$\frac{CL/F^{Trt}}{CL/F^{Con}}$	$\frac{AUC^{Trt}}{AUC^{Con}}$	$\frac{t_{1/2}{}^{Trt}}{t_{1/2}{}^{Con}}$
Fed* (47)	1.92	1.79	1.45 [R]	11.80	0.74	1.25 [R]	0.93 [R]
Fed#	1.45	1.18	1.56 [R]	14.40	0.74	1.24 [R]	0.77 [R]
Fed&	0.72	1.88	2.80 [R]	13.38	0.79	1.20 [R]	0.76 [R]
Mild hepatic dysfunction (48)	1.35	0.60	1.00 [R]	6.60 [R]	0.86[R]	1.15 [R]	1.30 [R]
Moderate hepatic dysfunction	1.35	0.95	1.50 [R]	6.60 [R]	0.43[R]	2.27 [R]	1.26 [R]

*Rivaroxaban 10 mg

Rivaroxaban 20 mg

& Rivaroxaban 4 x 5 mg

[R]: Ratios are calculated using reported values

rivaroxaban do not increase by more than 43% or decrease by more than 30% except for ritonavir, where *MAT* decreased but t_{max} did not. Generally, changes in t_{max} can be used to reflect changes absorption rate only if terminal half-life remains unchanged, as we have recently discussed in detail (17). The t_{max} value can be obtained by directly examining the concentration-time curve with understanding that it depends strongly on the sampling frequency (51) and can also be affected by the elimination half-life ($t^1/_2$) as previously discussed (17). *MAT* is a relatively more sensitive value that is only a function of the absorption rate, which directly reflects changes in absorption caused by inhibition, activation or induction of intestinal transporters. Our laboratory has previously recognized that the action of P-gp and BCRP inhibitors and inducers result in altered absorption rate because these transporters are highly expressed apically in the intestine and serve as an effective barrier to the intestinal absorption of numerous substrates (17). The absence of a corresponding change in t_{max} can be explained by the concomitant change in substrate elimination from the systemic circulation (52). However, none of the DDI studies included in this investigation reported changes in absorption rate in individual patients as the focus of those studies was on how drug exposure changed.

From our results, we report that among the 12 perpetrator drugs, including significant *in vitro* inhibitors of P-gp/BCRP, most of them did not markedly change *MAT* except for ritonavir and aspirin. Of the 8 P-gp and/or BCRP inhibitors only ritonavir resulted in more than a 30% *MAT* change (decreased by 52%), while the even more potent inhibitors such as ketoconazole (with I_{gut}/IC_{50} values of approximately 200 for BCRP and 500–2000 for P-gp) did not change *MAT*. Ritonavir is potentially a strong inhibitor of P-gp (I_{gut}/IC_{50} = 118–876) and BCRP (I_{gut}/IC_{50} = 504). Although our calculated *MAT* change for this interaction indicated increased absorption rate, the authors reported a 33% increase in t_{max} with a 21% increase in terminal half-life suggesting either a slight decrease in absorption rate or no change.

Concomitant dosing of aspirin exhibited the greatest increase in *MAT* (2.42-fold) with a comparable 2.0-fold increase in reported t_{max} (18), but no significant change in exposure (*AUC*). There are no reports of aspirin either inducing or inhibiting xenobiotic transporters. However, absorption time may change due to additional factors beyond transporter inhibition or induction, such as changes in gastric emptying rate, drug dissolution rate and permeability, as these processes can also be the rate-limiting steps during drug absorption (52). Aspirin can also prolong gastric emptying, which may have contributed to the observed 2.42-fold increase in *MAT*, as there is evidence of delayed time to peak blood-ethanol levels after treatment with low dose aspirin (53). The mechanism is attributed to the inhibition by aspirin of endogenous prostaglandins to produce macroscopic changes of the gastric mucosa (54, 55).

