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CME

Pharmacodynamic Effects of Cangrelor on Platelet $P2Y_{12}$ Receptor–Mediated Signaling in Prasugrel-Treated Patients

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CME Objective for This Article: At the completion of this article, the learner should be able to: 1) describe how the vasodilator-stimulated phosphoprotein (VASP) assay is used to determine the platelet reactivity index; 2) assess the in vitro $P2Y_{12}$ receptor inhibitory effects of cangrelor on platelets obtained from patients who are on maintenance prasugrel therapy; and 3) assess the in vitro $P2Y_{12}$ receptor inhibitory effects of prasugrel reloading doses on platelets obtained from patients who are on maintenance prasugrel therapy.

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Pharmacodynamic Effects of Cangrelor on Platelet $P2Y_{12}$ Receptor–Mediated Signaling in Prasugrel-Treated Patients

Objectives The purpose of this study was to assess the in vitro $P2Y_{12}$ receptor inhibitory effects of cangrelor on platelets from patients on maintenance prasugrel therapy treated with 2 reloading dose regimens.

Background Despite its more potent and rapid antiplatelet effects compared with clopidogrel, recent studies have shown variability in prasugrel-mediated $P2Y_{12}$ receptor inhibition, particularly in high-risk settings. Cangrelor is a potent intravenous $P2Y_{12}$ receptor inhibitor.

Methods A total of 60 patients with coronary artery disease on maintenance prasugrel (10 mg/day) therapy were randomized to a 30- or 60-mg reload of prasugrel. The platelet reactivity index (PRI), as assessed by whole-blood vasodilator-stimulated phosphoprotein, was measured with and without in vitro incubation of cangrelor (500 nM) at baseline, and at 1 and 4 h after reload.

Results In the absence of cangrelor, prasugrel reloading reduced PRI ($p < 0.001$ for both doses), although a 60-mg reload had greater platelet inhibition compared with a 30-mg reload at 4 h ($p = 0.001$). Cangrelor was associated with a reduction in PRI values during the overall study time course in patients reloaded with 30 mg ($p = 0.001$) and 60 mg ($p < 0.001$) of prasugrel. In patients reloaded with 30 mg prasugrel, cangrelor decreased PRI at each time point (baseline, $p < 0.001$; 1 h, $p = 0.013$; 4 h, $p = 0.001$). In patients reloaded with 60 mg prasugrel, cangrelor decreased PRI at baseline ($p < 0.001$) and 1 h ($p = 0.002$); levels of platelet reactivity comparable to those achieved with cangrelor were observed only at 4 h ($p = 0.325$). The intergroup comparisons with cangrelor were not significant at any time point.

Conclusions In patients on maintenance prasugrel therapy exposed to a reloading dose (30 or 60 mg) of prasugrel, in vitro cangrelor is associated with further platelet $P2Y_{12}$ receptor inhibitory effects. (J Am Coll Cardiol Intv 2014;7:426–34)

Prasugrel is a potent oral adenosine diphosphate (ADP) $P2Y_{12}$ receptor inhibitor approved for clinical use in patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) (1–3). Despite the more rapid and potent levels of platelet inhibition achieved compared with clopidogrel, pharmacodynamic (PD) studies have shown interindividual variability in prasugrel effects (4–8). Importantly, prasugrel-treated patients with high on-treatment platelet reactivity (HPR) are at increased risk of ischemic events (5). Delayed antiplatelet effects and high rates of HPR have been shown particularly in the early hours after prasugrel administration in patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary PCI (9,10). Similar PD findings also occur with ticagrelor (9–11). In patients on maintenance prasugrel therapy, prasugrel reloading is associated with enhanced platelet inhibition and reduced rates of HPR (7,12). However, several hours are required to achieve maximal antiplatelet effects (7,12). Ultimately, the use of orally-administered antiplatelet agents may be challenging in patients unable to swallow (e.g., patients who are sedated, intubated, or in shock, or those with nausea or vomiting). Overall, these observations support the need for intravenous antiplatelet therapies able to yield more prompt and potent platelet inhibitory effects, which are unlikely to be achieved with oral medications (8).

Cangrelor, a nonthienopyridine adenosine triphosphate analogue, is a potent intravenous direct-acting and reversible $P2Y_{12}$ receptor antagonist that has been shown to reduce ischemic complications, including stent thrombosis, in $P2Y_{12}$ inhibitor–naïve patients undergoing PCI (13,14). Previous investigations have reported that in vitro exposure of cangrelor on platelets from clopidogrel-treated patients is associated with potent $P2Y_{12}$ inhibitory effects (15–17). Whether cangrelor can exert rapid and further platelet inhibitory effects on platelets from patients treated with prasugrel is unknown. The aim of this PD investigation was to assess the in vitro effects of cangrelor on platelets from patients with coronary artery disease on maintenance prasugrel therapy treated with 2 reloading dose regimens.

