Pharmacodynamic Comparison of Prasugrel Versus Ticagrelor in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease

The OPTIMUS (Optimizing Antiplatelet Therapy in Diabetes Mellitus)-4 Study

BACKGROUND: Patients with diabetes mellitus (DM) are at increased risk of atherothrombotic events, underscoring the importance of effective platelet inhibiting therapies. Prasugrel and ticagrelor reduce thrombotic complications to a greater extent than clopidogrel. Subgroup analyses of pivotal clinical trials testing prasugrel and ticagrelor versus clopidogrel showed DM patients to have benefits that were consistent with the overall trial populations, although the magnitude of the ischemic risk reduction appeared to be enhanced with prasugrel. Whether these findings may be attributed to differences in the pharmacodynamic profiles of these drugs in DM patients remains poorly explored and represented the aim of this study.

METHODS: In this prospective, randomized, double-blind, double-dummy, crossover pharmacodynamic study, aspirin-treated DM patients (n=50) with coronary artery disease were randomly assigned to receive prasugrel (60 mg loading dose [LD]/10 mg maintenance dose once daily) or ticagrelor (180 mg LD/90 mg maintenance dose twice daily) for 1 week. Pharmacodynamic assessments were conducted using 4 different assays, including VerifyNow P2Y12, vasodilator-stimulated phosphoprotein, light transmittance aggregometry, and Multiplate, which allowed us to explore ADP- and non–ADP-induced (arachidonic acid-, collagen-, thrombin receptor-activating, peptide-induced) platelet signaling pathways. The acute (baseline, 30 minutes, and 2 hours post-LD) and maintenance (1 week) effects of therapy were assessed. The primary end point of the study was the comparison of P2Y12 reaction units determined by VerifyNow P2Y12 at 1 week between prasugrel and ticagrelor.

RESULTS: ADP- and non–ADP-induced measures of platelet reactivity reduced significantly with both prasugrel and ticagrelor LD and maintenance dose. P2Y_{12} reaction units defined by VerifyNow were similar between prasugrel and ticagrelor at 30 minutes and 2 hours post-LD. At 1 week, P2Y_{12} reaction units were significantly lower with ticagrelor than with prasugrel (52 [32–72] versus 83 [63–103]; least-square means difference: −31; 95% confidence interval, −57 to −4; P=0.022; primary end point). Pharmacodynamic assessments measured by vasodilator-stimulated phosphoprotein, light transmittance aggregometry, and Multiplate were similar between prasugrel and ticagrelor at each time point, including at 1 week. Rates of high on-treatment platelet reactivity were similar between groups with all assays at all time points.

CONCLUSIONS: In DM patients with coronary artery disease, ticagrelor exerts similar or greater inhibition of ADP-induced platelet reactivity in comparison with prasugrel in the acute and chronic phases of treatment, whereas the inhibition of measures of non–ADP-induced platelet reactivity was not significantly different between the 2 agents.

Prasugrel and ticagrelor are the 2 latest-generation P2Y12 receptor antagonists with important pharmacological advantages compared with clopidogrel. In particular, they are associated with enhanced platelet inhibition and greater reduction in atherothrombotic recurrences, albeit at the expense of increased bleeding, in patients with acute coronary syndrome. Subgroup analyses of pivotal clinical trials testing prasugrel and ticagrelor versus clopidogrel showed DM patients to have benefits that were consistent with the overall trial populations, although the magnitude of the ischemic risk reduction appeared to be heightened with prasugrel. Because the ischemic benefits of prasugrel and ticagrelor are mainly attributed to their enhanced platelet inhibitory effects, it has been suggested that these observations may be attributable to the PD differences between these 2 agents in DM patients. However, the comparative PD effectiveness of prasugrel and ticagrelor in the loading and maintenance phases of treatment in patients with DM remains poorly explored and represented the aim of this investigation.

METHODS

Study Design and Patient Population
The OPTIMUS-4 study (Optimizing Antiplatelet Therapy in Diabetes Mellitus-4) was a prospective, randomized, double-blind, double-dummy, crossover study aimed to compare the PD effects of both loading dose (LD) and maintenance dose (MD) regimens of prasugrel versus ticagrelor among DM patients with CAD on a background of aspirin therapy (NCT01852214). Patients were screened at the outpatient cardiology clinics of University of Florida Health-Jacksonville and were considered eligible for the study if they met all of the following inclusion criteria: (1) age between 18 and 74 years; (2) type 2 DM on treatment with oral hypoglycemic agents or insulin; (3) angiographically documented CAD (>50% stenosis in a major epicardial coronary vessel); and (4) on maintenance treatment with low-dose aspirin (81 mg once daily) for at least 30 days as per standard of care. DM status was defined according to American Diabetes Association criteria. Patients were excluded if any of the following criteria were present: (1) history of stroke, transient ischemic attack, or intracranial bleeding; (2) known allergies to prasugrel or ticagrelor; (3) weight <60 kg; (4) on treatment with a P2Y12 receptor antagonist or an oral anticoagulant in the previous 30 days; (5) clinical indication to be on treatment with a P2Y12 receptor antagonist or an oral anticoagulant in the previous 30 days; (6) hemoglobin A1c ≥ 10% within 3 months; (7) blood dyscrasia or bleeding diathesis; (8) active bleeding; (9) platelet count <80×103/mL; (10) hemoglobin <10g/dL; (11) hemodynamic instability; (12) creatinine clearance <30 mL/min; (13) hepatic dysfunction (baseline alanine aminotransferase >2.5 times the upper limit of normal); (14) sick sinus syndrome or atrioventricular block without a pacemaker; (15) treatment with drugs interfering with cytochrome P450 3A4 metabolism; and (16) pregnant or lactating females. The study complied with the Declaration of Helsinki, was approved by...
With the use of a computer-based randomization system, patients were assigned randomly (1:1) to receive either prasugrel or ticagrelor. Patients randomly assigned to prasugrel were treated with a 60 mg LD followed by 10 mg once daily MD (plus placebo-ticagrelor tablets twice daily); the MD of prasugrel (or placebo-prasugrel tablets) was initiated 24 hours after the LD. Patients randomly assigned to ticagrelor were treated with an 180 mg LD followed by 90 mg twice daily MD (plus placebo-prasugrel tablets only); the MD of ticagrelor (or placebo-ticagrelor) was started 12 hours after the LD. Randomized treatment was maintained for 1 week (7±2 days). After completion of the 1-week treatment period, patients discontinued the study medications for 2 to 4 weeks (wash-out period) and then crossed over to the alternate treatment, which was administered for 1 week. Aspirin 81 mg once daily was maintained throughout the study.

