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Coronary Artery Disease: The ONSET/OFFSET Study**

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Randomized Double-Blind Assessment of the ONSET and OFFSET of the Antiplatelet Effects of Ticagrelor Versus Clopidogrel in Patients With Stable Coronary Artery Disease The ONSET/OFFSET Study

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Background—Ticagrelor is the first reversibly binding oral P2Y₁₂ receptor antagonist. This is the first study to compare the onset and offset of platelet inhibition (IPA) with ticagrelor using the PLATO (PLATElet inhibition and patient Outcomes) trial loading dose (180 mg) with a high loading dose (600 mg) of clopidogrel.

Methods and Results—In a multicenter, randomized, double-blind study, 123 patients with stable coronary artery disease who were taking aspirin therapy (75 to 100 mg/d) received ticagrelor (180-mg load, 90-mg BID maintenance dose [n=57]), clopidogrel (600-mg load, 75-mg/d maintenance dose [n=54]), or placebo (n=12) for 6 weeks. Greater IPA (20 μ mol/L ADP, final extent) occurred with ticagrelor than with clopidogrel at 0.5, 1, 2, 4, 8, and 24 hours after loading and at 6 weeks ($P<0.0001$ for all); by 2 hours after loading, a greater proportion of patients achieved $>50\%$ IPA (98% versus 31%, $P<0.0001$) and $>70\%$ IPA (90% versus 16%, $P<0.0001$) in the ticagrelor group than in the clopidogrel group, respectively. A faster offset occurred with ticagrelor than with clopidogrel (4-to-72-hour slope [% IPA/h] -1.04 versus -0.48 , $P<0.0001$). At 24 hours after the last dose, mean IPA was 58% for ticagrelor versus 52% for clopidogrel ($P=NS$). IPA for ticagrelor on day 3 after the last dose was comparable to clopidogrel at day 5; IPA on day 5 for ticagrelor was similar to clopidogrel on day 7 and did not differ from placebo ($P=NS$).

Conclusions—Ticagrelor achieved more rapid and greater platelet inhibition than high-loading-dose clopidogrel; this was sustained during the maintenance phase and was faster in offset after drug discontinuation.

Clinical Trial Registration Information—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT00528411.
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Key Words: platelets ■ ticagrelor ■ clopidogrel ■ antiplatelet agents

Platelet activation by ADP is central to the development of atherothrombosis. The importance of the ADP-P2Y₁₂ receptor interaction has been demonstrated by the clinical benefits associated with the addition of clopidogrel to aspirin therapy in patients with acute coronary syndromes and patients treated with stents.^{1,2}

Clinical Perspective on p 2585

The antiplatelet effect of clopidogrel is slow in onset, variable, and irreversible, and approximately 15% to 30% of patients have been reported to be nonresponsive.¹⁻³ A 75-mg/d clopidogrel maintenance dose required at least 5 days and a 600-mg loading dose of clopidogrel required up

to 8 hours to achieve $\approx 50\%$ steady state of inhibition of ADP-induced platelet aggregation.^{1,4,5} Moreover, translational research studies have established a relationship between nonresponsiveness to antiplatelet drugs, high on-treatment platelet reactivity, and the occurrence of ischemic events in percutaneous coronary intervention patients.⁶⁻⁸ In addition, the slow offset of the antiplatelet effect due to irreversible P2Y₁₂ binding by the active thienopyridine metabolite is potentially problematic in the management of patients who are treated before coronary angiography and then require coronary artery bypass graft surgery or who need other unanticipated surgical procedures.⁹

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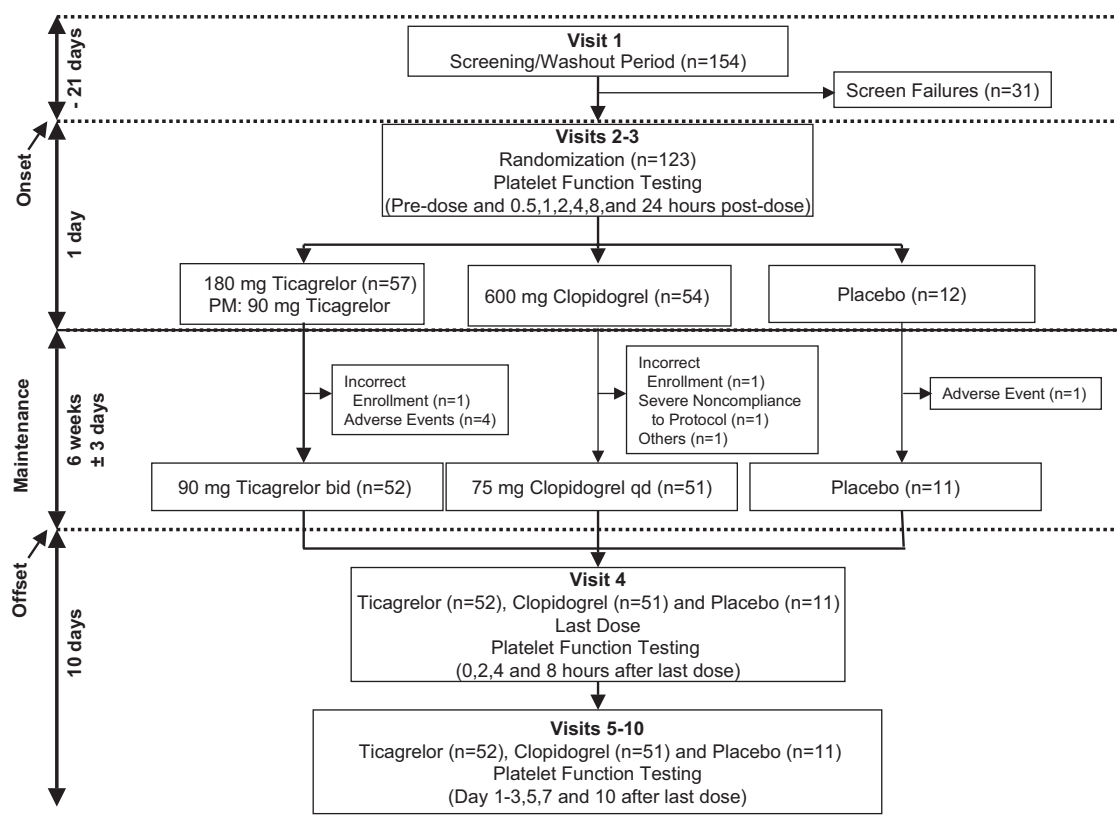