Squibb, sanofi-aventis, GlaxoSmithKline, Otsuka, Eli Lilly, Daiichi Sankyo, The Medicines Company, AstraZeneca, Evolva, and Gilead; and has received an Esther and King Biomedical Research Grant. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Methods

Study population and research design. This was a prospective, randomized PD study conducted in 60 patients on maintenance dual antiplatelet therapy with aspirin and prasugrel. All patients had a clinical indication to be on prasugrel as they all underwent PCI with an ACS (1–3). Patients were clinically stable at time of study entry. Patients were screened at the outpatient clinic of the Division of Cardiology of the University of Florida College of Medicine–UF Health Jacksonville. All patients were considered eligible for the study if they were between 18 and 74 years of age and were receiving treatment with aspirin (81 mg/day) and prasugrel (10 mg/day) for at least 14 days as part of their standard of care dual antiplatelet treatment regimen. Exclusion criteria for this study included active bleeding, previous cerebrovascular event, body weight <60 kg, 75 years of age or older, clinical instability after the index event, use of oral anticoagulation, platelet count <100 × 10^6/μl, hemoglobin <10 g/dl, creatinine >2 mg/dl; hepatic enzymes >2.5 times the upper limit of normal; and pregnant and lactating females were also excluded.

Using a computer-based randomization system, patients were randomized in a 1:1 fashion to either a 30- or 60-mg reload of prasugrel. Blood samples were collected at 3 time points: at baseline (while on maintenance prasugrel therapy), at 1 h and at 4 h after prasugrel reload. Blood sampling for PD analysis were collected by the antecubital vein in sodium citrate (0.105 *M*)-containing tubes using a 19-gauge needle. The first few milliliters of blood sampled were discarded to avoid spontaneous platelet activation. Baseline blood samples were collected 24 ± 4 h after the last maintenance dose of prasugrel in order to assess trough levels of platelet reactivity. PD assessments were performed with and without in vitro cangrelor (500 nM cangrelor) at each time point. Tubes were immediately incubated at 37°C in a water bath, and cangrelor was added to the whole blood and incubated for 5 min (17). The concentration of cangrelor used in vitro was chosen in line with previous investigations (15–17). This concentration approximates that of the mean steady-state plasma concentration of 484 nmol/l at the infusion dose of 4 μg/kg/min, which corresponds to the dose used in large-scale phase III clinical trial investigations (13,14,18,19). A flow diagram of the study design is shown in Figure 1. The study complied with the Declaration of Helsinki, and was approved by the Institutional Review Board of the University of Florida College of Medicine-Jacksonville, and all patients gave their informed written consent.

Abbreviations and Acronyms

ACS = acute coronary syndrome
ADP = adenosine diphosphate
ANCOVA = analysis of covariance
ANOVA = analysis of variance
HPR = high on-treatment platelet reactivity
MFI = mean fluorescence intensity
PCI = percutaneous coronary intervention
PD = pharmacodynamic
PGE1 = prostaglandin E1
PRI = platelet reactivity index
STEMI = ST-segment elevation myocardial infarction
VASP = whole-blood vasodilator-stimulated phosphoprotein
VASP-P = whole-blood vasodilator-stimulated phosphorylation

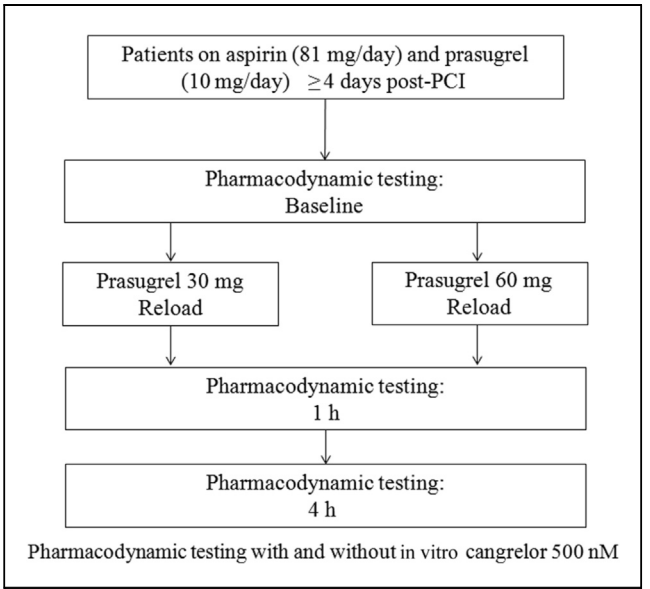

Figure 1. Study Design

PCI = percutaneous coronary intervention.

