Grapefruit juice markedly increases the plasma concentrations and antiplatelet effects of ticagrelor in healthy subjects

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The P2Y₁₂ antagonist ticagrelor is a promising option for the treatment of acute coronary syndrome due to its efficacy and reversible mode of antiplatelet action.
- Ticagrelor is metabolized mainly by CYP3A enzymes.
- Grapefruit juice is an inhibitor of intestinal CYP3A4 but the role of the intestine and the effect of grapefruit juice in the extensive first pass metabolism of ticagrelor is unknown.

WHAT THIS STUDY ADDS

- This study shows that ticagrelor exposure (plasma area under the concentration–time curve, AUC) is more than doubled by grapefruit juice, leading to an enhanced and prolonged antiplatelet effect of ticagrelor.
- The results indicate that intestinal CYP3A4-mediated first pass metabolism is important to ticagrelor pharmacokinetics and that grapefruit juice has an inhibitory effect on it.
- Care is warranted if grapefruit juice or other CYP3A4 inhibitors are used concomitantly with ticagrelor.

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AIM

This study examined the effects of grapefruit juice on the new P2Y₁₂ inhibitor ticagrelor, which is a substrate of CYP3A4 and P-glycoprotein.

METHODS

In a randomized crossover study, 10 healthy volunteers ingested 200 ml of grapefruit juice or water thrice daily for 4 days. On day 3, they ingested a single 90 mg dose of ticagrelor.

RESULTS

Grapefruit juice increased ticagrelor geometric mean peak plasma concentration (C_{max}) to 165% (95% confidence interval 147, 184%) and area under the concentration–time curve ($AUC(0,\infty)$) to 221% of control (95% confidence interval 200, 245%). The C_{max} and $AUC(0,34\text{ h})$ ($P < 0.05$) but not the $AUC(0,\infty)$ of the active metabolite C12490XX were decreased significantly. Grapefruit juice had a minor effect on ticagrelor elimination half-life prolonging it from 6.7 to 7.2 h ($P = 0.036$). In good correlation with the elevated plasma ticagrelor concentrations, grapefruit juice enhanced the antiplatelet effect of ticagrelor, assessed with VerifyNow® and Multiplate® methods, and postponed the recovery of platelet reactivity.

CONCLUSIONS

Grapefruit juice increased ticagrelor exposure by more than two-fold, leading to an enhanced and prolonged ticagrelor antiplatelet effect. The grapefruit juice–ticagrelor interaction seems clinically important and indicates the significance of intestinal metabolism to ticagrelor pharmacokinetics.

Introduction

Antiplatelet drugs like clopidogrel and ticagrelor that target the P2Y₁₂ adenosine diphosphate (ADP) receptor are widely used to prevent arterial thrombosis and improve prognosis, in particular after acute coronary syndrome with angioplasty and stenting [1]. The clinical effects of clopidogrel vary widely among individuals, for example due to genetic factors and drug–drug interactions reducing the formation of the active metabolite of clopidogrel. In this regard, the newer P2Y₁₂ receptor inhibitors ticagrelor and prasugrel seem to offer an improvement [2]. Ticagrelor binds to the P2Y₁₂ receptor reversibly, which differentiates it from the prodrugs clopidogrel and prasugrel, the effect of which is based on irreversible, long lasting binding of an active metabolite to the P2Y₁₂ receptor [3–5].

Ticagrelor undergoes extensive metabolism by CYP3A4 and CYP3A5 to two major metabolites, C124910XX and C133913XX. The O-deethylated metabolite C124910XX has a similar P2Y₁₂-inhibitory activity as the parent compound ticagrelor, but its significance to the clinical efficacy is limited [3, 6, 7]. Because of first pass metabolism, the oral bioavailability of ticagrelor averages only 36%. Its elimination half-life ($t_{1/2}$) is about 7 h and it is administered twice daily [6, 8, 9]. Consistent with the role of CYP3A enzymes in ticagrelor metabolism, ketoconazole and diltiazem have increased ticagrelor peak plasma concentrations ($C_{\max}$) and area under the plasma concentration–time curve (AUC), and rifampicin has decreased ticagrelor $C_{\max}$ and AUC, as reported by the manufacturer of ticagrelor [9]. However, the contribution of the intestine to ticagrelor first pass metabolism is unknown. This information would be important because the extent and time relationships of drug interactions caused by, for example, enzyme inhibitors can depend on the route of inhibitor administration (oral or intravenous) [10] and on the contribution of the intestine to the first pass metabolism of the victim drug [11].

