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Coadministration of Ticagrelor and Ritonavir: Toward Prospective Dose Adjustment to Maintain an Optimal Platelet Inhibition Using the PBPK Approach

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Ticagrelor is a potent antiplatelet drug metabolized by cytochrome (CYP)3A. It is contraindicated in patients with human immunodeficiency virus (HIV) because of the expected CYP3A inhibition by most protease inhibitors, such as ritonavir and an increased bleeding risk. In this study, a physiologically based pharmacokinetic (PBPK) model was created for ticagrelor and its active metabolite (AM). Based on the simulated interaction between ticagrelor 180 mg and ritonavir 100 mg, a lower dose of ticagrelor was calculated to obtain, when coadministered with ritonavir, the same pharmacokinetic (PK) and platelet inhibition as ticagrelor administered alone. A clinical study was thereafter conducted in healthy volunteers. Observed PK profiles of ticagrelor and its AM were successfully predicted with the model. Platelet inhibition was nearly complete in both sessions despite administration of a fourfold lower dose of ticagrelor in the second session. This PBPK model could be prospectively used to broaden the usage of ticagrelor in patients with ritonavir-treated HIV regardless of the CYP3A inhibition.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Antiplatelet ticagrelor is contraindicated in patients with HIV taking ritonavir due to CYP3A inhibition. Administration of clopidogrel or prasugrel may lead to treatment inefficacy because their bioactivation is reduced by ritonavir.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study evaluated the usefulness of PBPK modeling in a prospective dose-adjustment of ticagrelor in patients treated with ritonavir to avoid their PK interaction while maintaining ticagrelor optimal efficacy.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ The PK profile of ticagrelor and the interaction with ritonavir was reliably predicted by the model. The calculated reduced dose of ticagrelor allowed minimizing this interaction while the platelet inhibition remained unchanged. This study represents a nice example of a tailored medicine using the PBPK approach to prospectively optimize drug therapy.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

☑ PBPK can be prospectively used to broaden the usage of ticagrelor in patients with ritonavir-treated HIV. This study introduced a starting point toward the prediction of a safe and efficacious dose of ticagrelor in untested interaction scenarios.

Ticagrelor is the first drug of a new nonthienopyridine oral antiplatelet agent category that is an analog of nucleoside resembling ADP in structure. The parent compound is active and the hepatic metabolism generates one active metabolite (AM), AR-C124910XX, with the same activity compared to the parent drug. Guidelines recommend ticagrelor in addition to aspirin for all patients with non-ST elevation acute coronary syndrome at moderate to high ischemic risk, whereas prasugrel is only recommended in patients proceeding to percutaneous coronary intervention. Moreover, some studies suggest exclusive beneficial characteristics of ticagrelor in relation to its adenosine-like chemical structure.[1]

Because of the remarkable progress in human immunodeficiency virus (HIV) infection therapies, the mortality rate of patients is close to the uninfected population. Findings of recent studies suggest that HIV infection itself contributes to the advent of atherosclerosis regardless of cardiovascular risks. Additionally, some of the protease inhibitors' side effects, such as hypertension,

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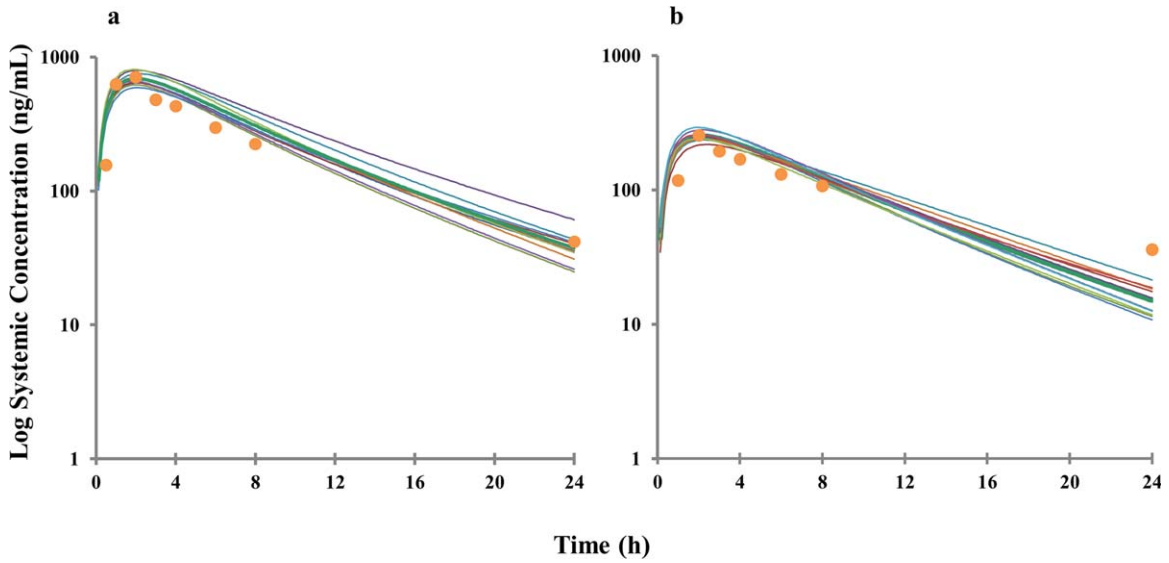