Whole-blood vasodilator-stimulated phosphoprotein. The whole-blood vasodilator-stimulated phosphoprotein (VASP) assay was used to determine the platelet reactivity index (PRI) according to standard protocols (12,16). VASP was performed before and after in vitro incubation with 500 nM cangrelor at each time point. In brief, whole-blood vasodilator-stimulated phosphoprotein phosphorylation (VASP-P) was measured by quantitative flow cytometry using commercially-available labeled monoclonal antibodies (Biocytex Inc., Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels after challenge with prostaglandin E1 (PGE1) and PGE1 + ADP. PGE1 increases VASP-P levels through stimulation of adenylate cyclase. ADP binding to purinergic receptors leads to inhibition of adenylate cyclase; thus, the addition of ADP to PGE1-stimulated platelets reduces levels of PGE1-induced VASP-P. The PRI was calculated as follows: [(MFI PGE1) − (MFI PGE1+ADP)/(MFI PGE1)] × 100%. A reduced PRI is indicative of greater inhibition of the $P2Y_{12}$ signaling pathway (12,16). Absolute levels as well as absolute changes or delta (defined as the difference with and without cangrelor) of PRI, were calculated.

Sample size calculation and study endpoints. The primary endpoint of the study was the comparison of PRI before and

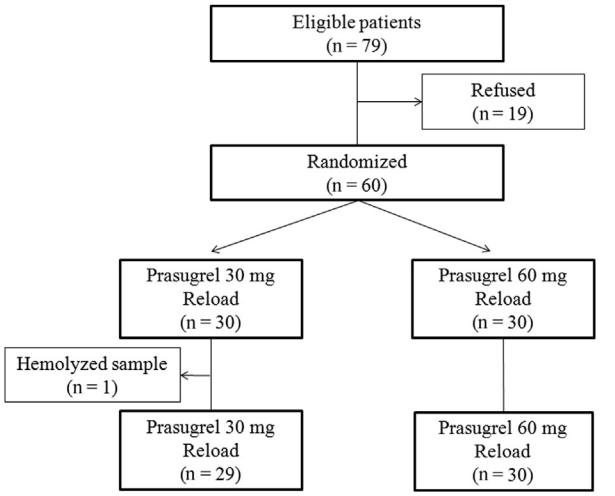

Figure 2. Subject Disposition

after in vitro incubation with cangrelor at baseline, while patients were on maintenance prasugrel 10 mg/day. Assuming an SD of 20% and a dropout rate of ~10%, we would be able to detect a difference of 10% in baseline PRI values before and after in vitro incubation with cangrelor with 60 patients, with a 95% power and a 2-tailed alpha value of 0.05. A cutoff of 10% absolute change in PRI was chosen as this has been associated with a 44% relative reduction of thrombotic events in patients undergoing PCI (20). Other endpoints included intergroup comparisons (prasugrel 30 mg vs. 60 mg) of PRI in the presence and absence of cangrelor during the overall study time course and at each time point; intragroup comparisons (prasugrel 30 mg vs. prasugrel 30 mg plus cangrelor; prasugrel 60 mg vs. prasugrel 60 mg plus cangrelor) of PRI during the overall study time course and at each time point; and intergroup comparisons of the absolute changes (delta) in PRI levels (before and after in vitro incubation with cangrelor) for each group during the overall study time course and at each time point.

Statistical analysis. Conformity to the normal distribution was evaluated for continuous variables with the Kolmogorov-Smirnov test. For baseline characteristics, continuous variables are expressed as mean ± SD, and categorical variables are expressed as frequencies and percentages. The chi-square or Fisher exact test (if the expected value in any cell was <5) was used to compare categorical variables between the 2 groups. A repeated-measures analysis of variance (ANOVA) model was used to evaluate intragroup comparisons and the overall difference between groups, using Bonferroni approach to correct for multiple comparisons. An analysis of covariance (ANCOVA) method with a general linear model, using the baseline value of platelet reactivity as a covariate, was used to evaluate all between-group comparisons. A 2-tailed p value <0.05 was considered to indicate a statistically significant difference for all of the analyses performed. Results are reported as least-square mean ± SE for the previously detailed analyses. Statistical analysis was performed using SPSS version 21.0 (SPSS Inc., Chicago, Illinois).