Investigators, laboratory personnel, and patients were blinded to treatment assignments. Prasugrel, ticagrelor, and placebo tablets were encapsulated and distributed by our institutional pharmacy to guarantee the blind. In particular, patients randomly assigned to prasugrel received a LD with six 10-mg prasugrel tablets plus 2 placebo-ticagrelor tablets followed by MD with prasugrel 10 mg once daily plus placebo-ticagrelor twice daily. Patients randomly assigned to ticagrelor received a LD with two 90-mg ticagrelor tablets plus 6 placebo-prasugrel tablets followed by MD with ticagrelor 90 mg twice daily plus placebo-prasugrel once daily. Compliance to treatment was assessed by pill count and patient interview. Blood sampling for PD testing was performed at a total of 8 time points using 4 different assays as described below. A flow diagram of the study is represented in Figure 1.

**Blood Sampling and PD Testing**

Blood sampling for PD testing was performed at 4 time points for each study sequence (total of 8 time points): baseline (before randomization), 30 minutes, and 2 hours after LD (to assess the acute PD effects associated with the LD), and after 1 week (7±2 days) of randomized MD treatment (to assess the chronic PD effects associated with the MD). To ensure measurement of trough levels of platelet reactivity, the 1-week blood sample was collected 12 hours after the last MD of ticagrelor or placebo-ticagrelor, and 24 hours after the last dose of prasugrel or placebo-prasugrel and aspirin.

PD testing was performed using 4 different assays: (1) VerifyNow P2Y12 point-of-care testing (VN-P2Y12); (2) whole blood vasodilator-stimulated phosphoprotein (VASP); (3) light transmittance aggregometry (LTA); (4) multiple electrode aggregometry (MEA). In brief, the VN-P2Y12 assay (Accriva) measures platelet-induced aggregation as an increase in light transmittance and reports results in P2Y12 reaction units (PRUs). VASP was measured by quantitative flow cytometry measures platelet-induced aggregation as an increase in light transmittance. MEA was assessed in whole blood by the Multiplate analyzer (Dynabyte Medical), as described previously. This instrument can perform up to 5 parallel aggregometry measurements assessing the change in impedance caused by the adhesion of platelets onto sensor units formed by silver-covered electrodes. Agonists were selected to explore ADP- and non–ADP thromboxane A2, collagen, and thrombin-mediated pathways of platelet aggregation. These included ADP 6.4 μmol/L, with and without prostaglandin E1 9.4 mmol/L, arachidonic acid 0.5 mmol/L, collagen 3.2 μg/mL, and thrombin–receptor–activating peptide 32 μmol/L. The mean values of 2 independent determinations are expressed as the area under the curve of the aggregation tracing and quantified by an area under the curve of arbitrary units (AUs × minute).

In line with expert consensus, high on-treatment platelet reactivity (HPR) was defined as follows: PRU >208 (VN-P2Y12), PRI >50% (VASP), MPA >59% (LTA 20 μmol/L ADP), MPA >46% (LTA 5 μmol/L ADP), or area under the curve >460 (MEA – ADP). Because LTA results with 5 μmol/L ADP were consistent with 20 μmol/L ADP, these are reported in the online-only Data Supplement Figure I.

**Sample Size Calculation and Study End Points**

The primary end point of the study was the comparison of PRU determined by VN-P2Y12 at 1 week between prasugrel and ticagrelor. Because there were no preliminary data available at the time of study design, we chose an arbitrary sample of 50 patients. Assuming a common standard deviation of 20 PRU and a =10% rate of invalid results because of hemolysis or drop-out, this sample would allow us to detect an absolute reduction of 10 PRU with prasugrel in comparison with ticagrelor after 1 week of randomized treatment with a 90% power and 2-sided α=0.05. This approach is in line with the recommendation for pilot investigations. A 10 PRU absolute difference was chosen as a reference value because, in a large meta-analysis, a 4% increase in cardiac events occurred for every 10-U increase in PRU. Additional assessments included PD comparisons at all time points using all assays, and comparisons of HPR rates. Adverse events, including ischemic and bleeding events, during the study period were recorded. Bleeding events were classified according to the Bleeding Academic Research Consortium definition.