Figure 1 Observed and simulated concentration-time profile of ticagrelor (**a**) and its active metabolite (**b**) following administration of a single dose of ticagrelor 200 mg in 10 trials of six healthy male volunteers. Circles represent mean concentrations observed by Teng *et al*.[13] (2010) and the thin lines represent mean concentration profile for each simulated trial. The bold line represents the mean value for the 10 simulated trials. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hyperlipidemia, or insulin resistance, increase the risk of cardio-arterial diseases in patients infected with HIV.[2–4] Ritonavir, a protease inhibitor used in the treatment of HIV infection, is used in combination with other antiretroviral drugs as a pharmacokinetic (PK) enhancer. Ritonavir increases the bioavailability via inhibition of the metabolism of other drugs. In spite of its potential benefits, ticagrelor is contraindicated in patients with HIV because of the expected interaction with ritonavir and potential bleeding risk.[5,6] Some studies have demonstrated modulation of ticagrelor's antiplatelet activity as a consequence of a PK drug-drug interaction (DDI). For instance, coadministration of rifampicin and a single 180 mg dose of ticagrelor resulted in 86% and 73% decrease in area under the curve (AUC) and peak plasma concentration (C_{max}) of ticagrelor. Accordingly, the inhibition of platelet activity (IPA) dropped more rapidly in the DDI arm (87% 12 hours after ticagrelor intake in the control group vs. 63% in the rifampicin group).[7] In a recent study, intravenous morphine reduced the AUC of a single 180 mg oral dose of ticagrelor by 36% and delayed the time to achieve maximal plasma concentration for 2 hours. The placebo group showed a significantly lower platelet activity compared with morphine-treated patients.[8] Furthermore, coadministration of grapefruit juice and a single 90 mg dose of ticagrelor resulted in 165% and 221% increase in AUC and C_{max} of ticagrelor, respectively, and enhanced significantly the platelet inhibition by the latter. This data suggest that regular grapefruit juice consumption can predispose patients to ticagrelor side effects, such as bleeding, dyspnea, and hyperuricemia if standard doses are administered. Moreover, by increasing ticagrelor half-life, grapefruit juice may delay the platelet recovery, which can be critical prior to a planned surgery.[9] Based on the relationship between ticagrelor's plasma concentration and platelet inhibition, as ritonavir increases ticagrelor bioavailability, administration of a lower dose of ticagrelor may lessen the impact of this DDI while maintaining optimal efficacy, if similar exposures of ticagrelor and AM can be achieved. To assess a DDI, physiologically based pharmacokinetic (PBPK) modeling is one of the recommended strategies by the US Food and Drug Administration guidelines as a link between preclinical and clinical studies.[10] *In vitro* to *in vivo* extrapolation and simulation is the first step toward prediction when integral information is not available.

In this study, a PBPK model for ticagrelor and AM was created based on *in vitro* and *in vivo* parameters. On the basis of a simulated interaction between ticagrelor 180 mg and ritonavir 100 mg in the Simcyp simulator, a lower dose of ticagrelor was calculated aiming to obtain, when coadministered with ritonavir, the same PK profile and platelet inhibition as ticagrelor administered alone. A clinical study was conducted in healthy volunteers to validate the calculated dose.

RESULTS

Simulations

Ticagrelor's initial model development and adjustment for $f_{m\text{-}3A}$. The refined model predicted similar AUC and C_{max} values for ticagrelor and AM following administration of a single dose of ticagrelor 200 mg as compared to the reference published clinical study.[11] Observed and simulated PK profiles are presented in **Figure 1**.

As a second step, ticagrelor's $f_{m\text{-}3A}$ value was challenged to quantitatively predict the impact of ketoconazole 200 mg on PK of ticagrelor and AM in healthy virtual volunteers. To evaluate the model's performance, a $R_{predicted/observed}$ value was calculated as below[12]:

$R_{predicted/observed}$ = model predicted ratio/clinically observed ratio

Concerning ticagrelor, the observed $R_{predicted/observed}$ value was 1.1 and 0.9 for AUC and C_{max}, respectively. Regarding AM, 1.3 and 0.9 were, respectively, observed for AUC and C_{max}.