Figure 1. Study design. bid Indicates twice daily; qd, once daily.

Ticagrelor (formerly AZD6140) is the first reversibly binding oral, direct-acting P2Y₁₂ receptor antagonist. Clinical pharmacology and early dose-finding studies suggested a faster onset and greater inhibition of platelet aggregation (IPA) with ticagrelor than with clopidogrel.^{10–12} Although the clinical efficacy of ticagrelor has been studied extensively in PLATO (A Study of Platelet Inhibition and Patient Outcomes), a comprehensive characterization of its antiplatelet onset and offset effect profile in a statistically powered comparison with clopidogrel has not been conducted in patients with coronary artery disease (CAD).¹³ Moreover, ticagrelor has not been compared with high-loading-dose clopidogrel in patients. Therefore, the present study was designed to determine the onset and offset of the antiplatelet effect of ticagrelor with the PLATO trial dose compared with high-loading-dose clopidogrel and placebo in stable CAD patients given background aspirin therapy.

Methods

Study Design and Subjects

The ONSET/OFFSET study was a multicenter, randomized, double-blind, double-dummy, parallel-group study. The study was performed in accordance with standard ethical principles; written consent was obtained from all patients. Patients ≥18 years of age with documented stable CAD who were undergoing aspirin therapy (75 to 100 mg/d) were enrolled in 8 investigational sites in the United States and the United Kingdom between October 2007 and March 2009. Exclusion criteria were a history of acute coronary syndrome within 12 months of screening; any indication (eg, atrial fibrillation, prosthetic heart valve, or coronary stent) for antithrombotic therapy (eg, warfarin, clopidogrel, or aspirin dose other than 75 to 100 mg/d

during the study period); congestive heart failure; left ventricular ejection fraction <35%; forced expiratory volume in the first second or forced vital capacity below the lower limits of normal; bleeding diathesis or severe pulmonary disease; pregnancy; current smoking; concomitant therapy with moderate or strong cytochrome P450 3A inhibitors, substrates, or strong cytochrome P450 3A inducers; platelet count <100 000/mm³; hemoglobin <10 g/dL; hemoglobin A1c ≥10%; history of drug addiction or alcohol abuse in the past 2 years; need for nonsteroidal antiinflammatory drug; or creatinine clearance <30 mL/min.

The total duration of the study was ≈10 weeks (Figure 1). Randomization numbers were prepared by AstraZeneca (Wilmington, Del). After a screening period of up to 21 days (visit 1), patients were randomized at visit 2 in balanced blocks (6 patients in each block) to ensure 1:1:1 randomization to clopidogrel, ticagrelor, and placebo treatment. The goal was 50 patients per treatment group. After 12 placebo patients had been randomized, the remaining patients were randomized to ticagrelor or clopidogrel in a 1:1 ratio. Randomization numbers were assigned sequentially as patients became eligible. An initial loading dose of ticagrelor (180 mg), clopidogrel (600 mg), or placebo was given after randomization at visit 2 followed by a maintenance administration (90 mg of ticagrelor or placebo) in the evening with a 12-hour interval between dosing. Patients then received maintenance treatment for 6 weeks (ticagrelor 90 mg BID, clopidogrel 75 mg/d, or placebo), followed by a 10-day drug-offset period during which patients received a final dose of the study drug on the first day of the offset period (time ≈0 hours). To ensure blinding of the treatments, matching placebo ticagrelor tablets and placebo clopidogrel capsules were provided. Each treatment group consisted of the same combination of matching active and placebo tablets/capsules, so medications provided for each treatment group were identical in appearance.

Patients fasted ≥8 hours before all visits, and all patients received concomitant aspirin (75 to 100 mg/d). Eligible patients undergoing clopidogrel therapy before screening underwent a 14-day minimum washout period before randomization. Compliance was measured by

the amount of medication returned at the respective visits. Bleeding was defined according to the PLATO criteria.¹⁴ The frequency of patients with dyspnea was determined.