Table 1. Baseline Characteristics

Variable	Prasugrel 30 mg (N = 30)	Prasugrel 60 mg (N = 30)	p Value
Age, yrs	54.9 ± 9.4	56.3 ± 8.7	0.563
Male	23 (76.7)	23 (76.7)	>0.999
BMI, kg/m^2	33.6 ± 9	32.1 ± 6.3	0.476
Race			0.326
Caucasian	24 (80)	27 (90)	
African American	5 (16.7)	2 (6.7)	
Hispanic	0	1 (3.3)	
Asian	1 (3.3)	0	
Hypertension	28 (93.3)	25 (83.3)	0.424
Dyslipidemia	28 (93.3)	27 (90)	>0.999
Smoking	9 (30)	9 (30)	>0.999
Diabetes mellitus	6 (20)	9 (30)	0.371
Previous MI	26 (86.7)	25 (83.3)	>0.999
Previous CABG	0	5 (16.7)	0.052
Medications			
Beta-blockers	27 (90)	26 (86.7)	>0.999
ACEI/ARB	26 (86.7)	29 (96.7)	0.353
Statins	30 (100)	28 (93.3)	0.269
PPI	5 (16.7)	7 (23.3)	0.684
CCB	4 (13.3)	7 (23.3)	0.506
Nitrates	7 (23.3)	11 (36.7)	0.260
LVEF >50%	20 (66.7)	24 (80)	0.511
Creatinine clearance, ml/min	114.6 ± 52	122.4 ± 38	0.524
Hematocrit, %	42.1 ± 3.2	40.8 ± 4.3	0.233
Platelet count, 1,000/mm^3	223.3 ± 48.6	223.1 ± 52.7	0.986

Values are mean ± SD or n (%).

ACEI/ARB = angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; BMI = body mass index; CABG = coronary artery bypass graft surgery; CCB = calcium channel blockers; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PCI = percutaneous coronary intervention; PPI = proton pump inhibitor.

Results

Patient population. Between July 2011 and March 2013, a total of 79 patients meeting study inclusion criteria were identified. Of these, 19 declined to participate; therefore, a total of 60 patients on maintenance prasugrel 10 mg/day therapy were randomly assigned to a reload with either 30 mg (n = 30) or 60 mg (n = 30) of prasugrel. One patient in the 30-mg reload group had all samples hemolyzed and was thus not available for analysis. Patient disposition is summarized in Figure 2. Baseline characteristics of the study population are summarized in Table 1. There were no differences in baseline characteristics between groups (Table 1).

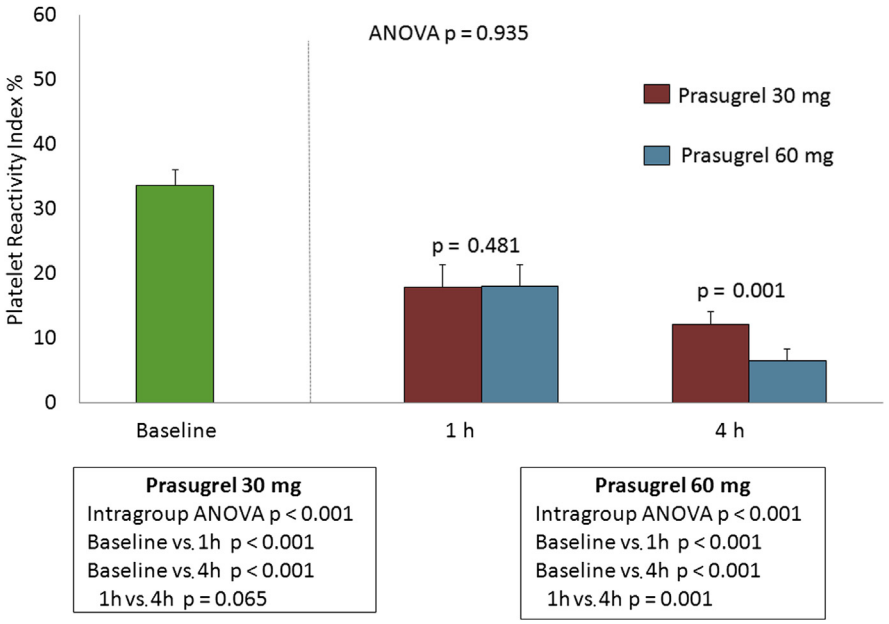

Figure 3. Comparison of Platelet Reactivity Values in a 30- and 60-mg Prasugrel Reloading Dose in the Absence of Cangrelor

Platelet reactivity index expressed as percentages measured by whole-blood vasodilator-stimulated phosphoprotein in the absence of cangrelor before and after reload with 30 or 60 mg prasugrel. Data are presented as least square means. **Error bars** indicate SE. The baseline histogram represents aggregate data of the 2 groups. Analysis of variance (ANOVA) p value refers to the overall difference in platelet reactivity index between the 30- and 60-mg reloading doses. Analysis of covariance (ANCOVA) p values are provided for intergroup comparisons at each time point. Intragroup comparisons are specified at the **bottom of the figure**. ANOVA p value indicates the overall intragroup difference across time points.