Grapefruit juice is often consumed by individuals over 45 years of age, an age group in which the use of cardiovascular medications such as antiplatelet agents is common [12]. Grapefruit juice is known for its ability to inhibit intestinal CYP3A4 via mechanism-based inactivation of CYP3A4 by its furanocoumarin constituents [13]. A single glass of grapefruit juice or one whole grapefruit has sufficient potency to cause a clinically significant pharmacokinetic interaction [13, 14]. Accordingly, grapefruit juice markedly increases the plasma concentrations of several CYP3A4 substrates such as felodipine, simvastatin and buspirone [15–17]. On the other hand, grapefruit juice can also dramatically decrease the plasma concentrations of some drugs, for example those of aliskiren, celiprolol and fexofenadine [18–22]. This effect is presumed to be due to inhibition of the organic anion transporting polypeptide influx transporters OATP1A2 and/or OATP2B1 by grapefruit

juice constituents in the intestine [23, 24]. Some of these drugs, for example aliskiren, are also CYP3A4 substrates. Ticagrelor is a substrate for the P-glycoprotein transporter, but the possible roles of OATP-transporters in ticagrelor pharmacokinetics are unknown. We therefore investigated the effects of grapefruit juice on ticagrelor pharmacokinetics and pharmacodynamics in a randomized crossover study in healthy volunteers. Functional effects were assessed with a turbidimetric optical ADP P2Y₁₂ receptor-specific platelet aggregation assay [25], which accurately reflects the effects of ticagrelor, and with two other whole blood platelet function tests. We evaluated the significance of the intestinal metabolism of ticagrelor and the clinical relevance of a possible pharmacokinetic interaction.

Methods

Subjects

Ten healthy volunteers (four women and six men) were enrolled in the study after giving written informed consent. Their health was confirmed by medical history, clinical examination, laboratory tests and a standardized bleeding risk questionnaire. All participants had normal blood platelet counts and haematocrit values. None was on any continuous medication nor was a tobacco smoker. The mean $\pm$ SD age was 22 ± 3 years (range 19–27 years), height 177 ± 9 cm (157–187 cm) and weight 75 ± 14 kg (57–95 kg).

Study design

The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District, and the Finnish Medicines Agency, Fimea. To investigate the effect of grapefruit juice on ticagrelor pharmacokinetics and antiplatelet efficacy, a randomized crossover study with two phases and a washout period of 2 weeks was designed. The participants ingested either 200 ml of normal strength grapefruit juice (Valio greipitäysmehu; Valio, Helsinki, Finland) or water three times a day at 08.00 h, 12.00 h and 20.00 h for 4 days to achieve nearly maximal intestinal CYP3A4 inhibition. On day 3 after an overnight fast, they ingested a single 90 mg dose of ticagrelor (Brilique®; AstraZeneca, London, UK) with 200 ml of grapefruit juice or water at 09.00 h. Standardized meals were served 4, 8 and 11 h post-dose. Use of grapefruit products, apple and orange juice and alcohol was prohibited during the study. Use of all other drugs was prohibited for 1 week before ticagrelor administration and for at least 72 h thereafter.

Ethylenediaminetetraacetic acid, citrate (109 mM sodium citrate, 3.2%) and hirudin (at $25 \mu\text{g ml}^{-1}$) blood samples (2.7–9 ml each) were collected prior to and up to 34 h after ticagrelor ingestion according to a time schedule. For pharmacokinetic assays, plasma was separated within 30 min and stored at -70°C until analysis. For the whole

blood platelet function tests, all studies were performed within 2 h of sampling.

Determination of ticagrelor and C124910XX concentrations

The plasma concentrations of ticagrelor and its C124910XX metabolite were measured using an API 3000 LC/MS/MS system (AB Sciex, Toronto, ON, Canada) [26, 27]. After a simple protein precipitation of plasma samples by acetonitrile, the chromatographic separation was achieved on an ACE 3 C-18 PFP column (100 × 2.1 mm i.d.) using a mixture of 10 mM ammonium formate (channel A) and acetonitrile (channel B) as mobile phase. The flow rate was set at 190 $\mu\text{L min}^{-1}$ and the gradient profile was set as follows: linear increase from 40% B to 90% B over 4 min followed by 1.5 min at 90% B, and 14 min re-equilibrium to starting mobile phase composition. The mass spectrometer was operated in negative MRM mode using transitions m/z 521 to 361 for ticagrelor and m/z 477 to 361 for C124910XX, respectively. The lower limit of quantification was 1.0 ng mL^{-1} for ticagrelor and the assay was linear over the standard curve range of 1–2500 ng mL^{-1} . The linearity of the C124910XX detector response was confirmed by use of sample dilutions and a signal-to-noise ratio 10:1 was used as the lower limit of quantification for C124910XX. The between-day coefficient of variation was <10% at relevant concentrations ($n = 4$).

Platelet function testing

The antiplatelet activity of ticagrelor was tested with VerifyNow® P2Y₁₂ assay, a turbidimetric optical detection system (Accumetrics, San Diego, CA, USA) in citrate anticoagulated whole blood samples collected at 0, 4, 12, 24 and 34 h after dosing [25, 28]. ADP is used to activate platelet P2Y₁₂ receptors and to induce aggregation on fibrinogen-coated microbeads. The change in optical signal is measured and expressed as P2Y₁₂ reaction units (PRU). In the control channel, platelets are activated with thrombin receptor activating peptides, and the baseline maximum aggregation is measured. Comparing the two channels, the drug induced inhibition of P2Y₁₂-mediated platelet aggregation is given as percentage of maximum aggregation. According to our own validations, the intra-assay coefficient of variation was 6.5% with the VerifyNow P2Y₁₂ test (two repeated samples from 20 donors, including both medicated and nonmedicated individuals).