**Statistical Analysis**

For baseline characteristics, continuous variables are expressed as mean±standard deviation, and categorical variables are expressed as frequencies and percentages. Conformity to the normal distribution was evaluated for continuous variables with the Kolmogorov-Smirnov test. Treatment effects were evaluated comparing the functional parameters observed in the overall patient population after prasugrel treatment with those achieved after ticagrelor regardless of the sequence. All statistical comparisons of platelet function for the primary end point and secondary end points with continuous variables were conducted using a linear mixed-effect model with treatment group, sequence, period, and treatment-group-by-period as fixed effects, patient as a random effect, and baseline value of the corresponding platelet function test as a covariate. Exploratory analyses were conducted to compare platelet reactivity levels as percent change in light transmittance.
between prasugrel and ticagrelor in each period, measured as PRU and PRI, using an analysis of variance method with a general linear model. The comparisons of rates of HPR were conducted using the McNemar test. A 2-tailed \( P \) value of <0.05 was considered to indicate a statistically significant difference for all the analyses performed. Platelet reactivity results are reported as least-square means (LSM) (95% confidence interval [CI]) for the above detailed analyses. Statistical analysis was performed using SPSS version 22.0 software (SPSS Inc.).

All analyses of platelet function and HPR were conducted on the PD population, which was defined as all randomly assigned subjects who received study drug, successfully completed at least 1 treatment period of the study, and had valid data for the primary end point (PRU at 1 week). Safety analyses were conducted on the safety population, which included all patients exposed to at least 1 dose of the study drug. The corresponding author had full access to all the data in the study and assumes responsibility for the accuracy and completeness of the data and all the analyses, and for the fidelity of this report to the trial protocol, as well.

**RESULTS**

**Patient Population**

Between February 2013 and July 2015, a total of 61 DM patients with CAD on aspirin treatment agreed to participate in the study; 11 patients were excluded, and thus a total of 50 patients were assigned randomly (prasugrel, n=26; ticagrelor, n=24). The randomized cohort was exposed to at least 1 dose of study medication and represented the safety population. Overall, 4 patients withdrew from the study before the completion of the first treatment period: 1 initially randomly assigned to prasugrel (dyspnea, n=1) and 3 initially randomly assigned to ticagrelor (dyspnea, n=2; withdrawal of consent, n=1). Thus, a total of 46 patients met criteria to be included in the PD population. Demographic and baseline characteristics of the PD population are summarized in the Table. No ischemic or the Bleeding Academic Research Consortium type 2 to 5 bleeding events were observed in the safety population during the overall study time course. Two patients (prasugrel, n=1; ticagrelor, n=1) had Bleeding Academic Research Consortium type 1 bleeding. Dyspnea was reported in 9 (18%) patients treated with ticagrelor and in 3 (6%) treated with prasugrel. Dyspnea led to study drug discontinuation in 3 patients treated with ticagrelor and 1 patient treated with prasugrel. Patient disposition is summarized in Figure 2.

**Pharmacodynamic Findings**

**ADP-Induced Platelet Reactivity**

At baseline, while on aspirin therapy, platelet reactivity was not significantly different between groups with all 4 assays. After administration of the LD, ADP-induced platelet reactivity decreased over time in both groups and with all assays (\( P < 0.001 \) for all assays). In intragroup comparisons, the reduction in platelet reactivity for both prasugrel and ticagrelor was evident as early as at 30 minutes, reaching statistical significance for some but not all assays (VN-P2Y12 [prasugrel, \( P = 0.393 \); ticagrelor, \( P = 0.055 \)]; VASP [prasugrel, \( P = 0.006 \); ticagrelor, \( P = 0.064 \)]; LTA [prasugrel, \( P = 0.001 \); ticagrelor, \( P = 0.041 \)]; MEA ADP [prasugrel, \( P = 0.002 \); ticagrelor, \( P = 0.010 \)]), and was sustained at 2 hours (\( P < 0.001 \) for all assays) up to 1-week (\( P < 0.001 \) for all assays). In the intergroup comparisons platelet reactivity measured by VN-P2Y12 (Figure 3A) was not significantly different between prasugrel and ticagrelor at 30 minutes (\( P = 0.222 \)) and was numerically lower in ticagrelor-treated patients at 2 hours post-LD (LSM difference, –22; 95% CI, –48 to 3; \( P = 0.086 \)). The primary end point of PRU defined by VN-P2Y12 after 1 week of MD treatment showed significantly lower levels with ticagrelor in comparison with prasugrel (52 [32–72] versus 83 [63–103]; LSM difference, –31; 95% CI, –57 to –4; \( P = 0.022 \)). There was no treatment-group-by-period interaction (\( P = 0.944 \)). PRI measured by VASP was not significantly different between prasugrel and ti-
MPA measured by LTA with 20 μmol/L ADP (Figure 4A) and platelet aggregation measured by MEA with ADP stimuli (Figure 4B) and with ADP plus prostaglandin E₁ (Figure II in the online-only Data Supplement) showed results similar to those defined by VASP. An exploratory analysis comparing PRU levels between prasugrel and ticagrelor in each period showed no significant differences in PRU values between prasugrel and ticagrelor at each time point of each period, with the exception of 1-week PRU in period 2 that was significantly lower in patients receiving ticagrelor (LSM difference, −47; 95% CI, −83 to −12; P=0.010; Figure 5A). The exploratory analysis comparing PRI levels between prasugrel and ticagrelor in each period showed no significant differences in platelet reactivity at each time point of each period (Figure 5B).