PD effects of prasugrel reloading without cangrelor. PRI values were similar between groups at baseline ($p = 0.378$). Moreover, in the intergroup analysis comparing a 30- and 60-mg prasugrel reload, PRI values were not significantly reduced during the overall time course between the 2 reloading dose regimens (ANOVA, $p = 0.935$). PRI at 4 h after a 60-mg reload was markedly reduced compared with a 30-mg reload (ANCOVA, $p = 0.001$), but not at 1 h (ANCOVA, $p = 0.481$) (Fig. 3). In the intragroup analysis, prasugrel reloading significantly reduced PRI levels after 1 and 4 h with both 30- and 60-mg reloading doses ($p < 0.001$ for both doses by ANOVA). There was greater platelet inhibition between 1 and 4 h in the 60-mg group, but not in the 30-mg group (Fig. 3).

In vitro PD effects of cangrelor after a 30-mg prasugrel reload. In patients receiving a 30-mg reload of prasugrel, in vitro cangrelor was associated with enhanced additive $P2Y_{12}$ receptor inhibition. In particular, PRI values were significantly reduced during the overall time course (ANOVA, $p = 0.001$) and at each time point compared with PRI values without cangrelor (baseline, $p < 0.001$; 1 h, $p = 0.013$; 4 h, $p = 0.001$) (Fig. 4A). Moreover, intragroup comparisons showed cangrelor to be associated with further inhibition in addition to that with prasugrel across time points (ANOVA, $p < 0.001$), with a significant difference in PRI levels between baseline and 1 h ($p = 0.002$) and between baseline and 4 h ($p < 0.001$); there was a nonsignificant reduction in PRI between 1 and 4 h ($p = 0.401$) (Fig. 4A).

In vitro PD effects of cangrelor after a 60-mg prasugrel reload. In patients receiving a 60-mg reload of prasugrel, in vitro incubation with cangrelor was associated with a significant difference in PRI values during the overall study time course (ANOVA, $p < 0.001$). Cangrelor markedly reduced PRI levels at baseline ($p < 0.001$) and at 1 h ($p = 0.002$) after a 60-mg prasugrel reload. However, 4 h after a 60-mg prasugrel reload, cangrelor led to a nonsignificant reduction in PRI ($p = 0.325$) (Fig. 4B). Intragroup comparisons showed cangrelor to be associated with enhanced platelet inhibitory effects across time points (ANOVA, $p = 0.001$) and a further significant reduction in PRI between baseline and 4 h ($p = 0.005$) as well as between 1 and 4 h ($p = 0.016$); there was a nonsignificant reduction in PRI between baseline and 1 h ($p = 0.124$) (Fig. 4B).

In vitro PD effects of cangrelor in patients reloaded with prasugrel 30 mg versus 60 mg. After in vitro incubation with cangrelor, PRI values were similar between the 30- and 60-mg prasugrel reload groups at baseline. In the intergroup analysis, absolute PRI levels (ANOVA, $p = 0.373$) (Fig. 5A) and absolute changes (delta) in PRI (ANOVA, $p = 0.290$) (Fig. 5B) were not significantly reduced between

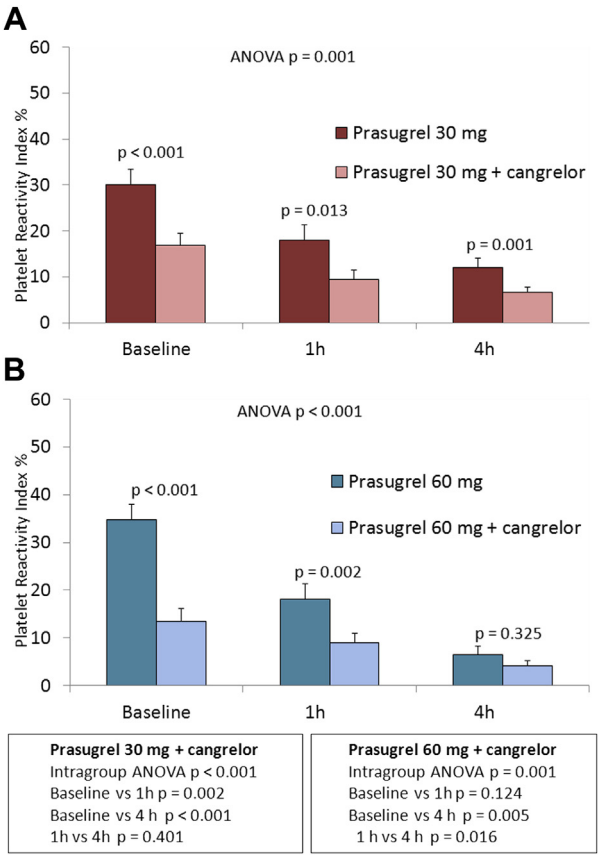

Figure 4. Platelet Reactivity Values Across Time Points After a 30- and 60-mg Prasugrel Reloading Dose in the Presence and Absence of Cangrelor