ADP-induced Multiplate® platelet aggregation (Dyna-byte®, Munich, Germany), based on an impedance method, was tested in hirudin anticoagulated whole blood samples collected at 0, 4, and 24 h after dosing [29]. In whole blood, ADP-activated platelets (ADP at 6.5 μM and 2.0 μM) aggregate on sensor surfaces and increase the electrical resistance which is continuously measured and converted into arbitrary aggregation units (AU). The area under the AU–time curve is calculated for each sample (AU min). With the Multiplate ADP-test the intra-assay coefficient of varia-

tion was 16% (three repeated samples from five donors including both medicated and non-medicated individuals).

As a third analysis of platelet function in whole blood, PFA-100® (Siemens, NY, USA) with Innovance® PFA P2Y cartridges was used [30]. In this blood flow based method, the closure time is measured to determine collagen- and shear force-induced platelet aggregation in the presence of ADP. Samples were collected 0, 4, 24 and 34 h after administration of ticagrelor. In our laboratory, the intra-individual variability was found to be 7% when ADP was used to activate the platelets (three repeated samples from eight non-medicated donors).

For the VerifyNow® and Multiplate® test results, baseline (i.e. just before administration of ticagrelor), minimum and average values were used as the primary pharmacodynamic variables. The average values were calculated by dividing the area under the effect vs. time curve from time 0 to the last time point (0–24 h or 0–34 h) by the corresponding time interval.

Pharmacokinetics

The following pharmacokinetic parameters were characterized for ticagrelor and C124910XX: C_{max} , time to C_{max} (t_{max}), $t_{1/2}$, AUC(0,34 h) and AUC(0,∞). MK-Model, version 5.0 (Biosoft, Cambridge, UK) was used to perform pharmacokinetic calculations with non-compartmental methods. The elimination constant (λ_z) was calculated by linear regression analysis of the terminal log-linear part of the plasma drug concentration–time curve. The $t_{1/2}$ was calculated by the equation $t_{1/2} = \ln 2 / \lambda_z$. The AUC values were calculated by a combination of the linear and log-linear trapezoidal rules with extrapolation to infinity, when appropriate, by division of the last measured concentration by λ_z .

Statistical analysis

The number of subjects was estimated to be sufficient to detect a 30% difference in the AUC(0,∞) of ticagrelor between the water and grapefruit juice phases, with a power of 80% (α level 5%). To analyze the data, SPSS 20 (SPSS Inc., Chicago, IL, USA) software was used. The results are expressed as geometric means with geometric coefficients of variation (C_{max} , $t_{1/2}$, AUC), means $\pm$ SD (PRU, % inhibition, AU min), medians with range (t_{max}) or geometric means with 90% confidence intervals (CIs) (plasma concentration–time curves). The C_{max} , $t_{1/2}$ and AUC data were logarithmically transformed before statistical analysis. The grapefruit juice and water phases were statistically compared by repeated measures analysis of variance with treatment phase and treatment sequence as factors. The t_{max} data were analyzed with the Wilcoxon signed rank test. Differences were considered statistically significant when $P < 0.05$, and corresponding 95% CIs were calculated for the ratio to control or the difference to control. In addition, a sigmoidal E_{max} model ($E_t = [E_{\text{max}} \times C_t^\gamma] / [EC_{50}^\gamma + C_t^\gamma]$) was fitted to the relationship between the plasma concentration of ticagrelor and the inhibition of platelet aggregation

in the optical aggregometry in the whole group in both study phases separately by means of non-linear regression analysis.

Results

The plasma concentrations of ticagrelor were markedly increased by the daily intake of grapefruit juice (Figure 1). Grapefruit juice increased the $C_{\max}$ of plasma ticagrelor to 165% (95% CI 147, 184%, $P < 0.001$) and its $AUC(0,\infty)$ to 221% of control (95% CI 200, 245%, $P < 0.001$) (Figure 1, Table 1). The $C_{\max}$ of ticagrelor was reached later in the grapefruit juice phase (median, 3 h) than during the water (control) phase (1.5 h; $P = 0.011$). Grapefruit juice also prolonged the elimination half-life ($t_{1/2}$) of ticagrelor from 6.7 to 7.2 h ($P = 0.036$).

The $C_{\max}$ and $AUC(0,34\text{ h})$ of C124910XX were decreased to 55% (95% CI 46, 65%, $P < 0.001$) and 86% of control (95% CI 76, 98%, $P = 0.03$), respectively, by grapefruit juice (Figure 1, Table 1). On the other hand, the $t_{1/2}$ of C124910XX was prolonged from 8.2 to 12.0 h ($P < 0.001$) and consequently the $AUC(0,\infty)$ remained unchanged. The $C_{\max}$ of C124910XX occurred later with grapefruit juice (median, 3 h) than with water (2 h; $P = 0.004$). Grapefruit juice decreased the C124910XX:ticagrelor $AUC(0,34\text{ h})$ ratio to 40% of control (95% CI 33, 48%, $P < 0.001$).