Rates of HPR markedly reduced over time with both prasugrel and ticagrelor, with no significant differences between the 2 agents at each time point and with all assays (Figure 6A through 6D). After 1 week of MD treatment, HPR assessed by VN-P2Y₁₂ was present in only 1 patient receiving prasugrel (2%) and 2 patients receiving ticagrelor (5%). Similar rates were shown with LTA with 20 μmol/L ADP (prasugrel, 0%; ticagrelor, 7%). HPR rates at 1 week were higher when assessed by VASP (prasugrel, 20%; ticagrelor, 19%) and MEA (prasugrel, 18%; ticagrelor, 19%).

Non–ADP-Induced Platelet Reactivity
Platelet aggregation induced by arachidonic acid reduced over time after prasugrel and ticagrelor administration. Compared with baseline (on aspirin monotherapy) arachidonic acid–induced aggregation was numerically reduced at 30 minutes and significantly reduced at 2 hours post-LD with both prasugrel and ticagrelor (P<0.001 for both), which was sustained after 1 week of MD treatment (prasugrel, P=0.023; ticagrelor, P=0.047). Thrombin receptor-activating peptide–induced aggregation was similar at 30 minutes but was significantly reduced at 2 hours after an LD of prasugrel or ticagrelor (P<0.001 for both) in comparison with baseline, and the effect was maintained at 1 week (P<0.001 for prasugrel, P=0.002 for ticagrelor). Collagen-induced platelet aggregation was numerically reduced at 30 minutes and significantly reduced at 2 hours after an LD of prasugrel or ticagrelor (P<0.001 for both) with all agonists (Figure 7A through 7C).

**DISCUSSION**

The present study is the first prospective, randomized, double-blind, double-dummy, crossover PD investigation comparing prasugrel versus ticagrelor during both the acute and maintenance phases of treatment, specifically conducted in patients with DM. The following findings...
were observed: (1) prasugrel or ticagrelor promptly (within 2 hours) achieved potent platelet inhibitory effects without significant PD differences between the prasugrel 60 mg and ticagrelor 180 mg LD regimens; (2) potent platelet inhibitory effects persisted after 1 week with both 10 mg once daily prasugrel and 90 mg twice daily ticagrelor MD during the maintenance phase of treatment, with ticagrelor achieving significantly lower levels of platelet reactivity when measured by PRU levels using VN-P2Y12 (primary end point); (3) there were no significant differences in platelet inhibitory effect of 10 mg once daily prasugrel and 90 mg twice daily ticagrelor MD during the maintenance phase of treatment as measured by VASP, LTA, and MEA; (4) HPR rates with prasugrel and ticagrelor were similar and markedly low as early as 2 hours after the LD and up to 1 week of MD treatment; (5) prasugrel and ticagrelor are associated with inhibitory effects on measures of non–ADP-induced, including thromboxane-, collagen-, and thrombin-, platelet reactivity.

DM is a pandemic currently affecting >150 million people worldwide and estimates suggest that this population will double during the next 20 years. Atherosclerotic macrovascular disease, including CAD, stroke, and peripheral arterial disease, accounts for the majority of morbidity and mortality associated with DM. Moreover, DM is a key determinant of recurrent cardiovascular events in patients with acute coronary syndrome or those undergoing percutaneous coronary intervention. Multiple factors, such as hyperglycemia, oxidative stress, endothelial dysfunction, platelet dysfunction, and abnormal coagulation factors, contribute to the prothrombotic milieu that characterizes DM. Importantly, increased platelet reactivity may account for inadequate response (ie, reduced platelet inhibitory effects) to oral antiplatelet agents, including the P2Y12 receptor antagonist clopidogrel, used for the prevention of ischemic events. Studies have consistently shown reduced antiplatelet effect of clopidogrel among DM patients in comparison with non-DM patients, which is mainly attributable to impaired pharmacokinetics, characterized by lower active metabolite levels, and only modestly attributed to an upregulation of the P2Y12 pathway. Prasugrel and ticagrelor have more favorable pharmacological profiles than clopidogrel, which translates into more prompt, potent, and predictable platelet inhibition. Subgroup analysis of patients with DM in the TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction 38) showed a reduction in ischemic events of prasugrel in comparison with clopidogrel (12.2% versus 17.0%; hazard ratio, 0.70; 95% CI, 0.58–0.85; P < 0.001), which was consistent with the overall trial findings (P interaction = 0.09), with no differences in major bleeding. Subgroup analysis of patients with DM in the PLATO trial (Study of Platelet Inhibition and Patient Outcomes) also showed that, in comparison with clopidogrel, ticagrelor reduced ischemic events (14.1% versus 16.2%; hazard ratio, 0.88; 95% CI, 0.76–1.03) consistently with the overall trial results (P interaction = 0.49), without differences in major bleeding.
ever, although the absolute risk reduction in ischemic events with ticagrelor was higher in DM patients than in non-DM patients (DM, 2.1%; non-DM, 1.8%), the magnitude of such ischemic benefit, which resulted in a 12% and 17% relative risk reductions in DM and non-DM patients, respectively, was not as marked as that observed with prasugrel.8,9 In fact, prasugrel therapy had notably high absolute reductions in ischemic events among DM patients in comparison with non-DM patients (DM, 4.8%; non-DM, 1.4%), which led to a 30% and 14% relative risk reductions in DM and non-DM patients, respectively.8,9 However, it is important to emphasize that such comparisons need to be interpreted with great caution given the broad differences between these studies. Nevertheless, these observations have questioned whether prasugrel and ticagrelor have different PD profiles in DM patients. However, comparative PD assessments between these 2 agents, selectively and comprehensively determined in DM patients to rule out potential differences, remained poorly explored until this investigation. Although DM patients have reduced generation of active metabolite and lower platelet inhibition than non-DM patients even when treated with prasugrel,7 the OPTIMUS-3 study showed that standard-dose prasugrel was able to achieve greater platelet inhibition than a double dose of clopidogrel in DM patients.15 Although no investigation has compared the PD effects of clopidogrel and ticagrelor specifically in patients with DM, a subgroup analysis of a randomized study showed that the superior PD efficacy of ticagrelor was consistent in DM patients.25 Indeed, our PD findings do not support a particular benefit in DM patients for prasugrel in comparison with ticagrelor.