Blood Sampling for Platelet Function Testing

Samples for platelet function testing were taken at predosing (0 hour) and after the first dose of study drug on visit 2, then throughout the onset period (0.5 to 24 hours after the first loading dose), at the start of the offset period (0 hour, visit 4), and throughout the 10-day offset period (2 to 240 hours after the last dose; online-only Data Supplement Table II).

Blood was collected from the antecubital vein into Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ) that contained 3.2% trisodium citrate for light-transmittance aggregometry and flow cytometry analyses and in 1 tube that contained 3.2% sodium citrate (Greiner Bio-One Vacuette North America, Inc, Monroe, NC) for VerifyNow measurements.

Light-Transmittance Aggregometry

Platelet aggregation induced by ADP (20 and 5 $\mu\text{mol/L}$), collagen 2 $\mu\text{g/mL}$, and arachidonic acid 2 mmol/L in platelet-rich plasma was assessed with a Chrono-log Optical Aggregometer (model 490-4D; Chrono-log Corporation, Havertown, Pa) as described previously.⁴ The assessment of 2 mmol/L arachidonic acid-induced aggregation was performed to evaluate the effects of aspirin.¹⁵ The final extent of aggregation, measured at 6 minutes after agonist addition, and the maximal extent of aggregation were expressed as the percent change in light transmittance from baseline, with platelet-poor plasma as a reference. IPA was calculated as follows, where PA is platelet aggregation, b is predosing, and t is postdosing:

$$\text{IPA}(\%) = 100\% \times \frac{\text{PA}_b - \text{PA}_t}{\text{PA}_b}$$

VerifyNow P2Y₁₂ Assay

VerifyNow is a turbidimetric-based system that measures platelet aggregation in whole blood.⁹ The instrument measures an optical signal, reported as P2Y₁₂ reaction units (PRU), and calculates the percent of inhibition based on iso-TRAP (thrombin receptor activating peptide)/protease-activated receptor (PAR)-4 activating peptide-induced aggregation as the baseline.

Vasodilator-Stimulated Phosphoprotein Phosphorylation Assay

The measurement of vasodilator-stimulated phosphoprotein phosphorylation (VASP-P) is a method of quantifying P2Y₁₂ receptor reactivity and reflects the extent of P2Y₁₂ receptor blockade (Biocytex Inc, Marseille, France).⁸ The platelet reactivity index (PRI) is calculated after measurement of VASP-P levels (mean fluorescence intensity [MFI]) determined by monoclonal antibodies after stimulation with prostaglandin (PG) E₁ (MFI_{PGE1}) and PGE₁ plus ADP (MFI_{PGE1+ADP}): PRI (%) = [(MFI_{PGE1}) - (MFI_{PGE1+ADP}) / (MFI_{PGE1})] × 100%.

Glycoprotein IIb/IIIa and P-Selectin Expression

ADP-stimulated (5 $\mu\text{mol/L}$, final concentration) expression of glycoprotein IIb/IIIa receptors and P-selectin was measured as described previously.⁴ The percent inhibition of baseline stimulated receptor expression was determined.

Primary End Points for Onset and Offset of IPA

The primary end point for onset was IPA (20 $\mu\text{mol/L}$ ADP, final extent) at 2 hours after the first dose; for offset, it was the slope of IPA between 4 and 72 hours after the last dose of study drug. Secondary pharmacodynamic end points were IPA (final and maximal extent), measured by 5- and 20- $\mu\text{mol/L}$ ADP- and 2- $\mu\text{g/mL}$ collagen-induced light-transmittance aggregometry; PRI; ADP-induced glycoprotein IIb/IIIa and P-selectin expression; and PRU and percent inhibition, measured by the VerifyNow P2Y₁₂ assay.

Sample-Size Calculation

In the DISPERSE study (Dose confirmation Study assessing anti-Platelet Effects of AZD6140 versus clopidogrel in non-ST-segment Elevation myocardial infarction), the variability (ie, SD) of IPA values at 2 hours after an initial 200-mg dose of ticagrelor (old formulation, comparable to 180 mg under the new formulation) was 12.3% (n=36).¹⁰ In the DISPERSE-2 study, the corresponding variability was 20.8% (n=7).¹¹ These 2 estimates were combined to give a weighted estimate of 13.9%. No patient data were available from the previous studies after a 600-mg loading dose of clopidogrel; however, with 50 patients per treatment group, it was calculated that there would be at least 91% power to detect mean differences in IPA of at least 15% between the 2 groups, with the assumption that the variability for the clopidogrel group was no more than double that for ticagrelor (14% versus 28%). The calculation also assumed a 5% significance level (2-sided).

For offset, estimates of the expected intercepts and slopes for each treatment group were obtained from the DISPERSE study.¹⁰ IPA data were available up to 24 hours after the last dose. A random coefficients model was fitted to the 4-, 8-, 12-, and 24-hour values and included fixed effects for treatment group, hour (ie, relative to last dose), and the treatment group-by-hour interaction and random coefficients for the patient and patient-by-hour interaction. The power to detect a given difference in slopes was calculated by simulation. Individual patient profiles of IPA were generated with the above estimates. With 50 patients per treatment group, there would be $\approx 90\%$ power to show a difference in slopes of -0.45 IPA%/h between therapies. The calculations assumed that the linear relationship in IPA offset would continue to the 72-hour time point.