(A) Comparison of platelet reactivity index (PRI) expressed as percentages (%) measured by whole-blood vasodilator-stimulated phosphoprotein across time points in the 30-mg prasugrel reload group in the presence and absence of cangrelor. **(B)** Comparison of PRI expressed as percentages measured by whole-blood vasodilator-stimulated phosphoprotein across time points in the 60-mg prasugrel group in the presence and absence of cangrelor. Data are presented as least square means. **Error bars** indicate SE. p values are provided for the intergroup comparisons. Analysis of variance (ANOVA) p value refers to the overall difference in PRI with and without cangrelor across time points in **A** and **B**. Intragroup comparisons are specified at the **bottom** of the figure.

the 2 groups during the overall time course. Similarly, there were no significant differences at any individual time point (Figs. 5A and 5B).

Discussion

The platelet $P2Y_{12}$ receptor is key in mediating platelet activation and aggregation processes (8,21–24). The present PD investigation was performed to elucidate the in vitro effects of cangrelor on $P2Y_{12}$-mediated signaling in patients treated with prasugrel maintenance therapy after reloading with either a 30- or 60-mg dose. Our PD investigation showed the following: 1) in vitro cangrelor is associated with enhanced platelet inhibition when added to platelets from patients on prasugrel maintenance therapy as well as when exposed to a reloading dose; 2) platelet inhibitory effects of in vitro cangrelor are immediate and faster than prasugrel reloading alone; and 3) levels of platelet reactivity to comparable those achieved with in vitro cangrelor were observed only 4 h after a 60-mg prasugrel reloading dose.

Adjunctive therapy with a platelet $P2Y_{12}$ receptor inhibitor is key for reduction of thrombotic risk in patients with ACS and undergoing PCI (1–3). Currently, only oral $P2Y_{12}$ receptor inhibitors are available for clinical use (8). Clopidogrel is the most widely used $P2Y_{12}$ receptor inhibitor and is characterized by nonuniform PD effects, a phenomenon associated with an increased risk of adverse outcomes (23–26). Prasugrel and ticagrelor are newer-generation $P2Y_{12}$ receptor inhibitors that are both characterized by a more rapid onset of action, greater potency, and more uniform PD effects compared with clopidogrel (8,27,28). These PD properties may explain the enhanced ischemic benefit, albeit at the expense of increased bleeding, with these agents compared with clopidogrel in ACS patients (27,28). However, despite these important advancements in oral $P2Y_{12}$ receptor–inhibiting strategies, prasugrel and ticagrelor have also recently both been found to have variability in PD response with the potential for worse clinical outcomes (4,5,7,9–11). Moreover, prasugrel and ticagrelor have consistently been shown to have a delayed onset of action in patients with STEMI undergoing primary PCI, requiring at least 2 h to exert their full antiplatelet effects and thus exposing these high-risk patients to an increased risk of thrombotic complications (9,10). Increasing the loading-dose regimen of these oral agents has been advocated, although the limited data thus far have not shown meaningful changes in antiplatelet effects (29,30). Inability to achieve adequate platelet inhibition with oral $P2Y_{12}$ receptor inhibitors is also a concern in other common clinical settings, such as patients unable to swallow oral medications (i.e., patients who are sedated, intubated, in shock, or those with nausea or vomiting). Furthermore, patients in certain high-risk settings such as STEMI and therapeutic hypothermia may also have impaired intestinal absorption and hepatic metabolism that can limit the pharmacological efficacy of oral $P2Y_{12}$ receptor inhibitors and increase the risk of stent thrombosis (31–33). These findings underscore the need for an intravenous agent with prompt, potent, and predictable antiplatelet properties.