Several studies have evaluated the comparative PD effectiveness of prasugrel and ticagrelor in different clini-
cal settings. Results were not consistent across studies, with some studies showing PD equipoise between the 2 agents and others showing more potent effects with ticagrelor. Nonetheless, data in patients with DM, who are characterized by a unique prothrombotic milieu, are still limited. A study by Laine et al assessing the acute effects of ticagrelor versus prasugrel in DM patients showed that ticagrelor was associated with lower PRI levels measured by VASP (6–18 hours after LD). A study by Alexopoulos et al assessing the chronic effects of these agents in DM patients showed that ticagrelor was associated with lower PRU levels measured by VN-P2Y12 (2–4 hours after MD). However, both studies did not show any differences in HPR rates. Indeed, our study results showing that prasugrel and ticagrelor reach overall nonsignificantly different levels of platelet inhibition in both the acute and chronic phases of treatment did not completely confirm these findings. This may be attributed to some methodological differences of these studies, such as the use of a single PD assay, the lack of double blinding, and the lack of evaluation of both the acute and maintenance phases of therapy. In our investigation, 4 different PD assays were used and performed at several time points to evaluate the PD effects of both the LD and MD regimens. Although our VN-P2Y12 data confirmed a difference in 1-week platelet reactivity and a trend toward lower PRU at 2 hours with ticagrelor, this may be attributable to assay-specific issues. In fact, no signal of reduced platelet reactivity with ticagrelor was shown with any of the 3 other ass-
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says, including LTA, which still represents the gold standard to measure platelet aggregation, or VASP, which is the most specific technique to evaluate P2Y12 receptor blockade, is not affected by aspirin, and does not take into account P2Y1 receptor activity. Moreover, given the pharmacological differences between these agents, with prasugrel an irreversible agent with once daily administration and ticagrelor a reversible agent with twice daily administration, in our study, PD assessments were conducted at very specific time points with relationship to timing of drug intake to enable an accurate comparative assessment.

The P2Y12 receptor signaling pathway plays a central role in atherothrombotic processes by amplifying platelet activation mediated by other receptors. This may explain why in vitro studies have shown that prasugrel and ticagrelor are effective in inhibiting markers of ADP- and non-ADP-induced platelet reactivity. In particular, the level of platelet inhibition achieved by combining aspirin and a potent P2Y12 receptor antagonist is no greater than that produced by the P2Y12 receptor antagonist alone. Our study confirms these in vitro findings by demonstrating in vivo that potent P2Y12 receptor blockade with both prasugrel and ticagrelor is also associated with inhibitory effects on measures of non-ADP-induced platelet reactivity. In particular, our study further expands on previous observations showing that, in DM patients, the addition of either prasugrel or ticagrelor on top of aspirin is able to significantly reduce thromboxane A2–mediated platelet reactivity, as assessed by assays sensitive to aspirin-induced effects (arachidonic acid- and collagen-induced aggregation), and platelet reactivity mediated by thrombin, as well, in both the acute and maintenance phases of treatment. These novel in vivo findings can indeed contribute to the enhanced antiplatelet efficacy of these agents, which may be noteworthy in patients with DM, given their upregulation of various platelet signaling pathways. In fact, recent data from the PEGASUS-TIMI 54 trial (Prevention of Cardiovascular Events in Patients with Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin–Thrombolysis In Myocardial Infarction 54), a secondary prevention study conducted in patients who experienced a prior (1–3 years) myocardial infarction, showed that prolonged treatment with ticagrelor, on a background of aspirin therapy, was associated with a reduction in mortality in patients with DM, albeit at the expense of increased bleeding. Although the PD substudy of this trial showed no effect of ticagrelor on the measurements of aspirin response, only 28% of patients had DM. Additional in-

Figure 6. Rates of high on-treatment platelet reactivity across time points after prasugrel and ticagrelor administration.