Statistical Analysis

Statistical analyses were performed by QDS (King of Prussia, Pa) with SAS (version 8.2). The analysis was an intention-to-treat analysis that included patients who were randomized to a treatment group, received at least 1 dose of study drug, and contributed interpretable postbaseline data. For all analyses, the primary comparison was made between the ticagrelor and clopidogrel treatments. Demographic data were compared between the 2 treatment groups with *t* test for numerical data or Fisher exact test for categorical data. The antiplatelet effect of ticagrelor compared with clopidogrel was analyzed by the Wilcoxon rank sum test (level of significance 0.05). The slopes of onset and offset were determined by a random coefficients model fitted to IPA values at 0.5, 1, and 2 hours after loading (onset) and 4, 8, 24, 48, and 72 hours after last dose (offset) and included fixed effects for treatment group, hour (relative to last dose or first dose), the treatment group-by-hour interaction, center, and center-by-treatment interaction, as well as random coefficients for the patient and patient-by-hour interaction. Difference of the slopes and 95% confidence intervals for primary comparisons of interest (ticagrelor versus clopidogrel) were calculated. The area under the effect curve from 0 to 8 hours after loading was determined for each treatment group. The mean time to maximum IPA was determined by the mean of each patient's time to reach his or her own maximum IPA.

The estimation for the time of IPA declining from 30% to 10% after the last dose was calculated with an IPA exponential decline-with-time model ($\text{IPA} = \text{IPA}_0 e^{-kt}$), where *t* is the time and *k* is the declining rate constant. Correlation analyses of IPA (20 $\mu\text{mol/L}$ ADP, final extent) versus IPA determined after stimulation by other agonists (final and maximal extent), PRI, the inhibition of stimulated glycoprotein IIb/IIIa and P-selectin expression, and percent inhibition and PRU as assessed by the VerifyNow test were performed with the Pearson product-moment correlation coefficient.

Results

Compliance, Demographics, and Baseline Characteristics

The number of patients enrolled at each center is listed in the online-only Data Supplement (Table I). Two centers, 1 in the

Table 1. Demographics

	Total Group (n=123)	Ticagrelor (n=57)	Clopidogrel (n=54)	Placebo (n=12)	Ticagrelor vs Clopidogrel, <i>P</i>
Demographics					
Age, y	64±9	62±9	65±8	64±8	0.07
Male, n (%)	93 (76)	43 (75)	40 (74)	10 (83)	0.24
Body mass index, kg/m ²	30±4	31±5	30±4	29±4	0.06
Ethnicity, n (%)					
White	108 (88)	51 (90)	48 (89)	9 (75)	0.92
Black	12 (10)	4 (7)	5 (9)	3 (25)	0.66
Other	3 (2)	2 (3)	1 (2)	0 (0)	0.59
Medical history, n (%)					
Family history of CAD	88 (72)	43 (75)	36 (67)	9 (75)	0.31
Hypertension	92 (75)	44 (77)	39 (72)	9 (75)	0.55
Hyperlipidemia	118 (96)	54 (95)	52 (96)	12 (100)	0.69
Diabetes mellitus					
HbA1c >6.0%	19 (15)	6 (11)	8 (15)	5 (42)	0.50
HbA1c ≤6.0%	8 (7)	6 (11)	2 (4)	0 (0)	0.15
Prior myocardial infarction	55 (45)	26 (46)	23 (43)	6 (50)	0.75
Prior coronary artery bypass graft	47 (38)	23 (40)	21 (39)	3 (25)	0.87
Prior percutaneous coronary intervention	93 (76)	41 (72)	41 (76)	11 (92)	0.63
Baseline medications, n (%)					
Statins	110 (89)	49 (86)	50 (93)	11 (92)	0.27
Angiotensin-converting enzyme inhibitors	20 (16)	10 (18)	8 (15)	2 (17)	0.70
β-blockers	90 (73)	39 (68)	42 (78)	9 (75)	0.27
Diuretics	42 (34)	20 (35)	18 (33)	4 (33)	0.84
Organic nitrates	18 (15)	6 (11)	12 (22)	0 (0)	0.10
Proton pump inhibitors	35 (29)	16 (28)	16 (30)	3 (25)	0.86
Calcium channel blockers	28 (23)	17 (30)	9 (17)	2 (17)	0.11
Baseline laboratory data					
White blood cells (×1000/mm ³)	6.5±1.6	6.6±1.8	6.4±1.4	6.6±1.5	0.52
Platelets (×1000/mm ³)	231±61	232±65	227±55	235±62	0.66
Hematocrit, %	42±4	42±3	42±4	42±4	1.0
Creatinine, μmol/L	91±22	91±24	89±21	88±26	0.82
LDL, mg/dL	68±48	72±55	63±45	81±31	0.35
HDL, mg/dL	34±25	32±26	32±25	44±25	1.0
Uric acid, μmol/L	382±83	381±83	377±82	406±88	0.80

HbA1c indicates hemoglobin A1c.