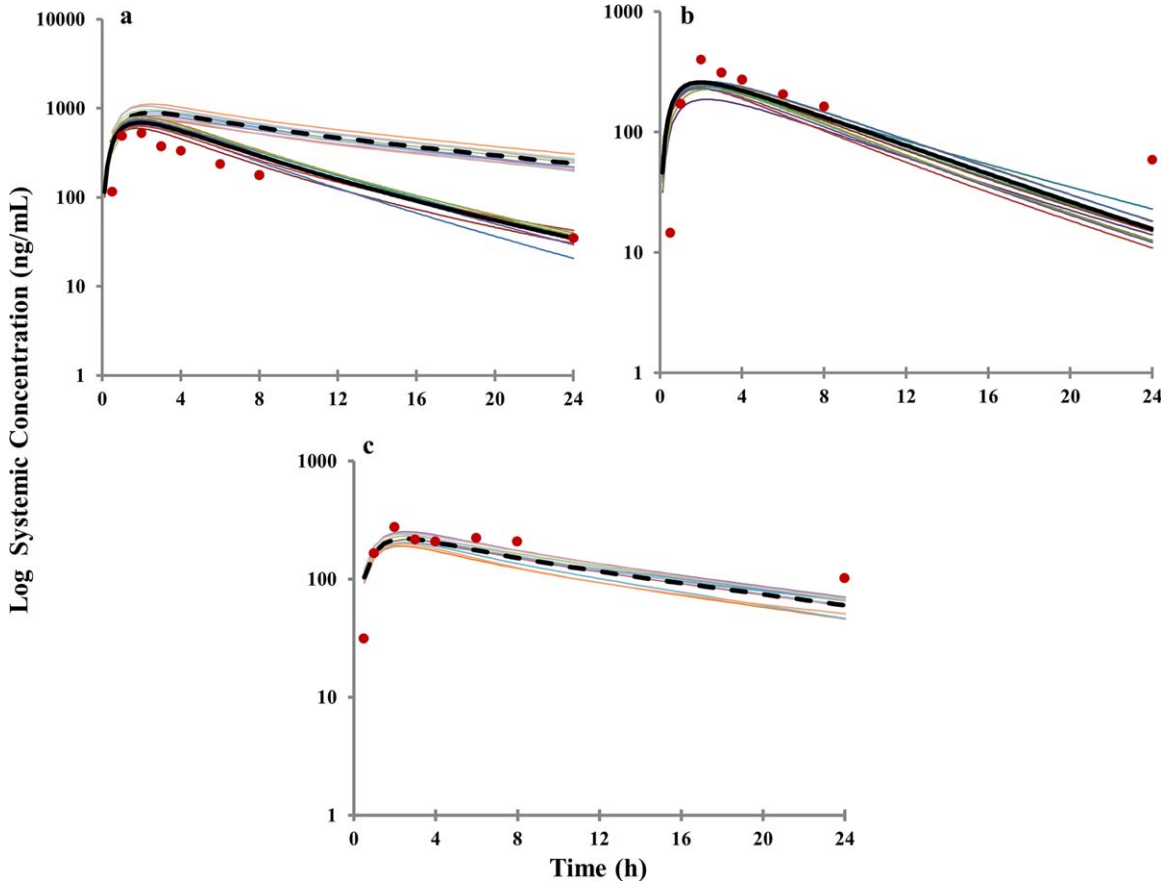

Figure 2 Concentration-time profiles of single dose ticagrelor 180 mg (**a**), its active metabolite (**b**), and ticagrelor 45 mg (**c**) in healthy male volunteers with (dashed line) and without (solid line) a single dose of ritonavir 100 mg. Fine lines represent mean concentration profile for each simulated trial ($n = 20$). The thick line represents the mean value for the 10 trials ($n = 200$). Circles represent mean concentrations observed at the first (**a** and **b**) and the second (**c**) session of the clinical study. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Simulation of DDI between ticagrelor and ritonavir. The PK interaction between a single dose of ticagrelor 180 mg and a single dose of ritonavir 100 mg was simulated in 10 trials of 20 healthy male volunteers to calculate ticagrelor's adjusted-dose (45 mg). As expected, the plasma concentration of ticagrelor 180 mg was markedly increased when coadministered with ritonavir (**Figure 2a**). A mean ± SD AUC ratio of 4.0 ± 1.7 and C_{max} ratio of 2.0 ± 0.4 were obtained for ticagrelor. Because ticagrelor

Table 1 Observed PK of ticagrelor and its active metabolite after administration of a single 180 mg dose of ticagrelor alone (session one) and a single 45 mg dose of ticagrelor coadministered with ritonavir 100 mg (session two) in 19 healthy male volunteers

		Ticagrelor 180 mg (95% CI)	Ticagrelor 45 mg + ritonavir 100 mg (95% CI)	Ratio (95% CI)
Ticagrelor	AUC (h × ng/mL)	4,100 (3,570–4,630)	5,550 (4,830–6,270)	1.36 (1.13–1.57)
	C_{max} (ng/mL)	650 (550–740)	280 (250–320)	0.44 (0.38–0.49)
	T_{max} (h)[a]	2 (1–4)	2 (2–8)	–
	$T_{1/2}$ (h)	6.37 (5.86–6.89)	14.3 (12.3–16.3)	2.31 (1.94–2.68)
	CL_F (L/h)	40.7 (35.6–45.7)	28.3 (20.0–36.7)	0.70 (0.58–0.83)
AM	AUC (h × ng/mL)	3,540 (3,220–3,860)	75.5 (54.3–96.7)	0.02 (0.01–0.03)
	C_{max} (ng/mL)	415 (366–464)	3.92 (2.73–5.11)	0.01 (0.00–0.01)

AM, active metabolite; AUC, area under concentration-time curve; CI, confidence interval; CL_F, oral clearance; C_{max}, maximal plasma concentration; T_{max}, time to achieve maximal plasma concentration; $T_{1/2}$, half -life.
Values are expressed as geometric means (95% confidence interval).
[a]Values expressed as median (range).

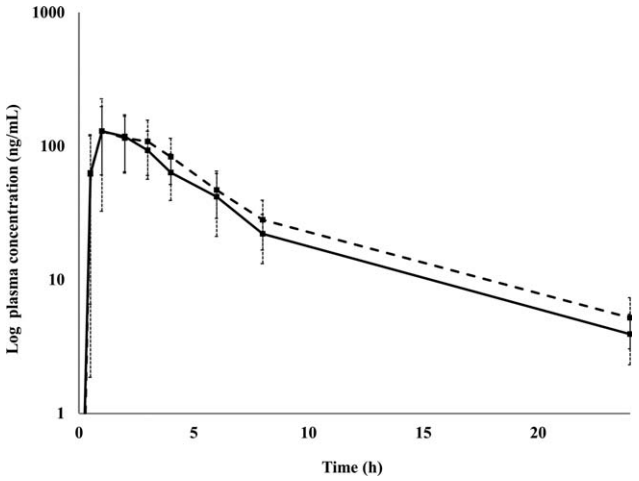

Figure 3 Observed mean concentration-time profile of a single dose fexofenadine 30 mg with (dashed line) and without (solid line) a single dose of ritonavir 100 mg in healthy male volunteers.

has shown a linear PK up to a 900 mg daily dose in published clinical studies,[11,13,14] the dose of 45 mg was calculated to be coadministered with a single ritonavir dose in order to obtain the same PK profile in the second session of the clinical study compared to ticagrelor 180 mg administered alone in the first session. Capsules were administered to volunteers at the second session of the clinical study 2 hours after the intake of 100 mg ritonavir (Norvir).