High on-treatment platelet reactivity was defined as P2Y12 reaction units (PRU) >208 (A), platelet reactivity index (PRI) >50% (B), maximal platelet aggregation (MPA) >59% (C), and area under the curve (AUC) >460 (D). Histograms represent rates. There are no significant differences between prasugrel and ticagrelor (P>0.10 for all comparisons).
sights on the clinical implications of ticagrelor therapy in DM patients will derive from the ongoing THEMIS study (Effect of Ticagrelor on Health Outcomes in Diabetes Mellitus Patients Intervention Study; NCT01991795), which is being conducted selectively in DM patients with documented CAD but without myocardial infarction. Ultimately, the PD findings from our study, along with previous investigations, support the central role of the P2Y12 receptor on amplifying platelet reactivity mediated by other signaling pathways by showing platelet inhibitory effects on various measures of platelet reactivity, including ADP- and non–ADP-induced. Such more comprehensive platelet inhibitory effect has questioned the need for long-term aspirin therapy in patients undergoing percutaneous coronary intervention when treated with more potent P2Y12 receptor blockade. Dropping aspirin therapy as a strategy to reduce bleeding complications without any trade-off in efficacy among patients treated with potent oral P2Y12 receptor inhibitors is currently being tested in several clinical trials, including in patients undergoing percutaneous coronary intervention (NCT02270242, NCT01813435).

Study Limitations
Our study was conducted using standard dosing regimens of prasugrel (60 mg LD/10 mg MD) and ticagrelor (180 mg LD and 90 mg twice daily MD) in patients with stable CAD, and it is unknown if our observations can be generalized to patients experiencing an acute coronary event. However, it would have been unethical to conduct a crossover study with a wash-out period in these patients who require dual-antiplatelet therapy. Moreover, our results cannot be extrapolated to lower dosing regimens of prasugrel (ie, 5 mg) and ticagrelor (ie, 60 mg). In our study, the PD effect of the LD was assessed 2

Figure 7. Non–ADP-induced platelet reactivity.
Comparisons of platelet reactivity across time points between prasugrel and ticagrelor measured by multiple electrode aggregometry (MEA) after stimuli with arachidonic acid (AA) (A), thrombin receptor–activating peptide (TRAP) (B), and collagen (COLL) (C). Data are presented as individual values. Solid lines with error bars indicate least-square means (95% confidence interval).
hours postdosing. Indeed, additional time points after LD would have allowed us to better define timing of peak platelet inhibitory effects. Our in vivo findings on the impact of more potent P2Y$_{12}$ receptor blockade on measures of non–ADP-induced platelet reactivity cannot be extrapolated to non-DM patients who do not have heightened platelet reactivity, which would perhaps not allow to unravel such a treatment effect. Moreover, markers of non–ADP-induced platelet reactivity were measured only by a single assay. Ultimately, our study was not powered for safety or efficacy; thus, no conclusions on the clinical comparisons between prasugrel and ticagrelor can be drawn, which is currently being investigated in an ongoing clinical trial.34

CONCLUSIONS
In DM patients with CAD, ticagrelor exerts similar or greater inhibition of ADP-induced platelet reactivity than prasugrel in the acute and chronic phases of treatment, whereas markers of non–ADP-induced platelet reactivity were not significantly different between the 2 agents. Such similar PD profiles translate into similarly low rates of HPR, a marker of risk for atherothrombotic recurrences. Results of ongoing clinical trials will provide further details on the role of potent P2Y$_{12}$ receptor inhibition in DM patients with CAD.

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REFERENCES


Supplemental Material
Supplemental Figure 1. Platelet reactivity measured by light transmittance aggregometry (LTA) following stimuli with ADP 5 µM. Comparisons of maximal platelet aggregation (MPA %) across time points between prasugrel and ticagrelor. The dashed line indicates threshold for high platelet reactivity. Data are presented as individual values. Solid lines with error bars indicate least-square means (95% confidence interval).

Supplemental Figure 2. Platelet reactivity measured by multiple electrode aggregometry (MEA) following stimuli with ADP and PGE₁. Comparisons of area under the curve (AUC) of the aggregation tracing across time points between prasugrel and ticagrelor. Data are presented as individual values. Solid lines with error bars indicate least-square means (95% confidence interval).
Welcome to Circulation on the Run. Your weekly podcast summary and backstage pass to the journal and its editors. I'm Dr. Carolyn Lam, Associate Editor from The National Heart Center and Duke National University of Singapore. Have you wondered which anti-platelet agent you should use in your patients with diabetes and coronary artery disease? Well, our feature paper deals with just this topic, so stay tuned, I'll be writing back with its author and associate editor. First, here's your summary of this week's journal: The first paper unravels novel peptides involved in atrial extracellular matrix remodelling in atrial fibrillation. This is work from first author Dr. Barallobre-Barreiro, corresponding author Dr Mayr from King's College London, and colleagues. They used novel mass spectrometry methods to analyze extracellular matrix in human atrial appendages from patients undergoing coronary artery bypass surgery.

Now, previous proteomic studies have examined the cellular proteome, but this is the first study to comprehensively characterize extracellular matrix proteins in human cardiac tissues, including the identification of glycosylation sites. They found extensive cleavage in the protein core of decorin which is a small leucine-rich proteoglycan that regulates collagen fibrillogenesis and a variety of other extracellular matrix cell signalling molecules. Decorin processing differed between human ventricles and atria and was altered in disease. It's C-terminus which is important for the interaction with connective tissue growth factor was predominantly detected in ventricles compared to atria. In contrast, atrial tissues from patients in persistent atrial fibrillation had higher levels of full length decorin, but also harbored a unique cleavage site that was not found in atrial appendages from patients in sinus rhythm. This unique cleavage site preceded the M-terminal domain of decorin and altered the binding capacity for myostatin, this altering muscle growth.