United States and 1 in the United Kingdom, enrolled most of the patients (n=43 and n=40, respectively). Fifty-two patients in the ticagrelor group, 51 in the clopidogrel group, and 11 in the placebo group completed the study. For the complete pharmacodynamic analysis set, there were 49 patients in the ticagrelor group, 44 in the clopidogrel group, and 10 in the placebo group. The overall compliance rate was >95% for each treatment group by drug count. The treatment code was not broken prematurely for any patient. The most common protocol deviations related to laboratory tests (18.5% for the ticagrelor group and 10.0% for the clopidogrel group), procedures/tests (14.8% and 12.0%, respectively), and informed consent issues (13.0% and 14.0%, respectively). Approximately 6% of patients in each group had treatment visits outside the

protocol window. The treatment groups were evenly balanced and consisted predominantly of white men between 41 and 83 years of age (Table 1).

Arachidonic Acid-Induced Aggregation

Overall, 96% and 98% of patients had baseline and end-of-study arachidonic acid-induced maximal platelet aggregation <20%, respectively.

Onset and Maintenance IPA

The primary end point for onset, IPA at 2 hours after loading (20 μmol/L ADP, final extent) was greater for ticagrelor than for clopidogrel (88% versus 38%, *P*<0.0001; Table 2). IPA was higher at 0.5 hours after loading with ticagrelor (41% versus 8%, *P*<0.0001) and at all times in the first 24 hours

Table 2. IPA (20 μ mol/L ADP) at 2 Hours After First Dose of Ticagrelor and Clopidogrel

	Ticagrelor (n=54)		Clopidogrel (n=50)		<i>P</i>	
	IPA, %	PA, %	IPA, %	PA, %	IPA, %	PA, %
Final extent	88 \pm 15	7 \pm 9	38 \pm 33	44 \pm 24	<0.0001	<0.0001
Maximum extent	65 \pm 17	23 \pm 10	25 \pm 23	55 \pm 18	<0.0001	<0.0001

PA indicates platelet aggregation.
Values are mean \pm SD.

after loading and in the maintenance phase ($P<0.0001$; Figure 2). Within 1 hour of ticagrelor loading, IPA was greater than the maximum IPA achieved after clopidogrel loading. In the ticagrelor group, IPA did not differ between 2 and 8 hours after loading, whereas in the clopidogrel group, IPA was greater at 8 hours than at 2 hours ($P=0.02$, repeated-measures ANCOVA model).

The mean time to maximum IPA in the ticagrelor group was 5.8 hours less and the area under the effect curve from 0 to 8 hours after loading (20 μ mol/L ADP, final extent) was higher than in the clopidogrel group (Table 3). The rate of onset (slope) of the antiplatelet effect curve as assessed by IPA (20 μ mol/L ADP, final extent) from 0 to 2 hours after the loading dose was greater in the ticagrelor group than in the clopidogrel group (43.57 versus 19.45 IPA %/h, $P<0.0001$; Table 4). By 2 hours after loading, a greater proportion of patients achieved $>50\%$ IPA (98% versus 31%, $P<0.0001$) and $>70\%$ IPA (90% versus 16%, $P<0.0001$) in the ticagrelor group than in the clopidogrel group, respectively. Concordant results were observed with the final and maximum extent of platelet aggregation (Table 2).

Offset of IPA

At the end of the 6 weeks of treatment, IPA (20 μ mol/L ADP, final extent) was significantly higher in the ticagrelor group

Table 3. IPA_{max}, TIPA_{max}, and AUEC₀₋₈ (20 μ mol/L ADP, Final Extent) at Onset

	Ticagrelor (n=54)	Clopidogrel (n=50)
IPA _{max} , %	93	58
TIPA _{max} , h	2.0	7.8
AUEC ₀₋₈ , % h	659	275

IPA_{max} indicates maximum IPA; TIPA_{max}, time to IPA_{max}; and AUEC₀₋₈, area under the effect curve from 0 to 8 hours after loading.

than in the clopidogrel group ($P<0.0001$; Figure 2); however, IPA did not differ between the groups at 24 and 48 hours after the last dose. The ticagrelor group had significantly lower IPA at 72 and 120 hours after the last dose ($P\leq 0.05$), and the IPA did not differ thereafter between the groups (Figure 2). The rate of offset (slope) of the antiplatelet effect curve as assessed by IPA (20 μ mol/L ADP, final extent) from 4 to 72 hours after the last dose, the primary end point for offset, was greater in the ticagrelor group than in the clopidogrel group (-1.04 versus -0.48 IPA %/h, $P<0.0001$; Table 4). The time required for IPA to decrease from 30% to 10% in the ticagrelor group was less than half that in the clopidogrel group (53.30 versus 116.20 hours, respectively; Table 5), and the time to reach 10% was nearly twice as long after clopidogrel discontinuation (109.19 versus 195.66 hours, respectively). IPA for ticagrelor on day 3 after the last dose was comparable to that for clopidogrel at day 5; IPA on day 5 for ticagrelor was similar to clopidogrel on day 7 and did not differ from placebo ($P=NS$).