Clinical study

A total of 20 healthy male volunteers with a mean age of 27 years (range, 21–43 years) were enrolled. Nineteen volunteers completed the study and one subject declined subsequently. All administered drugs were well-tolerated and no adverse events were reported during the study.

Pharmacokinetic assessments and phenotyping

Observed PK parameters of ticagrelor 180 mg administered alone and ticagrelor 45 mg coadministered with ritonavir 100 mg are summarized in **Table 1**. The observed mean AUC was 4,100 ng.h/mL (95% confidence interval [CI] = 3,570–4,630) for ticagrelor 180 mg vs. 5,550 ng.h/mL (95% CI = 4,830–6,270) for ticagrelor 45 mg coadministered with ritonavir. AUC of ticagrelor 45 mg coadministered with ritonavir was 36% higher than that of ticagrelor 180 mg alone. Thereby, the bioequivalence could not be claimed. Observed PK profiles of ticagrelor 180 mg and AM during the first session of the clinical trial were subsequently overlaid to the simulated data; the results are outlined in **Figure 2a** and **Figure 2b**, respectively. The simulation seems to describe the observed clinical data. The elimination of AM was slightly overestimated. Furthermore, ticagrelor 45 mg PK profile obtained at session two of the clinical study was overlaid to the simulated DDI with ritonavir. A good consistency between the observed and predicted PK was noticed (**Figure 2c**).

Expectedly, the observed plasma concentration of AM after ritonavir administration was insignificant due to extensive inhibition of cytochrome (CYP)3A. Likewise, the metabolic ratio of midazolam was negligible as compared to the first session (**Supplementary Figure S1** online). With regard to the efflux transporter P-glycoprotein (P-gp) phenotyping using fexofenadine, no significant difference was observed between the PK profile of fexofenadine administered with and without ritonavir (**Figure 3**).

Platelet inhibition assessments

All volunteers demonstrated platelet activities below the predefined cutoffs. Regarding the Platelet Reactivity Index (PRI) measured by the VAsodilator-Stimulated Phosphoprotein Assay (VASP) assay, a mean relative reduction from baseline (T_0) of 77% (95% CI = 74–79%) was observed 4 hours after a single 180 mg dose of ticagrelor as compared to 74% (95% CI = 69–80%) after a single 45 mg dose coadministered with ritonavir ($P = 0.34$). The PRI value after ticagrelor intake was 9.3% at the first session vs. 15.7% at the second session ($P = 0.10$).

With respect to the Platelet Reactivity Units (PRUs) obtained by VerifyNow assay, a mean reduction from baseline of 93% (95% CI = 89–96%) was observed 4 hours after a single 180 mg dose of ticagrelor as compared to 86% (95% CI = 81–92%) after a single 45 mg dose coadministered with ritonavir ($P = 0.17$). The absolute PRU value after ticagrelor intake was 12 PRU at the first session vs. 18 PRU at the second session ($P = 0.15$). Both regimens led to a potent and efficacious inhibition of the

Table 2 Observed antiplatelet activity of ticagrelor after administration of a single 180 mg dose of ticagrelor alone (session one) and a single 45 mg dose of ticagrelor coadministered with ritonavir 100 mg (session two) in 19 healthy male volunteers using VASP and VerifyNow tests

		Ticagrelor 180 mg (95% CI)	Ticagrelor 45 mg + ritonavir 100 mg (95% CI)	*P* value[a]
VASP	PRI (baseline)	89% (88–91%)	91% (89–93%)	0.09
	PRI (4h postdose)	9.3% (6.5–12%)	15.7% (10.2–21.2%)	0.10
	Reduction of PRI	77% (74–79%)	74% (69–80%)	0.34
VerifyNow	PRU (baseline)	258 (248–268)	239 (220–257)	0.12
	PRU (4h postdose)	11.8 (4.7–18.9)	18.1 (5.3–30.8)	0.15
	Reduction of PRU	93% (89–96%)	86% (81–92%)	0.17

PRI, Platelet Reactivity Index; PRU, platelet reactivity units; VASP, VAsodilator-Stimulated Phosphoprotein test.
[a]*P* value < 0.05: significant, *P* value < 0.01 highly significant. Values are expressed as geometric means (95% confidence interval).