Currently, glycoprotein IIb/IIIa inhibitors are the only antiplatelet agents clinically available for intravenous use and indeed have been shown to be advantageous in patients requiring immediate and potent platelet inhibition (34). Recently, Valgimigli et al. (35) showed that, in STEMI patients undergoing primary PCI, 60 mg prasugrel was associated with suboptimal platelet inhibition for at least 2 h

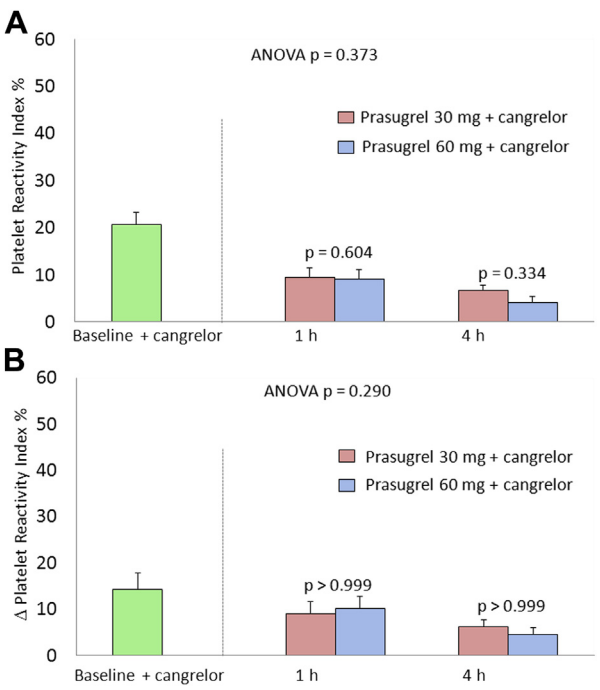

Figure 5. Comparison of Platelet Reactivity Values Between 30- and 60-mg Prasugrel Reloading Doses in the Presence of Cangrelor

(A) Comparison of platelet reactivity index (PRI) expressed as percentages measured by whole-blood vasodilator-stimulated phosphoprotein across time points between 30- and 60-mg prasugrel reloading dose regimens in the presence of cangrelor. The ANOVA p value refers to the overall difference in PRI between the 2 reloading doses. Analysis of covariance (ANCOVA) p values are provided for intergroup comparisons at each time point. **(B)** Absolute change or delta (Δ) between PRI values in the absence and presence of cangrelor in each arm (30- and 60-mg prasugrel reload) is provided. ANOVA p value refers to the overall difference between groups across time points. The baseline histogram represents aggregate data of the 2 groups. Data are presented as least square means. **Error bars** indicate SE. p values are provided for intergroup comparisons.

after dosing, whereas adding a bolus only of tirofiban not only obviated the need for an infusion but also led to nearly complete elimination of residual variability of platelet inhibition. However, glycoprotein IIb/IIIa inhibitor use is in decline, given the high risk of bleeding complications and the introduction of alternative antithrombotic treatment regimens that have been shown to have a more favorable safety profile, without any trade-off in clinical efficacy (34). Nevertheless, periprocedural thrombotic complications still remain a concern, highlighting the need for effective platelet-inhibiting strategies (8).

Cangrelor is a potent intravenous, direct-acting (no metabolism required) $P2Y_{12}$ receptor inhibitor with an immediate onset of action (36). Moreover, cangrelor has an ultra-short half-life (3 to 6 min), leading to a very rapid offset of action with return to baseline platelet function within 30 to 60 min (36). These PD properties may represent a potential treatment option in patients undergoing surgery and who cannot discontinue $P2Y_{12}$ inhibiting therapy (37,38). Further, cangrelor may represent an attractive treatment option in PCI patients in whom immediate and potent $P2Y_{12}$ receptor blockade is required. Although, 2 phase III clinical trials failed to meet the primary efficacy endpoint, likely attributed to the definition of the study endpoints, the primary efficacy endpoint was met in the recently reported CHAMPION PHOENIX (Effect of Platelet Inhibition with Cangrelor during PCI on Ischemic Events) trial (13,18,19). In this trial, conducted in 11,145 clopidogrel-naïve patients undergoing PCI, cangrelor significantly reduced the rate of ischemic events at 48 h, including stent thrombosis, with no significant increase in severe bleeding, irrespective of clinical presentation (stable angina, unstable angina/non-STEMI, STEMI) (13).

Concerns have emerged regarding the potential for drug interactions with oral $P2Y_{12}$ receptor inhibitors when using cangrelor. In an ex vivo PD investigation conducted in healthy volunteers, it was shown that if patients were administered clopidogrel during a cangrelor infusion, clopidogrel would be ineffective, given that its active metabolite would not bind with the $P2Y_{12}$ receptor while occupied by cangrelor (39). Clopidogrel PD was not affected when administered after cangrelor infusion, given its very rapid offset of action. Similarly, in vitro pre-incubation of blood with cangrelor before addition of active metabolites of clopidogrel or prasugrel reduced their ability to inhibit platelet aggregation (40). In contrast, cangrelor added after pre-incubation with the active metabolites of clopidogrel or prasugrel led to sustained platelet inhibition. Therefore, phase III clinical investigations reinforced that clopidogrel (the only oral $P2Y_{12}$ inhibitor used in these trials) should be administered only at the end of cangrelor infusion to be effective (13,18,19). The optimal transition strategy from cangrelor to prasugrel or ticagrelor is currently under investigation (Clinical Trial numbers NCT01852019 and NCT01766466).