VerifyNow P2Y₁₂ Assay

The greatest change in PRU from baseline in the ticagrelor group occurred within 2 hours after loading compared with 8 hours in the clopidogrel group (Figure 3). PRU was significantly lower in the ticagrelor group at all times in the first 24 hours after loading and during maintenance ($P<0.0001$). PRU was lower at 8 and 24 hours after the final dose in the

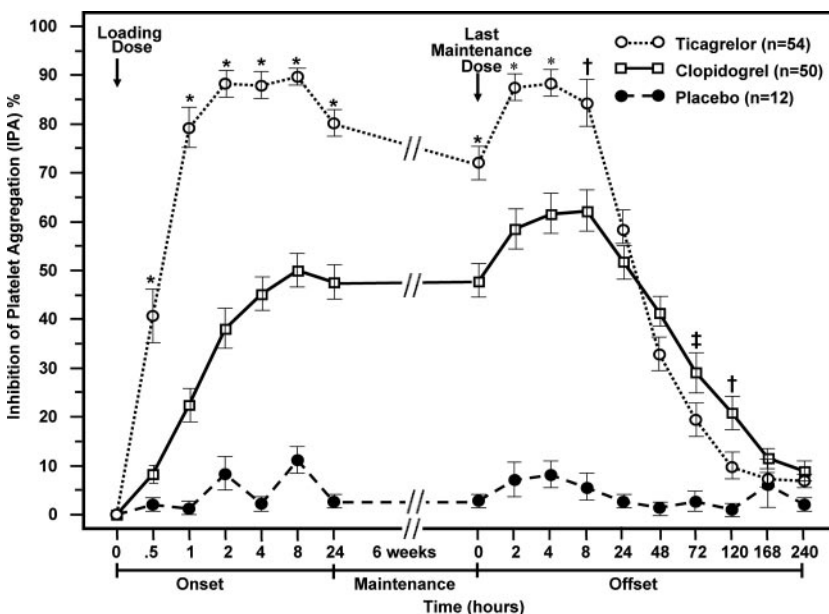


Figure 2. IPA (%; 20 μ mol/L ADP, final extent) by protocol time and treatment. Data are expressed as mean \pm SEM. * $P<0.0001$, † $P<0.005$, ‡ $P<0.05$, ticagrelor vs clopidogrel.

Table 4. Slope of Onset (0 to 2 Hours After Loading Dose) and Offset (4 to 72 Hours After Last Dose) Measured by IPA (20 μ mol/L ADP, Final Extent)

	Ticagrelor (n=54)		Clopidogrel (n=50)		Difference of Mean Slope (Ticagrelor–Clopidogrel)			<i>P</i>
	Intercept	Slope (IPA %/h)	Intercept	Slope (IPA %/h)	Estimate (IPA %/h)	Lower 95% CI	Upper 95% CI	
Onset								
Final extent	10.59	43.57	8.42	19.45	24.12	18.47	28.77	<0.0001
Maximum extent	6.75	31.44	5.56	11.98	19.47	15.34	23.59	<0.0001
Offset								
Final extent	94.00	−1.04	71.84	−0.48	−0.56	−0.71	−0.40	<0.0001
Maximum extent	59.78	−0.74	41.66	−0.29	−0.45	−0.60	−0.29	<0.0001

CI indicates confidence interval.

ticagrelor group ($P<0.0001$). At 48 hours and thereafter, PRU did not differ between groups.

Vasodilator-Stimulated Phosphoprotein Phosphorylation

The greatest change from baseline in PRI in the ticagrelor group occurred within 2 hours after loading compared with 8 hours in the clopidogrel group (Figure 4). PRI after the first loading dose and during maintenance was significantly lower, which indicates greater inhibition at all times in the ticagrelor group than in the clopidogrel group ($P<0.0001$). The PRI was lower at 8 and 24 hours after the final dose in the ticagrelor group ($P<0.005$ for both). At 48 hours and thereafter, there were no differences between the treatment groups.

Expression of Platelet Receptors

Platelet function, as measured by expression of glycoprotein IIb/IIIa and P-selectin receptors, demonstrated wide variability (Figures I and II in the online-only Data Supplement). The maximum antiplatelet effect of ticagrelor, as measured by both receptors, occurred within 2 hours of loading (2 versus 8 hours, $P=NS$) and was lower than in the clopidogrel group

at all times after loading and during maintenance ($P<0.05$). Receptor expression was more suppressed for ticagrelor at 0 and 24 hours after the final dose. At 48 hours and thereafter, there were no differences between treatment groups.

Correlation of IPA (20 μ mol/L ADP, Final Extent) With Other Pharmacodynamic Measurements

In both treatments groups, IPA (20 μ mol/L ADP, final extent) significantly correlated with other pharmacodynamic parameters ($P<0.0001$; Table III in the online-only Data Supplement). The strongest correlations for ticagrelor were with IPA (maximal extent) irrespective of the ADP concentration, inhibition (%), and PRU as measured by the VeriNow P2Y₁₂ assay and PRI.

Clinical Outcomes

Bleeding-related events occurred more frequently in the ticagrelor group (28.1%) than in the clopidogrel (13.0%) and placebo (8.3%) groups. There was 1 clinically relevant minor bleeding event in the placebo group; the remaining events were classified as minor (1 event in the ticagrelor group) or minimal. There were no major bleeding events. Five patients discontinued study treatment owing to an adverse event (4 treated with ticagrelor and 1 in the placebo group). Dyspnea judged by the investigator to be likely or possibly due to the study drug occurred in 25%, 4%, and 0% of patients in the ticagrelor, clopidogrel, and placebo groups, respectively (ticagrelor versus clopidogrel $P<0.01$). Three patients in the ticagrelor group stopped the study drug owing to dyspnea.