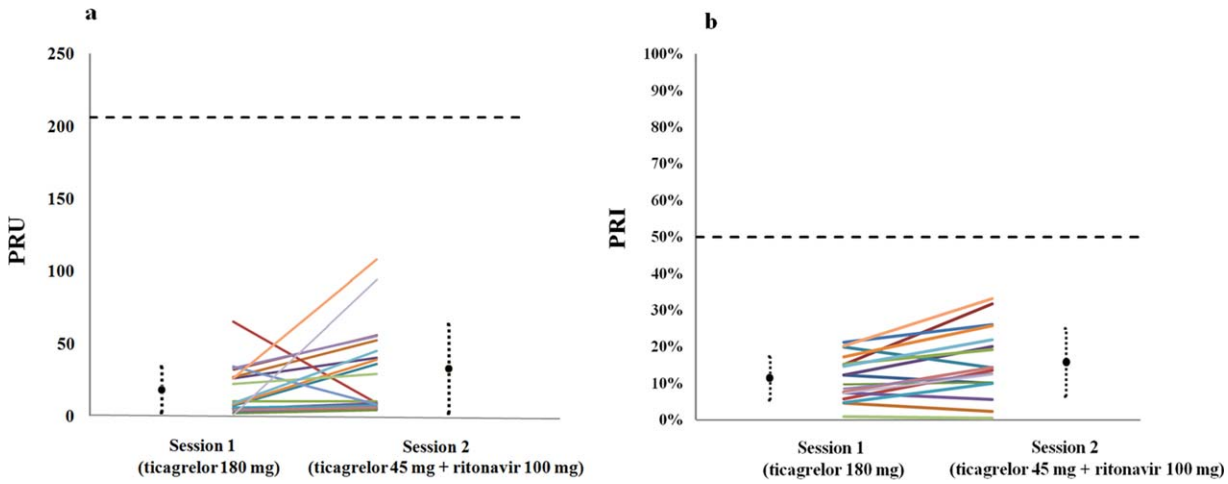

Figure 4 Observed platelet inhibition after administration of a single 180 mg dose of ticagrelor alone (session one) and a single 45 mg dose of ticagrelor coadministered with ritonavir 100 mg (session two) in healthy male volunteers, using VerifyNow (**a**) and VAsodilator-Stimulated Phosphoprotein (VASP) (**b**) assays. The dashed line represents the nonresponsiveness cutoff values (Platelet Reactivity Index [PRI] = 50% for VASP and $P2Y_{12}$ reaction unit [PRU] = 206 for VerifyNow tests). The *P* value of the bilateral *t* test = 0.10 (VASP) and 0.15 (VerifyNow). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

P_2Y_{12} receptor (**Table 2**). Mean observed platelet inhibition results are presented in **Figure 4**. Altogether, all volunteers presented a platelet inhibition below the cutoff value in both sessions.[15]

DISCUSSION

This study highlights the usefulness of modeling and simulation in a stepwise dose adjustment in case of a clinically relevant and/or unavoidable DDI situation. Dose recommendations supported by PBPK modeling have already been successfully realized for various compounds, such as macitentan and roxulitinib.[16,17] As a first step, a PBPK model was created for ticagrelor using *in vitro* and *in vivo* parameters. A baseline PK profile of ticagrelor was successfully refined before the model was used to predict any interaction. Relative contribution of CYP3A in the metabolism of ticagrelor was challenged and improved on the basis of an existing ketoconazole DDI clinical study.[18] Fraction of the drug escaping gut clearance (F_g) was estimated from a published clinical study with grapefruit juice.[9] To refine other parameters, such as the fraction unbound in the gut and the absorption rate constant (k_a), the Simcyp-provided sensitivity analysis was used. As expected, the final model predicted strong inhibition of CYP3A by a single 100 mg ritonavir dose and its impact on the PK of a single 180 mg ticagrelor dose in healthy volunteers. The mean simulated AUC ratio of four was obtained for the DDI between ticagrelor and ritonavir. As a result, a fourfold lower ticagrelor dose (i.e., 45 mg) was calculated to obtain the same PK profile, thus platelet inhibition for ticagrelor with and without ritonavir. During a clinical study, single doses of 180 mg ticagrelor and 45 mg ticagrelor in combination with ritonavir were administered to healthy volunteers. Observed ticagrelor AUC values for both sessions were comparable, with a mean AUC increase of 36% for ticagrelor 45 mg coadministered with ritonavir compared to 180 mg administered alone. This could be due to slight overestimation of $f_{m\text{-}3A4}$ in ticagrelor's model. In order to have higher confidence in the model, the latter should be tested against various clinical DDI studies with larger panel of CYP3A inhibitors and inhibition potencies. Because the AUC ratio between two sessions fell outside the 0.8–1.25 range, the bioequivalence could not be claimed. The average bioequivalence has been defined as the absence of significant difference in the rate and the extent of exposition to an active compound at its site of action.[19] Nonetheless, the platelet inhibition was revealed to be similar in both sessions and thus the clinical relevance of a 36% increase in ticagrelor's AUC in session two is doubtful. It is worth mentioning that the PK bioequivalence boundaries are too restrictive in terms of final effect in clinical practice.

A 50% lower C_{max} value was observed when the low-dose ticagrelor was administered during the second session. To evaluate the platelet inhibition in both sessions, VASP and VerifyNow assays were realized. These tests have been shown to be reliable and rapid for assessment of antiplatelet effect of ticagrelor and identifying potential on-treatment nonresponders. Results indicated that all volunteers had a platelet aggregation below the predetermined cutoff values (i.e., 206 PRU and 50% PRI) at both sessions, even though a fourfold lower dose of ticagrelor was administered during the second session. The relationship between the PK of ticagrelor and its platelet inhibition effect has already been demonstrated. It was observed that the IPA increased with plasma concentrations of ticagrelor and the inhibition achieved a plateau (90% IPA) when ticagrelor's concentration attained 200 ng/mL. [13] This association is not surprising as ticagrelor exerts its platelet inhibition by direct binding to P_2Y_{12} receptors, needing no bioactivation. The maximum IPA was observed 2 hours after ticagrelor intake and was maintained 8 hours postdose.[13,20] Because the observed C_{max} were considerably higher than 200 ng/mL at both sessions of our study, the platelet inhibition was still at its maximum level at the blood sampling time (i.e., 4 hours postdose). This might be reason why the 36% increase in AUC of ticagrelor at session two did not have significant impact on its platelet inhibition effect.