Grapefruit juice increased the $AUC(0,34\text{ h})$ of ticagrelor in all subjects, the effect being especially pronounced (three-fold increase) in one of the individuals (Figure 2).

The effect on the $AUC(0,34\text{ h})$ of C124910XX, however, varied between subjects (Figure 2, Table 1).

The single dose of ticagrelor resulted in near full inhibition of ADP-dependent platelet responses and only modest interindividual variation of the measured effects at 4 h. Compatible with the increased ticagrelor plasma concentrations, enhanced antiplatelet effects were observed during the grapefruit juice phase with both turbidimetric optical detection VerifyNow® and impedance aggregometry Multiplate® assays (Figure 3, Table 2). In the VerifyNow® P2Y₁₂ assay, the average platelet aggregation activity at 0–34 h after dosing, measured as P2Y₁₂ reaction units (PRU), was 101 (range 47–143) in the grapefruit juice phase and 151 (range 89–208) in the water phase ($P < 0.001$). The average inhibition of P2Y₁₂-mediated platelet aggregation was 59% (range 39–82%) and 42% (range 20–65%), respectively ($P < 0.001$). In the VerifyNow® assay, the peak of platelet inhibition at 4 h after 90 mg of ticagrelor was only modestly stronger in the grapefruit juice phase (12 PRU, i.e. 95%) than in the control phase (30 PRU, i.e. 89%, $P = 0.002$), but marked differences in platelet inhibition between the phases were observed thereafter (Figure 3). In 6.5 μM ADP-induced Multiplate®, the average area under the AU–time curve during the 0–24 h sampling interval was only 30 AU min (range 20–51 AU min) with grapefruit juice while it was 37 AU min (range 26–49 AU min) with water ($P = 0.006$; Table 2).

Platelet function was also assessed with the blood flow based PFA-100® analyzer, which reached a maximal effect at 4 h in both groups (>300 s closure time). In both study

Figure 1

Geometric mean (90% confidence interval) plasma concentrations of ticagrelor (A) and its metabolite C124910XX (B) in 10 healthy volunteers. The insets depict the same data on a semi-logarithmic scale. The volunteers ingested 200 ml of grapefruit juice or water three times a day for 4 days and a single 90 mg dose of ticagrelor on day 3. ● water phase; ○ GFJ phase

Table 1

Pharmacokinetic variables of ticagrelor and its metabolite C124910XX. Ten healthy volunteers ingested 200 ml of grapefruit juice or water three times a day for 4 days and a single 90 mg dose of ticagrelor on day 3

Variable	Water phase (control)	Grapefruit juice phase	Geometric mean ratio, GFJ : water, (95% CI)	P value
Ticagrelor				
C_{max} (ng ml ⁻¹)	497 (17)	818 (29)	1.65 (1.47, 1.84)	<0.001
t_{max} (h)	1.5 (1–3)	3 (1.5–3)		0.011
$t_{1/2}$ (h)	6.7 (14)	7.2 (18)	1.07 (1.01, 1.14)	0.036
AUC(0,34 h) (ng ml ⁻¹ h)	2745 (32)	5985 (37)	2.18 (1.98, 2.40)	<0.001
AUC(0,∞) (ng ml ⁻¹ h)	2826 (33)	6250 (40)	2.21 (2.00, 2.45)	<0.001
C124910XX				
C_{max} (CU)	137 (26)	75 (26)	0.55 (0.46, 0.65)	<0.001
t_{max} (h)	2 (1.5–3)	3 (3–4)		0.004
$t_{1/2}$ (h)	8.2 (15)	12 (36)	1.45 (1.24, 1.70)	0.001
AUC(0,34 h) (CU ml ⁻¹ h)	1194 (28)	1030 (20)	0.86 (0.76, 0.98)	0.027
AUC(0,∞) (CU ml ⁻¹ h)	1271 (30)	1232 (29)	0.97 (0.85, 1.10)	0.585
AUC(0,34 h) ratio (C124910XX : ticagrelor)	0.43 (29)	0.17 (37)	0.40 (0.33, 0.48)	<0.001

Data are given as geometric mean with geometric coefficient of variation (percentage), t_{max} as median with range. The ratios between the two phases are given with 95% CI. GFJ, grapefruit juice; CU, arbitrary concentration unit; CI, confidence interval; C_{max} , peak plasma concentration; t_{max} , time to C_{max} ; $t_{1/2}$, elimination half-life; AUC(0,34 h), area under the plasma concentration–time curve from 0 to 34 h; AUC(0,∞), area under the plasma concentration–time curve from time 0 to infinity.

Figure 2

The area under the plasma concentration–time curve (AUC(0,34 h) of ticagrelor (A), its active metabolite C124910XX (B) and the metabolite : ticagrelor AUC(0,34 h) ratio (C) in individual subjects following a single 90 mg dose of ticagrelor ingested in the water (control) (W) and the grapefruit juice (GFJ) phases

phases, the closure times exceeded the measuring range in most of the samples taken after ticagrelor dosing until the last time point (data not shown).