Discussion

This is the first study to comprehensively characterize the onset and offset of the antiplatelet effect of ticagrelor in a statistically powered comparison with clopidogrel, and it is the first comparison of ticagrelor with high-dose clopidogrel (600 mg) in stable CAD patients. The 3 major findings of the present study are as follows: (1) The onset of the antiplatelet effect of ticagrelor with the PLATO dosing regimen was rapid (a significant antiplatelet effect was observed within 30 minutes of loading) and markedly greater than with high-loading-dose clopidogrel; (2) the greater antiplatelet effect of ticagrelor was sustained during maintenance therapy; and (3) the offset effect for ticagrelor as determined by the rate of offset (slope) measured by aggregometry was significantly

Table 5. Time for IPA (20 μ mol/L ADP) to Decrease From 30% to 10%

	Ticagrelor (n=54)	Clopidogrel (n=50)
Final extent		
IPA ₀	94.92±2.01	63.71±2.07
Declining rate constant	0.021±0.00096	0.009±0.00084
Time when IPA=30%, h	55.88	79.66
Time when IPA=10%, h	109.19	195.66
Average time, h	53.30	116.20
Maximal extent		
IPA ₀	67.83±1.72	37.44±1.51
Declining rate constant	0.023±0.0012	0.008±0.00096
Time when IPA=30%, h	36.13	26.12
Time when IPA=10%, h	84.80	155.73
Average time, h	48.66	129.61

IPA₀ indicates IPA at beginning of offset; average time, time when IPA=30%–time when IPA=10%.

Data are presented as mean±SE. IPA data from 2 to 240 hours after last dose were used to fit the model.

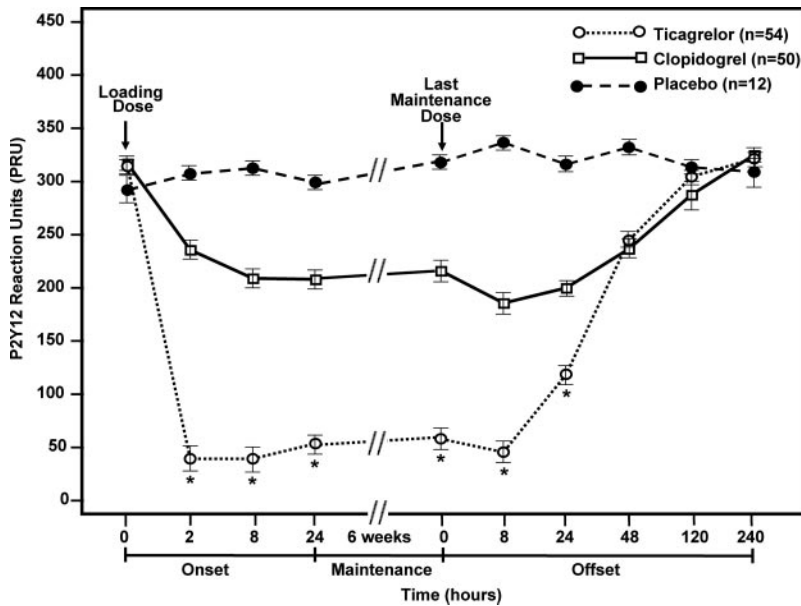


Figure 3. P2Y₁₂ reaction units (PRU) as assessed by the VerifyNow P2Y₁₂ assay by protocol time and treatment. Data are expressed as mean±SEM. * $P<0.0001$, ticagrelor vs clopidogrel.

faster than clopidogrel, and the residual antiplatelet effect of ticagrelor returned to baseline faster than clopidogrel.

Onset Pharmacodynamics

The pharmacodynamic response to the ticagrelor loading dose in the present study is consistent with the results of the DISPERSE and DISPERSE-2 studies.^{10,11} In those studies, the earliest platelet function assessment was at 2 hours after loading, and at that time, a maximal antiplatelet effect occurred. However, in the ONSET/OFFSET study, platelet aggregation was measured earlier after the loading dose (0.5- and 1-hour measurements), and within 1 hour, we observed a near-maximal response ($\approx 80\%$ inhibition). At 1 hour after loading, platelet inhibition induced by ticagrelor was ≈ 1.6 times greater than the maximal platelet inhibition induced by clopidogrel that occurred at 8 hours after loading. The significant antiplatelet effect observed within 30 minutes of loading indicates that ticagrelor may have

particular utility in the setting of ad hoc percutaneous coronary intervention, for which immediate inhibition is desired. The rapid onset of IPA after ticagrelor loading is consistent with the properties of a direct-acting P2Y₁₂ inhibitor, for which IPA is dependent on plasma drug concentrations.¹⁰