Table 3 Observed and simulated AUC of ticagrelor considered together with its active metabolite after administration of a single 180 mg dose of ticagrelor alone (session one) and a single 45 mg dose of ticagrelor coadministered with ritonavir 100 mg (session two) in healthy male volunteers

	Clinical $AUC_{ticagrelor+AM}$	Simulated $AUC_{ticagrelor+AM}$	Clinical/simulated
Session 1	7,850 (7,260–8,440)	8,000 (7,420–8,580)	0.98
Session 2	5,870 (5,140–6,590)	5,560 (4,920–6,190)	1.05
Ratio	0.75	0.69	–

AUC, area under concentration-time curve (h × ng/mL).
Ratio: AUC $session_2$/AUC $session_1$.
AUC values are expressed as means (95% confidence interval).

Because the ticagrelor model had never been tested *in vivo* and considering the safety of healthy volunteers, ticagrelor's AUC was considered alone for the dose calculation in our clinical study. Knowing that ticagrelor and AM are equipotent with regard to platelet inhibition, another reasonable approach could be comparing the sum of both compounds' AUCs at both sessions of the clinical study to estimate the global antiplatelet activity in the body. To this end, the AUC ratio was recalculated in a post-hoc analysis using the equation:

$$AUC\ ratio = AUC_{ticagrelor(session\ 2)} + AUC_{AM(session\ 2)} / AUC_{ticagrelor(session\ 1)} + AUC_{AM(session\ 1)}$$

Based on this equation, the observed AUC ratio was 0.75. Results are outlined in **Table 3**.

On the basis of the same strategy, the mean (±SD) simulated AUC ratio in case of coadministration of 180 mg ticagrelor with a single ritonavir dose was calculated as 3(±1). Therefore, a dose of 60 mg was retrospectively obtained for ticagrelor capsules to be administered with ritonavir at session two. According to the observed results for ticagrelor 45 mg and the difference of 75% between two sessions of the clinical study, an average bioequivalence could be expected in case of coadministration of ticagrelor 60 mg with ritonavir. Altogether, the observed/simulated AUC ratios were 0.98 and 1.05 for session one and session two, respectively, confirming the consistency of the PBPK model with the clinical observations.

The activity of the P-gp efflux transporter was evaluated using the PK profile of a low-dose fexofenadine as a probe substrate in both sessions. Given that ticagrelor is a substrate of P-gp, the inhibition of the latter transporter by ritonavir together with CYP3A inhibition may increase the bioavailability of ticagrelor in an extended manner. Surprisingly, ritonavir single dose had no effect on the PK of fexofenadine. It is worth mentioning that, in spite of wide utilization of fexofenadine in P-gp phenotyping, this compound has demonstrated to be the substrate of other transporters such as OATP1B1 and OATP2B1, and discrepant results have been observed.[21–23] Ritonavir inhibits various OATP transporters, such as OATP1B1, OATP1B3, and OATP2B1.[24–26] Therefore, caution should be taken when interpreting P-gp phenotyping results based on fexofenadine PK variation.

In this study, a well-stirred hepatic model and a perfusion-rate limited clearance were assumed. Results obtained in this study are restricted by the administration of single doses of ticagrelor and ritonavir. Considering a possible induction effect of ritonavir on various enzymes and transporters, a different DDI magnitude cannot be ruled out in clinical practice. On the other hand, it has been shown that HIV infection itself may modulate some enzymes' activity regardless of any treatment.[27,28]

Currently, the life expectancy of patients with HIV has significantly risen owing to the new antiretroviral drugs. Given their age and characteristic of their pathology, elderly infected patients are at high risk of atherothrombotic events and need proper treatment. Ticagrelor is recommended in all patients at moderate to high risk of ischemic events.[29] However, it is contraindicated in patients receiving strong CYP3A inhibitors, such as ritonavir, darunavir, and atazanavir, due to the inherent bleeding risk. To avoid this interaction, prescription of clopidogrel is suggested by various guidelines. Nonetheless, clopidogrel is a pro-drug that requires bioactivation by different CYPs including CYP3A. Inhibition of this isoenzyme in patients with HIV may lead to a lack of efficacy and high risk of cardiovascular events.[30] Prasugrel, another pro-drug inhibitor of platelet aggregation, could constitute an alternative to ticagrelor. Two main CYPs responsible for its metabolism are CYP3A, subject to the same interaction with ritonavir, and CYP2B6, subject to polymorphism and interindividual variability that may lead to a possible nonresponse in some patients.[31,32] No head-to-head comparative clinical study between ticagrelor and prasugrel is yet available.

This study introduced a starting point toward prediction of the safe and efficacious dose of ticagrelor in patients co-treated with ritonavir using PBPK modeling and simulation. This model can be prospectively used to broaden the usage of ticagrelor in patients with ritonavir-boosted HIV. Applications of the obtained results directly to patients require further model validation, including physiopathological factors and other co-medications. Additionally, the steady-state PK of all compounds should be assessed to obtain a reliable picture of the clinical scenario, including potential mechanism-based inhibition and induction properties of the perpetrator drug.

METHODS

Simulations

Ticagrelor's initial model development and adjustment for $f_{m\text{-}3A}$. A PBPK model was created for ticagrelor and AM based on *in vitro* and *in*

vivo parameters using Simcyp simulator version 14.2 (Simcyp Limited, Certara, Sheffield, UK). The parameters included absorption, distribution, metabolism, and excretion-related data in a three-compartmental model incorporating the liver, gut, and central compartment. The absorption and the distribution steps were described by a first order process and a minimal-PBPK model, respectively. Ticagrelor seems to be considerably distributed in body tissues.[5] Thus, full-PBPK model would be a proper choice to parameterize ticagrelor's distribution.