The cleaved decorin peptide antagonized myostatin, such that myostatin expression was decreased in atrial appendages of patients with persistent atrial fibrillation and in hearts of decorin-null mice. Furthermore, a synthetic peptide corresponding to this decorin region, those dependently inhibited the response to myostatin in cardiomyocytes and in perfused mouse hearts. This is clinically important because myostatin inhibition has been implicated as a substrate for atrial fibrillation. This study therefore provides first evidence that peptides generated from the cleavage of extracellular matrix proteins such as decorin, constitutes a local regulatory mechanism for growth factors in human cardiac tissue.

The next study looked at therapeutic hypothermia in patients with out of hospital cardiac arrest, and questioned if it may be most effective when induced early during cardiopulmonary resuscitation or CPR, in contrast to prior trials that looked at therapeutic hypothermia induced only after return of spontaneous circulation and hospital admission. This is the RINSE trial from Professor Bernard and colleagues from Ambulance Victoria Australia, which was a multi center randomized controlled trial which assigned adults with out of hospital cardiac arrest undergoing CPR to either a rapid intravenous infusion of up to two liters of cold saline, or standard care. The primary outcome measure was survival at hospital discharge. Secondary end points included return of spontaneous circulation.
The trial was unfortunately closed early at forty-eight percent of the recruitment target, due to changes in temperature management protocols at the major receiving hospitals. Still, a total of one thousand, one hundred and ninety-eight patients were randomized. Six hundred and eighteen to therapeutic hypothermia during CPR, and five hundred and eighty to standard pre-hospital care. Overall there was no difference in outcomes at discharge. In patients with an initial shockable cardiac rhythm there was lower rate of return of spontaneous circulation in patients who received cold saline compared with standard care. Thus, although this trial was stopped early, the data suggests that induction of mild therapeutic hypothermia using a rapid infusion of large volume intravenous cold saline during CPR did not affect outcomes at hospital discharge and may in fact cause harm in the subset of out of hospital cardiac arrest patients who present with shockable rhythm.

The last study provides the first generalizable risk score for sudden cardiac death among American adults from the general population without a history of cardiovascular disease. This large study from Dr. Deo of University of Pennsylvania, and colleagues, derived a sudden cardiac death prediction model using the Atherosclerosis Risk in Communities or ARIC cohort, and validated it in the Cardiovascular Health Study or CHS cohort. They found that the twelve independent risk factors in the ARIC study included age, male sex, African American race, current smoking, systolic blood pressure, use of [anti-hypotensive 00:06:00] medication, diabetes, serum potassium, serum albumin, HDI, estimated GFR, and QTc interval. Over a ten year follow up period this model combining these risk factors showed good to excellent discrimination for sudden cardiac death risk. In fact the model slightly outperformed that of the 2013 ACC AHA pooled cohort risk equations.

Finally, they also showed in the echocardiographic sub-cohort that a left ventricular ejection fraction less than fifty percent was present in only 1.1 percent of these participants and did not enhance sudden cardiac death prediction. This study importantly contributes to the distinguishing of sudden cardiac death risk across the general population, and the results can help target future strategies aimed at sudden cardiac death prevention for the highest risk subgroups in the American general population. That does it for the summaries. Now for our feature paper.

For our feature paper today we are discussing the super important issue of anti-platelet therapy in type 2 diabetes with coronary artery disease. Joining me today are the corresponding author, Dr. Dominick Angiolillo from the University of Florida College of Medicine - Jacksonville, as well as Dr. Gabriel Steg, Associate Editor from Paris, France. Welcome gentlemen.

Dominick: Thanks for having us.

Gabriel: Hello.

Carolyn: Dominick, I'd really like to start with you. Your paper entitled the OPTIMUS-4 Study, is really a study of the pharmacodynamic comparison of Prasugrel versus Ticagrelor in these patients with type 2 diabetes and coronary artery disease. The whole question is,
what was the rationale to look at the pharmacodynamics?

Dominick: As the title of the study says, OPTIMUS-4, it means that there was an OPTIMUS-1, 2 and 3 in the past, which means that there's a lot of thought that went into this and a lot of background information. The rationale for this specific study was that we’re all well aware of the fact that patients with diabetes have high platelet reactivity, which may be one of the reasons why they have a higher risk of recurrent atherothrombotic events. Therefore, the need to define ways to optimize their anti-platelet effects, their levels of platelet inhibition. In this specific study we took an approach of looking at the novel, although we cannot call them novel nowadays, but the newer P2Y12 receptor inhibitors Prasugrel and Ticagrelor. Looking at them in a head to head comparison from a pharmacodynamic standpoint to see if one drug would be superior than the other, again, in terms of a platelet inhibitory effect.

This is the rationale, and just to expand a little bit on this, there's been a perception, again I want to underscore a 'perception' that based on subgroup analysis of the larger clinical trials, that Prasugrel is a superior drug for patients with diabetes. We do know that there's a benefit also with Ticagrelor compared with Clopidogrel, although the absolute risk reductions in the studies led to a perception that Prasugrel would be a better drug. We said to ourselves, "Well, we’re never going to have a large scale head to head clinical comparison, why don’t we do a head to head pharmacodynamic comparison to see if there are any differences?" This was the overall rationale for conducting this specific study.

Carolyn: That really sets a background perfectly. Tell us about the main findings.

Dominick: The main finding was as follows, we conducted a very detailed pharmacodynamic study, this was a prospective randomized double-blind double-dummy crossover study, with all patients on the background of aspirin therapy. We looked at platelet reactivity, using a variety of assays, I like to say it in every possible salsa that you can imagine. The primary end point which is platelet reactivity at one week into two drugs, using an [ADP 00:10:00] specific assay, actually showed that Ticagrelor was superior to Prasugrel in terms of platelet inhibitory effects. That was the only time point where it was shown, but the study was actually designed to show the opposite, so it was a very interesting finding, while with all the other time points there were no differences between the platelet inhibitory effects between the two drugs.

