Coadministration of ticagrelor and ritonavir: Toward prospective dose adjustment to maintain an optimal platelet inhibition using the PBPK approach

MARSOUSI, Niloufar, et al.

Abstract

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.

Reference


PMID : 27264793
DOI : 10.1002/cpt.407

Available at: http://archive-ouverte.unige.ch/unige:86304

Disclaimer: layout of this document may differ from the published version.
Coadministration of Ticagrelor and Ritonavir: Toward Prospective Dose Adjustment to Maintain an Optimal Platelet Inhibition Using the PBPK Approach

N Marsousi¹,², CF Samer¹,³, P Fontana⁴,⁵, JL Reny⁵,⁶, S Rudaz²,³, JA Desmeules¹,²,³ and Y Daali¹,²,³

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 clopidogrel or prasugrel in 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,
hyperlipidemia, or insulin resistance, increase the risk of cardio-
arterial diseases in patients infected with HIV.\textsuperscript{2–4} Ritonavir, a pro-
tease 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 inhibi-
tion of the metabolism of other drugs. In spite of its potential ben-
fits, ticagrelor is contraindicated in patients with HIV because of
the expected interaction with ritonavir and potential bleeding
risk.\textsuperscript{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
\((C_{\text{max}})\) 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).\textsuperscript{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.\textsuperscript{8} Furthermore,
coadministration of grapefruit juice and a single 90 mg dose of
ticagrelor resulted in 165% and 221% increase in AUC and \(C_{\text{max}}\) 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.\textsuperscript{9} Based on the relationship between ticagrelor’s
plasma concentration and platelet inhibition, as ritonavir increases
ticagrelor bioavailability, administration of a lower dose of ticagre-
lor 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.\textsuperscript{10} In vitro to in vivo extrapolation and simula-
tion 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 simu-
lated 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 \(f_{\text{m-3A}}\). The
refined model predicted similar AUC and \(C_{\text{max}}\) values for ticagre-
lor and AM following administration of a single dose of ticagrelor
200 mg as compared to the reference published clinical study.\textsuperscript{11}
Concerning ticagrelor, the observed \(R_{\text{predicted/observed}}\) value was
1.1 and 0.9 for AUC and \(C_{\text{max}}\), respectively. Regarding AM, 1.3
and 0.9 were, respectively, observed for AUC and \(C_{\text{max}}\).

![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.\textsuperscript{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.]

Figure 1.
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 $C_{\text{max}}$ 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

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

AM, active metabolite; AUC, area under concentration-time curve; $C_{\text{L}}$, confidence interval; $CL_{\text{L}}$, oral clearance; $C_{\text{max}}$, maximal plasma concentration; $T_{\text{max}}$, 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).
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).

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 (T0) 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 platelet.

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

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

PRI, Platelet Reactivity Index; PRU, platelet reactivity units; VASP, VAsodilator-Stimulated Phosphoprotein test.

aP value < 0.05: significant, P value < 0.01 highly significant. Values are expressed as geometric means (95% confidence interval).
P2Y12 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 (Fg) 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 (ka), 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 \( \text{F}_{\text{RAU}} \) 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\text{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 P2Y12 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\text{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.
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:

\[
\text{AUC ratio} = \frac{\text{AUC}_{\text{ticagrelor}(\text{session } 2)} + \text{AUC}_{\text{AM}(\text{session } 2)}}{\text{AUC}_{\text{ticagrelor}(\text{session } 1)} + \text{AUC}_{\text{AM}(\text{session } 1)}}
\]

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-boostered 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_{m3A}. A PBPK model was created for ticagrelor and AM based on in vitro and in vivo results. The model was adjusted for clinical observations using PBPK modeling and simulation.

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Simulated</th>
<th>Clinical/simulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{ticagrelor + AM}</td>
<td>AUC_{ticagrelor + AM}</td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>7,850 (7,260–8,440)</td>
<td>8,000 (7,420–8,580)</td>
</tr>
<tr>
<td>Session 2</td>
<td>5,870 (5,140–6,590)</td>
<td>5,560 (4,920–6,190)</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.75</td>
<td>0.69</td>
</tr>
</tbody>
</table>

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).
**CLINICAL STUDY**

**Study population**

Healthy male volunteers between 18 and 60 years old with a body mass index between 18 and 27 kg/cm² 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](http://www.clinicaltrials.gov) (trial identifier NCT02435563).

**Study design and treatment**

This 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 P2Y12 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

---

**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

---

**ARTICLES**

**CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 100 NUMBER 3 | SEPTEMBER 2016**
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. 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 antplatelet agents, such as ticagrelor, is the whole blood aggregometry method where results are expressed as IPA%. However, this method is time-consuming and requires long sample preparation steps. 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 P2Y12 activity. Likewise, VASP assay is a flow cytometric technique measuring specific inhibition of P2Y12 receptor. A PRI >50% obtained by this test is a reliable index of high platelet reactivity and an insufficient antplatelet 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.

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 [PGE1+ADP])/MFI [PGE1] × 100 where MFI is the median fluorescence intensity of samples incubated with PGE1 or PGE1 and ADP. A PRI >50% obtained by this test is an index of high platelet reactivity and an insufficient exposure in most studies.

VerifyNow P2Y12 Assay
Whole blood sample tubes were mixed gently after withdrawal. The VerifyNow P2Y12 assay was performed within 4 hours of blood collection using single-use cartridges. The VerifyNow P2Y12 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.

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%. 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.

ACKNOWLEDGMENTS
Simcyp Limited., a Certara Company, is gratefully acknowledged for an academic license for the Simcyp Population-based Simulator and providing user support. The authors also wish to thank the Clinical Research Centre of the Geneva University Hospitals. The clinical study was supported by the Swiss National Science Foundation (FNRS 32003B-156471) and the division of Anesthesiology, Pharmacology, and Intensive Care (APSI) of the Geneva University Hospitals.

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., N.M., C.F.S., P.F., J.-L.R., S.R., and J.D. wrote the manuscript. Y.D., N.M., C.F.S., P.F., J.-L.R., S.R., and J.D. provided user support. The authors also wish to thank the Clinical Research Centre of the Geneva University Hospitals. The clinical study was supported by the Swiss National Science Foundation (FNRS 32003B-156471) and the division of Anesthesiology, Pharmacology, and Intensive Care (APSI) of the Geneva University Hospitals.


43. Bidet, A. et al. VerifyNow and VASP phosphorylation assays give similar results for patients receiving clopidogrel, but they do not always correlate with platelet aggregation. Platelets 21, 94–100 (2010).