With regard to metabolism, using intrinsic clearances obtained from an *in vitro* phenotyping study did not allow covering the oral clearance of ticagrelor observed in the reference clinical study (data not shown).[11,33] Therefore, intrinsic hepatic clearances ($CLu_{H,\ int}$) were back-calculated from the observed oral clearance combining bottom-up and top-down approaches. To this end, Eqs. 1 and 2 were applied using the retrograde calculation in the simulator[34]:

$$CL_{met} = \frac{CL - CL_R}{B:P} \quad (1)$$

$$CLu_{H,int} = \frac{Q_H \times CL_{met}}{f_{u,B}(Q_H - CL_{met})} \quad (2)$$

where CL is the systemic plasma clearance (L/h), CL_{met} is the hepatic metabolic clearance in blood (L/h), CL_R is the renal clearance (L/h), B:P is the blood to plasma partition ratio, Q_H is the hepatic blood flow (L/h), and $f_{u,B}$ is the fraction unbound in blood. A well-stirred model and a perfusion-limited clearance were assumed in the model.

In order to verify and optimize the robustness of the model, the latter was challenged to quantitatively predict the magnitude of two interactions and results were compared to a published clinical trial. To this end, a DDI clinical study on ticagrelor and ketoconazole was chosen to adjust the CYP3A contribution in metabolism of ticagrelor.[18] In this study, ketoconazole 200 mg was administered b.i.d. in healthy volunteers for 14 days and a single dose of ticagrelor 90 mg was given on day 4. Value of $f_{m\text{-}3A}$ was set to 0.8 in the retrograde mode based on the similarity of predicted and observed AUC ratios for ticagrelor. In the final model, two pathways were considered for ticagrelor's CYP3A mediated metabolism in order to separate the formation of ticagrelor's active (27%) and inactive (55%) metabolites and to obtain the same PK profile as well as the same DDI magnitude for the active metabolite, in accordance with previously published data.[11,33] Remaining clearance was assigned as undefined additional pathways.

Furthermore, a published clinical study on ticagrelor and grapefruit juice interaction was used to refine ticagrelor's F_g considering the selective inhibition of intestinal CYP3A by the latter. [9] In this study, grapefruit juice was administered daily to healthy volunteers for 4 days and a single dose of ticagrelor 90 mg was administered on day 3. An AUC ratio of 2.2 was observed. In the simulator, the intestinal content of CYP3A was set to zero in order to reproduce the inhibition by grapefruit juice. The F_g and the fraction unbound in the gut of 0.5 and 0.35 were obtained using sensitivity analysis of the simulator. Because the overall bioavailability of ticagrelor is known to be approximately 35% and based on the equation $F = F_a \times F_h \times F_g$, with an F_h of approximately 80% and an F_g of 50%, as predicted above, the F_a value was estimated as 78%.[11] F is the total oral bioavailability, F_a is the fraction absorbed, and F_h and F_g are fractions of the drug escaping hepatic and gut clearances, respectively. Ticagrelor's human jejunum permeability ($P_{eff\ man}$) was calculated by the simulator using gut permeability (P_{app}) value of 15×10^{-6} cm/s, generated from an *in vitro* experience carried out on Caco-2 cell line (data submitted for publication). For details of ticagrelor and AM input parameters, please see **Supplementary Table S1** online. All the equations describing the PBPK model have been widely published and discussed.[34–36]

Simulation of DDI between ticagrelor and ritonavir

Ten trials of 20 virtual healthy male volunteers were simulated. The dynamic mode was chosen to link the plasma concentration of ticagrelor to that of ritonavir in a time-dependent manner. The reliability of the ritonavir Simcyp file was verified using published DDI clinical trials with CYP3A substrates, such as midazolam, triazolam, and zolpidem. Reference clinical studies and simulation results are summarized in **Supplementary Document S2** online. The model input parameters of used CYP3A substrates as well as ritonavir are depicted in **Supplementary Documents S3 and S4** online, respectively. Because ticagrelor has shown a linear PK up to 900 mg daily doses in published clinical studies,[13,14] the simulated $AUC_{ticagrelor\ with\ ritonavir}/AUC_{ticagrelor}$ was used as the reduction factor to calculate a new ticagrelor dose to administer with ritonavir during the second session of the clinical study.

CLINICAL STUDY

Study population

Healthy male volunteers between 18 and 60 years old with a body mass index between 18 and 27 kg/cm^2 were eligible to participate in the study. Volunteers were under no medication and were asked to abstain from drinking grapefruit juice. The study protocol has been reviewed and approved by the independent ethics committee of Geneva University Hospitals as well as the Swiss Agency for Therapeutic Drugs (Swissmedic). All participants provided written informed consent prior to study enrolment. Protocol conception and trial conduct were performed in accordance with the Declaration of Helsinki ethical principles and the Good Clinical Practice guidelines of the International Congress of Harmonization. The trial is registered at http://www.clinicaltrials.gov (trial identifier NCT02435563).

Study design and treatment

This study was an open-label, before-after trial design. It aimed to obtain the same PK profile for a single dose of ticagrelor 180 mg administered alone (session one) and an adjusted-dose of ticagrelor coadministered with a single dose of 100 mg ritonavir (session two). A dosage of 180 mg of ticagrelor was chosen as it is the prescribed loading dose in clinical practice. The primary endpoint of the study included PK assessment for ticagrelor in both sessions. The secondary objective consisted of platelet activity evaluation and its consistency with the PK profile of ticagrelor at both sessions. The study was conducted in the Clinical Research Centre of Geneva University Hospitals. Two sessions were separated by a washout period of at least 3 weeks.