The relationship between the plasma concentration of ticagrelor and the inhibition of platelet aggregation in the optical aggregometry (VerifyNow® P2Y₁₂) test was similar during both the water and the grapefruit juice phase. The concentrations producing 50% inhibition of platelet aggregation (EC_{50}) were 44.9 ng ml⁻¹ and 51.6 ng ml⁻¹, respectively (Figure 4).

No signs of bleeding were observed in any of the subjects during the study.

Discussion

This study shows that regular intake of grapefruit juice markedly increases the plasma concentrations of ticagrelor and enhances its antiplatelet effects. Grapefruit juice increased the mean C_{max} of ticagrelor by about 70% and more than doubled its AUC. In addition, the C_{max} of the C124910XX metabolite of ticagrelor was decreased, while its AUC(0,∞) was unaffected. These changes were accompanied by a markedly enhanced and prolonged antiplatelet effect. The effect of grapefruit juice on ticagrelor concentrations was comparable with that of diltiazem

Figure 3

Platelet function after a single 90 mg dose of ticagrelor in the control (water) and grapefruit juice phases, as measured with optical aggregometry VerifyNow® (A) and impedance based ADP-included platelet aggregation Multiplate® assay (B). Results are given as mean $\pm$ SD platelet inhibition and mean $\pm$ SD area under the platelet aggregation units–time curve (AU min), respectively. ● water phase; ○ GFJ phase

Table 2

Antiplatelet effect of ticagrelor. Ten healthy volunteers ingested 200 ml of grapefruit juice or water three times a day for 4 days and a single 90-mg dose of ticagrelor on day 3

Variable	Water phase (control)	Grapefruit juice phase	Difference, GFJ – water, (95% CI)	P value
VerifyNow®				
PRU baseline	284 $\pm$ 29	267 $\pm$ 35	–17 (–38, 4)	0.102
PRU minimum	30 $\pm$ 23	12 $\pm$ 19	–18 (–27, –9)	0.002
PRU average	151 $\pm$ 40	101 $\pm$ 33	–50 (–60, –40)	<0.001
Platelet inhibition average (%)	42 $\pm$ 13	59 $\pm$ 14	18 (14, 22)	<0.001
Multiplate®, 6.5 μM ADP				
AU baseline	73 $\pm$ 15	74 $\pm$ 16	1 (–10, 12)	0.843
AU minimum	15 $\pm$ 5	13 $\pm$ 6	2 (–8, 3)	0.297
Platelet aggregation average (AU min)	37 $\pm$ 8	30 $\pm$ 10	–7 (–12, –3)	0.006
Multiplate®, 2.0 μM ADP				
AU baseline	56 $\pm$ 17	59 $\pm$ 16	3 (–6, 11)	0.484
AU minimum	13 $\pm$ 5	10 $\pm$ 5	3 (–8, 3)	0.299
Platelet aggregation average (AU min)	28 $\pm$ 8	21 $\pm$ 7	–7 (–13, –1)	0.027

Data are given as mean $\pm$ SD, the differences of the results between the two phases are given with mean (95% CI). GFJ, grapefruit juice phase; CI, confidence interval; PRU, P2Y₁₂ reaction units; AU, aggregation units.

(240 mg once daily) that increased ticagrelor $C_{\max}$ by 69% and AUC to 2.7-fold of control [9].

Grapefruit juice leads to strong inactivation of the CYP3A4 enzyme in the intestinal wall and has been shown to influence the pharmacokinetics of several drugs [13–17, 31–33]. In the present study, the $C_{\max}$ of C124910XX and the ratio of the AUC(0,34 h) of C124910XX to that of parent ticagrelor were markedly decreased by grapefruit juice. This implies that grapefruit juice reduced metabolite C124910XX production and indicates that inhibition of the first-pass metabolism of ticagrelor is the principal mecha-

nism of the observed interaction. Furthermore, given that the bioavailability of ticagrelor is about 36% in the absence of comedications, the 2.2-fold increase in ticagrelor AUC by grapefruit juice suggests that the first-pass metabolism of ticagrelor occurs mainly in the intestinal wall. There is also evidence for a weak effect of grapefruit juice on hepatic CYP3A4, as demonstrated by the effect of repeated doses of grapefruit juice on the $t_{1/2}$ of some drugs such as triazolam and oxycodone [32, 34]. Our data with slightly increased $t_{1/2}$ of ticagrelor are consistent with these observations.