Verapamil has the potential to inhibit P-gp according to its I_{gut}/IC_{50} ratio, which would result in a decreased *MAT* ratio. However, co-administration of verapamil resulted in the counterintuitive 1.33-fold increase in *MAT*in healthy volunteers, but no change in patients with mild renal function. With respect to transporters, increase in *MAT* may be caused by 1) induction or activation of efflux transporters or 2) inhibition of intestinal uptake transporters. Our laboratory has previously demonstrated the potential for P-gp to be activated (56), although we did not find any literature supporting P-gp activation by verapamil. Further, no literature evidence exists for involvement of uptake transporters in rivaroxaban disposition. However, it has been demonstrated that in addition to inhibitory effects on CYP3A4 and P-gp, verapamil can also induce CYP3A4 (57). Considering that the expression of CYP3A4 and P-gp are regulated by the same transcription factor PXR, P-gp may also be induced by verapamil (58). In the healthy volunteer study, verapamil was dosed for 10 days

(120 mg, day 1; 240 mg, day 2; 360 mg, days 3–10), therefore the potential for P-gp induction may be a possibility. Verapamil is a calcium-channel blocker, in a class of drugs that exert their effect by inhibiting calcium entry into cells, which can potentially decrease gastric smooth muscle contraction and delay gastric emptying. Studies in preclinical species have demonstrated delayed gastric emptying caused by verapamil (59), and the results in humans reported a slight decrease in gastric emptying rate (60).

Another confounding study is the DDI interaction with macitentan in the presence and absence of additional SJW co-administration (36). Of the 11 drug DDI studies with rivaroxaban in Table I, macitentan is the only perpetrator to cause both *MAT* and t_{max} to decrease close to 30%, but there is no effect on *AUC* and *CL/F*. Dhillon (61), summarizing macitentan published DDI studies, reports that although macitentan is a CYP3A4 substrate, showing changes in macitentan kinetics with strong inhibitors and inducers of CYP3A, *in vitro* studies of macitentan "did not have clinically relevant inhibitory or inducing effects on CYP enzymes" nor inhibit hepatic or renal transporters including P-gp. However, "*in vitro* macitentan inhibits the breast cancer resistance protein at clinically relevant intestinal concentrations". These findings are in concordance with the DDI results listed in Table I (36). Further addition of SJW, yields no change in the *MAT* decrease, but increases the observed ratio of t_{max} change to 0.95, with an observed 35% increase in *CL/F* and a 26% decrease in $t½$. Chronic use of SJW (12 days in the present study) can markedly induce CYP3A4 metabolism and can also induce the expression of P-gp (36, 37, 62). Although the decrease in *AUC* of rivaroxaban in the presence of chronic SJW and macitentan was only on average 26% and no clinically relevant change in endothelin antagonism was observed, the authors recommend (36) that "the combination of SJW with rivaroxaban should be avoided." In the present analysis, it is possible that an intestinal transporter interaction is occurring, but it is difficult to confirm its relevance considering the other studies analyzed here.

Our study has limitations. First, estimation of *MAT* from computer fitting of mean concentration time curves rather than from individual pharmacokinetic profiles of the study population may be confounding as we have previously noted (17). Second, some drugs are absorbed relatively quickly after oral administration; therefore, depending on the design of the clinical study, if the pharmacokinetic curve does not have enough points in absorption phase, this will affect the integrity of the calculated absorption rate constants.

CONCLUSIONS

The analyses here indicate that inhibition of intestinal efflux transporters have minimal, clinically insignificant effects on rivaroxaban absorption, which is consistent with the concern that *in vitro* measures may not always translate to clinically significant *in vivo* relevance. However, the data here for rivaroxaban are not as consistent as our previous analysis of apixaban data (7). We emphasize, particularly for BDDCS Class 2 poorly soluble drugs, the need to consider gastric emptying, drug dissolution and other factors related to absorption rate when utilizing *MAT* changes to evaluate the involvement of intestinal transporters in drug disposition.

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