In the morning of the first session after an overnight fast, volunteers took a 180 mg dose of commercialized ticagrelor (Brilique). At the same time, 30 mg of fexofenadine (Telfast) as well as 100 μg of midazolam (Midazolam Sintetica) were administered in order to assess the activity of CYP3A and P-gp, respectively. Venous blood samples were taken in EDTA tubes (Vacutainer) to assess baseline PK parameters of ticagrelor, the active metabolite and fexofenadine, prior to ticagrelor administration (time zero) and at the following postdosage times: 30 minutes, 1, 2, 3, 4, 6, and 24 hours. The venous blood sample collected 1 hour after midazolam intake was used for CYP3A phenotyping in each session. Supplementary blood samples collected on citrate-containing tubes (Vacuette and Vacutainer) before and 4 hours after ticagrelor intake were used to assess the antiplatelet activity of ticagrelor by the VASP and the VerifyNow P_2Y_{12} assays, respectively. In the second session, volunteers took a tablet of commercialized ritonavir 100 mg (Norvir) at home 2 hours before the assigned time of ticagrelor intake. Ticagrelor low-dose

capsules were manufactured from commercialized ticagrelor tablets (Brilique) by the pharmacy of the Geneva University Hospitals. The second session was conducted the same way as the first session.

Pharmacokinetic assessments and phenotyping

Plasma was obtained after centrifugation of blood samples at 2,000 rpm (2,750 g-force) for 10 minutes and aliquots were conserved at -80°C until analysis. The analysis of samples was performed using fully validated methods for ticagrelor, AM, fexofenadine. and ritonavir by liquid chromatography coupled with a triple-quadrupole mass spectrometer. CYP3A phenotype was assessed using metabolic ratio of midazolam (OH-midazolam/midazolam) 1 hour after intake of a single 100 µg dose. Midazolam and OH-midazolam were analyzed using a previously validated analytical method.[37,38] The PK profile of fexofenadine was assessed in the same way to evaluate the P-gp activity in the presence and absence of ritonavir. For details of the quantification methods and instruments please see **Supplementary Document S1** online (data submitted for publication).

Platelet inhibition assessments

The historical gold standard method to measure the pharmacodynamic effect of antiplatelet agents, such as ticagrelor, is the whole blood aggregometry method where results are expressed as IPA%.[5,14] However, this method is time-consuming and requires long sample preparation steps.[39,40] On the other hand, VerifyNow is a new aggregometry measurement method with the advantage of being fully automated, simple, and quick and it can be used as a point-of-care test for monitoring the antiplatelet activity of P_2Y_{12} inhibitors. Likewise, VASP assay is a flow cytometric technique measuring specific inhibition of P_2Y_{12} receptor. A PRI >50% obtained by this test is a reliable index of high platelet reactivity and an insufficient antiplatelet exposure and efficacy in most studies. A good correlation between results obtained by these different platelet tests has been observed. However, using multiple tests generate more consistent results.[41–45]

VASP assay

Whole blood sample tubes were mixed gently after withdrawal. VASP phosphorylation analysis was performed within 24 hours of blood collection using Platelet VASP kit (Stago, Zürich, Switzerland), according to the manufacturer's instructions. The PRI was calculated by the equation PRI = (MFI [PGE1]-MFI [PGE_1+ADP]/MFI [PGE_1] × 100 where MFI is the median fluorescence intensity of samples incubated with PGE_1 or PGE_1 and ADP. A PRI >50% obtained by this test is an index of high platelet reactivity and an insufficient exposure in most studies.[15,46,47]

VerifyNow $P2Y_{12}$ Assay

Whole blood sample tubes were mixed gently after withdrawal. The VerifyNow $P2Y_{12}$ assay was performed within 4 hours of blood collection using single-use cartridges. The VerifyNow $P2Y_{12}$ system was used for measuring platelet aggregation via light transmittance variations. Results were displayed as absolute PRU and inhibition percentage (calculated as baseline value-PRU/baseline value × 100). Different cutoff values of 206 to 240 have been used in various studies with regard to absolute PRU. The cutoff of PRU ≥206 was used in our case as the most conservative cutoff to define a high platelet reactivity.[41,42,48,49]

Data analysis

Average bioequivalence was assessed on ticagrelor's AUC. With an alpha error of 5% and an expected intrasubject coefficient of variation of 20% for ticagrelor's AUC, a statistically relevant number of volunteers was calculated to be at least 19 in order to claim bioequivalence with a power of 80%.[50] The PK parameters of ticagrelor and AM were calculated using a standard noncompartmental method by WinNonLin version 6.2.1 (Pharsight, Mountain View, CA, USA). The comparison between two AUC values was expressed by the geometric mean ratio. If the asymptotic 95% CI around the geometric mean ratio of ticagrelor adjusted-dose administered by ritonavir, and ticagrelor 180 mg alone fell within bioequivalence limits of 0.80 to 1.25, average bioequivalence would be claimed.

Additional Supporting Information may be found in the online version of this article.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

N.M. wrote the manuscript. Y.D., N.M., C.F.S., P.F., J.-L.R., S.R., and J.D. designed the research. Y.D., N.M., and C.F.S. performed the research. N.M. analyzed the data. Y.D. and N.M. contributed new reagents/analytical tools.

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