Figure 4

Relationship between inhibition of platelet aggregation, as measured by VerifyNow® P2Y₁₂ test, and the individual plasma concentrations of ticagrelor. A sigmoidal $E_{\max}$ model (equation given in figure) was fitted to the data for both the water phase (●) and the grapefruit phase (○) using non-linear regression analysis. Water phase: $E_{\max} = 96.6\%$, $EC_{50} = 44.9 \text{ ng ml}^{-1}$, $\gamma = 1.2$; GFJ phase: $E_{\max} = 96.8\%$, $EC_{50} = 51.6 \text{ ng ml}^{-1}$, $\gamma = 1.5$

Importantly, the increase in ticagrelor plasma concentrations by grapefruit juice was shown to have functional consequences by markedly enhanced antiplatelet effects in whole blood. In both the optical and the impedance based ADP P2Y₁₂ receptor-specific platelet aggregation assays, grapefruit juice increased the average inhibition of platelet aggregation after ticagrelor dosing. In both assays, the 90 mg ticagrelor dose induced a near maximal inhibitory effect at 4 h post-dose, coinciding with the peak plasma concentration of ticagrelor in both study phases. However, grapefruit juice postponed the recovery of platelet reactivity, and pronounced differences between the study phases were observed in the VerifyNow® starting from 12 h after ticagrelor administration. Accordingly, when ticagrelor was taken with water, inhibition of platelet activity remained in the clinical target range (PRU < 235) [35] in all subjects until 12 h, but only in six subjects until the 24 h and 34 h time points (data not shown). In contrast, when ticagrelor was administered with grapefruit juice, inhibition of platelet activity remained in the target range in all subjects until 24 h, and in all subjects but one even up to 34 h. Despite substantial interindividual variability in the relationship between ticagrelor concentration and platelet response in VerifyNow®, the relationship was nearly identical in the control and grapefruit juice phases in the whole group (Figure 4) as well as in individual subjects (data not shown). Accordingly, as grapefruit juice considerably reduced the plasma concentrations of C124910XX during the first 4 h after ticagrelor ingestion, this active metabolite does not seem to modify significantly the concentration–effect relationship of ticagrelor in the present setting.

The single 90 mg dose of ticagrelor had a strong effect on all the three whole blood platelet aggregation assays around the time of ticagrelor peak concentration in plasma. The optical and impedance based ADP P2Y₁₂ receptor-specific platelet aggregation assays demonstrated individual variability in the antiplatelet effect of ticagrelor, as well as the effect of grapefruit juice, even during the first 24 h after ticagrelor administration. Thus, these two assays are suitable for monitoring the antiplatelet effect of ticagrelor after a single dose, and most likely also during multiple dosing in the clinical situation. On the other hand, the closure times in the blood flow based assay exceeded the measuring range until 34 h post-dose in most subjects, i.e. this assay was not able to show any individual variability in response, while it sensitively detected even the small interference of platelet function caused by ticagrelor at 34 h post-dose. Thus, this assay may be suitable for testing the recovery of platelet function, e.g. when invasive clinical interventions are planned after discontinuation of ticagrelor treatment.

It is remarkable that all the individuals obtained a near full inhibition of ADP-induced aggregation lasting for at least 12 h, but the platelet activity recovered individually. Based on present data, the rate of recovery is determined by both the individual plasma concentrations, i.e. the $t_{1/2}$ of ticagrelor, and by the individual concentration–effect relationship, which demonstrated about 10-fold interindividual variability even in this homogenous group of 10 healthy volunteers.

Ticagrelor is typically used as a regular, twice daily, long term medication. According to pharmacokinetic principles, the observed two to three-fold increase in the total AUC of ticagrelor after a single dose translates to an equal increase in its steady-state plasma concentrations during multiple dosing. Furthermore, based on the pharmacodynamic results, it can be extrapolated that when the typical 90 mg twice daily dosing of ticagrelor is used with regular consumption of grapefruit juice, a high proportion of the patients would have over 90% inhibition of the ADP-specific platelet function in the VerifyNow test throughout the 12 h dosing interval. The present data suggest that regular intake of grapefruit juice may predispose patients to drug-specific adverse effects if typical therapeutic doses of ticagrelor are used. It should be noted that interindividual variation in the extent of the grapefruit juice–ticagrelor interaction can be considerable. The greatest individual increase in ticagrelor exposure in our homogenous group of healthy young subjects was a more than three-fold increase in the AUC of ticagrelor. Age, gender, drug interactions and concomitant diseases can affect platelet function, as well as the pharmacokinetics and pharmacodynamics of ticagrelor [36]; these individual factors may thus modify the magnitude of the grapefruit juice–ticagrelor interaction and its clinical consequences in individual patients. A further caveat is that by increasing ticagrelor exposure, grapefruit juice can prolong the

recovery of platelets after ticagrelor dosing, which can be of significance when preparing for surgery [5], or during management of bleeding complications. The risk for major bleeding, while low, is common to all therapeutic platelet inhibitors, including ticagrelor [37, 38]. Ticagrelor has also other clinically significant adverse effects, such as dyspnoea and hyperuricaemia [39]. These adverse effects are occasionally seen with therapeutic doses, but our results suggest that the risk of such effects may increase if grapefruit juice or other strong CYP3A4 inhibitors are used concomitantly with ticagrelor.

In conclusion, grapefruit juice markedly increases the plasma concentrations and antiplatelet effect of ticagrelor, most likely by inhibition of the CYP3A4-mediated first pass metabolism of ticagrelor mainly at the intestinal level. The concomitant use of ticagrelor and grapefruit juice is best to be avoided.