There is limited experience with understanding the effects of cangrelor when added to platelets already exposed to an oral $P2Y_{12}$ receptor inhibitor. Previous investigations have shown that the addition of therapeutic concentrations of cangrelor in vitro before and after clopidogrel administration enhances $P2Y_{12}$ inhibitory effects (15–17). The novelty of our investigation is that it expands our understanding of the in vitro PD effects of cangrelor by assessing patients on prasugrel therapy. In vitro addition of cangrelor provides a significant reduction in PRI before and after prasugrel reloading in patients already on maintenance prasugrel therapy, indicating that not all $P2Y_{12}$ receptors have been occupied by binding with the active metabolite of orally-administered prasugrel. These findings suggest that even though prasugrel is a potent $P2Y_{12}$ receptor inhibitor, it is not able to rapidly inhibit platelet reactivity to

the degree observed with cangrelor. However, it should also be noted that after in vitro cangrelor, PRI levels at baseline, while patients were on maintenance prasugrel therapy, were not as low as those observed 1 and 4 h after prasugrel reload. This finding is indicative of an additive effect over time with a prasugrel reload. It may be argued, however, that previous in vitro studies with cangrelor conducted in stable cardiovascular patients treated with clopidogrel showed lower levels of platelet reactivity (16). Nevertheless, our study population comprised patients who had experienced an ACS and thus were more likely to have a hyper-reactive platelet phenotype and who were exposed to reloading doses of a more potent antiplatelet agent (i.e., prasugrel), which can explain the changes in PRI over the course of the study. Indeed, higher in vitro concentrations of cangrelor (10 μM), as used by Aleil et al. (16), could have led to more potent inhibition of PRI, even at baseline. However, such a high concentration of cangrelor would not be reflective of that which will be potentially used in clinical practice and likely increase the risk of bleeding complications (23).

Although a 60-mg reload is able to achieve more potent antiplatelet effects than a 30-mg reload, confirming a previous investigation (12), levels of platelet reactivity comparable to those achieved with cangrelor are achieved only 4 h after a 60-mg prasugrel reload in patients already on maintenance therapy. Indeed, although a reloading dose regimen of prasugrel led to significant changes in platelet reactivity as soon as 1 h in this investigation, clinical studies have shown that in higher-risk settings, such as STEMI, this may require longer time frames (9,10,35). Immediate platelet inhibition is desired at the time of coronary intervention. Our study showed that cangrelor was associated with an ~15% further absolute reduction in PRI in patients on maintenance prasugrel therapy, which was consistent over time, irrespective of the prasugrel loading-dose regimen. Of note, for each 10% absolute reduction in PRI, a 44% relative reduction in thrombotic events in patients undergoing PCI has been shown (20). Whether cangrelor can exert similar effects in ticagrelor-treated patients remains to be established.

Study limitations. The main limitation of the present investigation is derived from its very design, since in vitro conditions make the results of this study exploratory, and ex vivo PD studies are warranted to confirm these findings. Moreover, although this study was conducted in patients who experienced an ACS, platelet reactivity was assessed remotely from their ACS presentation. Thus, the PD findings with a combination of prasugrel and cangrelor warrant confirmation in the setting of ACS patients undergoing PCI. Indeed, the forthcoming HORIZONS AMI II study (Harmonizing Outcomes With Revascularization and Stents in Acute Myocardial Infarction) trial, in which cangrelor will be used in a large cohort of patients with STEMI undergoing primary PCI treated with a novel generation $P2Y_{12}$ inhibitor, will be pivotal for a better understanding of these effects. Further, platelet reactivity was assessed only with VASP-PRI. Indeed, adding additional PD assays would have corroborated our study findings, although VASP-PRI is most specific to assess $P2Y_{12}$ receptor–mediated signaling (16,24). Further, although previous investigations have identified variability in response to prasugrel (4–8), the sample size of our study did not allow us to explore contributors to this phenomenon.

Conclusions

In patients on maintenance prasugrel therapy exposed to a reloading dose regimen (30 or 60 mg) of prasugrel, in vitro cangrelor is associated with further platelet $P2Y_{12}$ receptor inhibitory effects as assessed by VASP-PRI. Levels of platelet reactivity comparable to those achieved with cangrelor were observed 4 h after a 60-mg prasugrel reload, making cangrelor an attractive strategy if more prompt antiplatelet effects are required. Ex vivo studies in patients undergoing PCI are warranted to confirm these in vitro findings.

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Key Words: cangrelor ■ platelet reactivity ■ prasugrel.

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