The other thing that we did look at, which gives a little bit of a novelty to this study is, we went beyond just looking at ADP induced effects, which is the target for these two drugs, we looked at other signalling pathways which one would not believe to be necessarily affected by P2Y12 inhibitors, and we found these also to be reduced by both drugs to a similar extent.

Carolyn: Fascinating. I'm going to get to your second point a bit later. First, that first finding that surprisingly Ticagrelor appeared to perform better using one of the specific assays and so on, I'd really like Gabriel's opinion there. What do you think is the overall clinical implications or what was the message that the editorial board was hoping to get across
to the audience? Because I noticed you invited an editorial as well, a beautiful one written by Dr. [Star-ee-an 11:36] Parker. What was the thinking behind that?

Gabriel: I think this is really a very important paper and I'm delighted that Dominick Angiolillo and his team submitted it to Circulation, in fact to be frank, we invited that paper after seeing his presentation at the ACC earlier this year. The reason that paper caught everybody's attention in the editorial board was that it's addressing a frequent and deadly disease, diabetes, that kills really patients with cardiovascular disease. There's a critical issue in the treatment because of the limitations of Clopidogrel because of the increased platelet reactivity in diabetics, and there's tremendous interest in the novel P2Y12 inhibitors Prasugrel and Ticagrelor, and of course any hint of differences between these agents has major clinical implications. In addition, I think I can state that Dominick's team is really one of the premiere international teams looking at this exact issue, platelet reactivity in diabetics. What they did was really state of the art rigorous clinical investigation by a highly skilled team, looking rigorously at a double blind crossover designed four different assays looking at platelet function and platelet response, looking both at the effect of a loading dose and the maintenance dose.

To me, the message is not a minute difference between the treatments, in fact I think that even though it's the primary outcome and it does show a slightly greater response with Ticagrelor than with Prasugrel, the overall most of the other assays at the other time points show a consistent good response with both agents. To us, and to me, the message is that the novel agents are clearly superior to Clopidogrel as we've seen in the clinical trials, but they are fairly consistent in their benefit, and it's reassuring to see this not in healthy volunteers but in actual patients with stable coronary artery disease. I think it was really important to show that. Certainly platelet reactivity doesn't summarize entirely the effects of any drug, and there might be platelet independent effects of Ticagrelor mostly and possibly Prasugrel, but I think on the platelet side, I think that this paper really nails it.

Carolyn: I read that editorial and really agree that that puts everything in perspective really well. I particularly like the figure that accompanied the editorial. In case any of our listeners out there don't really remember all the different pathways and how Prasugrel and Ticagrelor and Clopidogrel are metabolized differently, I'd really refer everyone to that figure as well. Just want to pick up on one of the points that both of you mentioned, and that is the non ADP induced platelet reactivity that Prasugrel and Ticagrelor both seem to have an affect on and so on, and if they're so effective, Dominick, is there still a role for aspirin co-administration with these new anti-platelet agents?

Dominick: The study clearly puts a little bit more beef, let's put it this way, to some of the ongoing clinical studies looking at whether we need aspirin in the patients treated with one of these newer P2Y12 receptor inhibitors. There are a series of ongoing studies out there. There's a laundry list, so I'm not going to go into the details. It does highlight that maybe when you have ultimate P2Y12 blockade, which is a key signalling pathway and blocks other responses by virtue of the fact that there's an interplay between this pathway and others, maybe you do not need this additional anti-platelet agent such as aspirin, which we know there's associated with potential bleeding particularly gastrointestinal side
effects.

Having said that, this is not something that I'm advocating at time, but what I am saying is that we'll need to look into the results of the clinical trials. I believe that this study is an additional piece of evidence from an ex vivo standpoint to prior in vitro studies showing that aspirin is not associated with additional platelet inhibitory effects, at least not substantial platelet inhibitory effects. One can say that you may get away with just one of these newer agents. Again, this is based on pharmacodynamic findings, let's just wait for the clinical trial results.

Carolyn: I think that's so fairly put, and I learnt so much just listening to this conversation. Thank you so much for joining me today. Any last words from Gabriel?

Gabriel: Yeah, I'd like to make a couple of points as an Associate editor for Circulation. The first one is, this paper was picked up when we saw Dominick's team's presentation at the ACC, and I think it exemplifies that we really want to pick up the best science from the meetings, either before the meetings and publish it simultaneously as much as possible, but sometimes also at the meetings, so expect to see Circulation Editors at your presentations and maybe you'll seduce them enough with your science that we'll get good science submitted to the journal. The other aspect to it is also that I think with the new editorial board there's really a focus on trying to make the journal very international in it's approach, and I think it's fitting that I am Associate Editor from Europe and I think there's no more international a scientist than Dominick Angiolillo who's not only a good friend but also has been trained in Italy, has practiced in Spain, and now works in the US. I think he embodies how science transcends boundaries and borders. I think there's a definite international outlook to Circulation, and we're looking for great science from anywhere in the world, not solely the US.

Carolyn: Thank you so much Gabriel. Thank you so much Dominick. Thank you listeners for listening today, you've been listening to Circulation on the Run. Don't forget to join us next week for more summaries and highlights.