Competing Interests

All authors have completed the Unified Competing Interest form of ICMJE (available on request from the corresponding author) and declare no support from any organization for the submitted work; RL has participated in an advisory board of AstraZeneca, no other financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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REFERENCES

- 1 Dorsam RT, Kunapuli SP. Central role of the P2Y₁₂ receptor in platelet activation. *J Clin Invest* 2004; 113: 340–5.
- 2 Abergel E, Nikolsky E. Ticagrelor: an investigational oral antiplatelet treatment for reduction of major adverse cardiac events in patients with acute coronary syndrome. *Vasc Health Risk Manag* 2010; 6: 963–77.
- 3 VAN Giezen JJ, Nilsson L, Berntsson P, Wissing BM, Giordanetto F, Tomlinson W, Greasley PJ. Ticagrelor binds to human P2Y₁₂ independently from ADP but antagonizes ADP-induced receptor signaling and platelet aggregation. *J Thromb Haemost* 2009; 7: 1556–65.
- 4 Husted S, Emanuelsson H, Heptinstall S, Sandset PM, Wickens M, Peters G. Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y₁₂ antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin. *Eur Heart J* 2006; 27: 1038–47.
- 5 Storey RF, Bliden KP, Ecob R, Karunakaran A, Butler K, Wei C, Tantry U, Gurbel PA. Earlier recovery of platelet function after discontinuation of treatment with ticagrelor compared with clopidogrel in patients with high antiplatelet responses. *J Thromb Haemost* 2011; 9: 1730–7.
- 6 Zhou D, Andersson TB, Grimm SW. *In vitro* evaluation of potential drug-drug interactions with ticagrelor: cytochrome P450 reaction phenotyping, inhibition, induction, and differential kinetics. *Drug Metab Dispos* 2011; 39: 703–10.
- 7 Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA, PLATO Investigators, Freij A, Thorsen M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2009; 361: 1045–57.
- 8 Teng R, Oliver S, Hayes MA, Butler K. Absorption, distribution, metabolism, and excretion of ticagrelor in healthy subjects. *Drug Metab Dispos* 2010; 38: 1514–21.
- 9 AstraZeneca. Brilinta Prescribing Information. 2011. Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022433s000lbl.pdf (last accessed 22 November 2012).
- 10 Ahonen J, Olkkola KT, Neuvonen PJ. Effect of route of administration of fluconazole on the interaction between fluconazole and midazolam. *Eur J Clin Pharmacol* 1997; 51: 415–9.
- 11 Quinney SK, Malireddy SR, Vuppalaanchi R, Hamman MA, Chalasani N, Gorski JC, Hall SD. Rate of onset of inhibition of gut-wall and hepatic CYP3A by clarithromycin. *Eur J Clin Pharmacol* 2012. DOI 10.1007/S.00228-012-1339-x.
- 12 Johannot L, Somerset SM. Age-related variations in flavonoid intake and sources in the Australian population. *Public Health Nutr* 2006; 9: 1045–54.
- 13 Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, Brown MB, Guo W, Watkins PB. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest* 1997; 99: 2545–53.
- 14 Bailey DG, Dresser GK, Kreeft JH, Munoz C, Freeman DJ, Bend JR. Grapefruit-felodipine interaction: effect of unprocessed fruit and probable active ingredients. *Clin Pharmacol Ther* 2000; 68: 468–77.
- 15 Bailey DG, Spence JD, Munoz C, Arnold JM. Interaction of citrus juices with felodipine and nifedipine. *Lancet* 1991; 337: 268–9.
- 16 Lilja JJ, Kivisto KT, Backman JT, Lamberg TS, Neuvonen PJ. Grapefruit juice substantially increases plasma concentrations of buspirone. *Clin Pharmacol Ther* 1998; 64: 655–60.
- 17 Lilja JJ, Kivisto KT, Neuvonen PJ. Grapefruit juice-simvastatin interaction: effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther* 1998; 64: 477–83.
- 18 Tapaninen T, Neuvonen PJ, Niemi M. Grapefruit juice greatly reduces the plasma concentrations of the OATP2B1 and CYP3A4 substrate aliskiren. *Clin Pharmacol Ther* 2010; 88: 339–42.

- 19 Greenblatt DJ. Analysis of drug interactions involving fruit beverages and organic anion-transporting polypeptides. *J Clin Pharmacol* 2009; 49: 1403–7.
- 20 Bailey DG. Fruit juice inhibition of uptake transport: a new type of food-drug interaction. *Br J Clin Pharmacol* 2010; 70: 645–55.
- 21 Ieiri I, Doi Y, Maeda K, Sasaki T, Kimura M, Hirota T, Chiyoda T, Miyagawa M, Irie S, Iwasaki K, Sugiyama Y. Microdosing clinical study: pharmacokinetic, Pharmacogenomic (SLCO2B1) and interaction (grapefruit juice) profiles of celirolol following the oral microdose and therapeutic dose. *J Clin Pharmacol* 2012; 52: 1078–89.
- 22 Lilja JJ, Backman JT, Laitila J, Luurila H, Neuvonen PJ. Itraconazole increases but grapefruit juice greatly decreases plasma concentrations of celirolol. *Clin Pharmacol Ther* 2003; 73: 192–8.
- 23 Satoh H, Yamashita F, Tsujimoto M, Murakami H, Koyabu N, Ohtani H, Sawada Y. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab Dispos* 2005; 33: 518–23.
- 24 Bailey DG, Dresser GK, Leake BF, Kim RB. Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. *Clin Pharmacol Ther* 2007; 81: 495–502.
- 25 Jeong YH, Bliden KP, Antonino MJ, Park KS, Tantry US, Gurbel PA. Usefulness of the VerifyNow P2Y12 assay to evaluate the antiplatelet effects of ticagrelor and clopidogrel therapies. *Am Heart J* 2012; 164: 35–42.
- 26 Beaudry F, Yves Le Blanc JC, Coutu M, Ramier I, Moreau JP, Brown NK. Metabolite profiling study of propranolol in rat using LC/MS/MS analysis. *Biomed Chromatogr* 1999; 13: 363–9.
- 27 Mistri HN, Jangid AG, Shrivastav P. Sensitive and rapid method to determine trimetazidine in human plasma by liquid chromatography/tandem mass spectrometry. *J AOAC Int* 2008; 91: 562–71.
- 28 Lordkipanidze M, Pharand C, Nguyen TA, Schampaert E, Diodati JG. Assessment of VerifyNow P2Y12 assay accuracy in evaluating clopidogrel-induced platelet inhibition. *Ther Drug Monit* 2008; 30: 372–8.
- 29 Velik-Salchner C, Maier S, Innerhofer P, Streif W, Klingler A, Kolbitsch C, Fries D. Point-of-care whole blood impedance aggregometry versus classical light transmission aggregometry for detecting aspirin and clopidogrel: the results of a pilot study. *Anesth Analg* 2008; 107: 1798–806.
- 30 Koessler J, Kobsar AL, Rajkovic MS, Schafer A, Flierl U, Pfoertsch S, Bauersachs J, Steigerwald U, Rechner AR, Walter U. The new INNOVANCE(R) PFA P2Y cartridge is sensitive to the detection of the P2Y(1)(2) receptor inhibition. *Platelets* 2011; 22: 19–25.
- 31 Lilja JJ, Kivisto KT, Neuvonen PJ. Grapefruit juice increases serum concentrations of atorvastatin and has no effect on pravastatin. *Clin Pharmacol Ther* 1999; 66: 118–27.
- 32 Lilja JJ, Kivisto KT, Backman JT, Neuvonen PJ. Effect of grapefruit juice dose on grapefruit juice-triazolam interaction: repeated consumption prolongs triazolam half-life. *Eur J Clin Pharmacol* 2000; 56: 411–5.
- 33 Libersa CC, Brique SA, Motte KB, Caron JF, Guedon-Moreau LM, Humbert L, Vincent A, Devos P, Lhermitte MA. Dramatic inhibition of amiodarone metabolism induced by grapefruit juice. *Br J Clin Pharmacol* 2000; 49: 373–8.
- 34 Nieminen TH, Hagelberg NM, Saari TI, Neuvonen M, Neuvonen PJ, Laine K, Olkkola KT. Grapefruit juice enhances the exposure to oral oxycodone. *Basic Clin Pharmacol Toxicol* 2010; 107: 782–8.
- 35 Price MJ, Endemann S, Gollapudi RR, Valencia R, Stinis CT, Levisay JP, Ernst A, Sawhney NS, Schatz RA, Teirstein PS. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. *Eur Heart J* 2008; 29: 992–1000.
- 36 Teng R, Mitchell P, Butler K. Effect of age and gender on pharmacokinetics and pharmacodynamics of a single ticagrelor dose in healthy individuals. *Eur J Clin Pharmacol* 2012; 68: 1175–82.
- 37 Becker RC, Bassand JP, Budaj A, Wojdyla DM, James SK, Cornel JH, French J, Held C, Horrow J, Husted S, Lopez-Sendon J, Lassila R, Mahaffey KW, Storey RF, Harrington RA, Wallentin L. Bleeding complications with the P2Y12 receptor antagonists clopidogrel and ticagrelor in the PLATElet inhibition and patient Outcomes (PLATO) trial. *Eur Heart J* 2011; 32: 2933–44.
- 38 Nijjer SS, Davies JE, Francis DP. Quantitative comparison of clopidogrel 600mg, prasugrel and ticagrelor, against clopidogrel 300mg on major adverse cardiovascular events and bleeding in coronary stenting: Synthesis of CURRENT-OASIS-7, TRITON-TIMI-38 and PLATO. *Int J Cardiol* 2012; 158: 181–5.
- 39 Storey RF, Becker RC, Harrington RA, Husted S, James SK, Cools F, Steg PG, Khurmi NS, Emanuelsson H, Cooper A, Cairns R, Cannon CP, Wallentin L. Characterization of dyspnoea in PLATO study patients treated with ticagrelor or clopidogrel and its association with clinical outcomes. *Eur Heart J* 2011; 32: 2945–53.