The present results are also concordant with the CLEAR PLATELETS (Clopidogrel Loading with Eptifibatide to Arrest the Reactivity of Platelets) and CLEAR PLATELETS-2 studies that examined the pharmacodynamic response to a 600-mg clopidogrel loading dose administered at the time of elective coronary artery stenting.^{5,6} The maximum antiplatelet effect from a 600-mg clopidogrel load on a background of aspirin therapy occurred at 6 to 8 hours after dosing, similar to the ONSET/OFFSET study. Overall, the pharmacodynamics measured by light-transmittance aggregometry were largely consistent with the results of VerifyNow and flow cytometry measuring VASP-P. The ONSET/OFFSET study

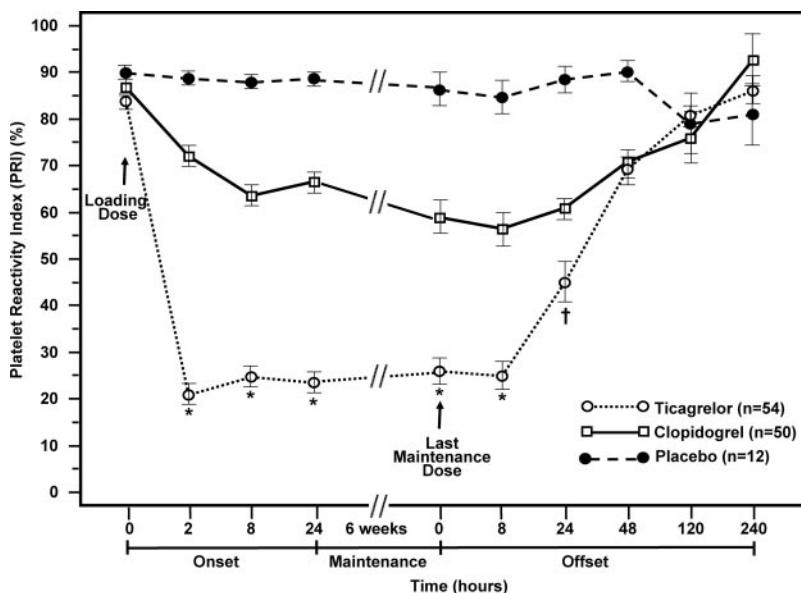


Figure 4. Platelet reactivity index (PRI, %) as assessed by VASP-P by protocol time and treatment. Data are expressed as mean±SEM. * $P<0.0001$, † $P<0.005$, ticagrelor vs clopidogrel.

was also the first prospective study to use the VASP-P and VerifyNow P2Y₁₂ assays to detect the antiplatelet properties of a direct-acting P2Y₁₂ inhibitor.

Offset Pharmacodynamics

Despite the greater antiplatelet effect of ticagrelor, IPA at 24 hours after the last dose was equivalent in ticagrelor- and clopidogrel-treated patients, which is indicative of a faster immediate offset of effect. These data suggest that patients who miss 1 dose of ticagrelor will have a level of platelet inhibition at 24 hours after the last dose that is equivalent to patients undergoing maintenance clopidogrel therapy. Platelet inhibition in the ticagrelor group was numerically less at 48 hours after the last dose and was significantly less at 72 and 120 hours. Thereafter, platelet inhibition was equivalent. However, the VASP-P and VerifyNow measurements demonstrated equivalent antiplatelet effects at 48 hours that persisted for 240 hours. Price et al⁹ measured the onset and offset of platelet inhibition by clopidogrel in healthy volunteers with the VerifyNow P2Y₁₂ assay. They demonstrated low platelet inhibition (median 12%) at day 5 of offset in the majority of subjects.⁹ The latter results are consistent with the present observations.

On the basis of the present IPA data, bleeding risk may be less in patients taken to surgery between 48 and 120 hours after cessation of ticagrelor therapy compared with clopidogrel therapy. Moreover, in support of the offset data in the present study, in the PLATO trial, coronary artery bypass graft–related bleeding was numerically lower in ticagrelor-treated patients than in clopidogrel-treated patients despite the recommendation that the study drug be withheld for 5 days in the clopidogrel group and for 24 to 72 hours in the ticagrelor group.¹³ The primary safety end points in PLATO did not differ between groups, but non–coronary artery bypass graft–related major bleeding by PLATO and TIMI (Thrombolysis In Myocardial Infarction) criteria were greater in the ticagrelor group. However, the lower number of coronary artery bypass graft bleeding events in the ticagrelor group appeared to counterbalance the increased non–coronary artery bypass graft–related major bleeding and drove the primary end point of major bleeding to be no different between groups. It is clear that further prospective studies are required to demonstrate the relation of bleeding to platelet function in patients treated with reversible versus irreversible P2Y₁₂ inhibitors, and at this time, the optimal ex vivo measurements to determine safety and efficacy remain uncertain.

Ticagrelor inhibits the P2Y₁₂ receptor by a noncompetitive mechanism toward ADP.¹⁶ With noncompetitive binding, the agonist cannot displace the drug from the receptor. Theoretically, increasing concentrations of ADP should not significantly alter the antiplatelet effect of ticagrelor.¹⁶ Moreover, direct P2Y₁₂ inhibitors may inhibit the externalized internal pool of P2Y₁₂ receptors that are not accessible during transient exposure to active thienopyridine metabolites.¹⁷ In addition to overall greater platelet inhibition, the latter mechanisms may also explain the lower occurrence of ischemic events associated with ticagrelor than with clopidogrel therapy in the PLATO trial.

Study Limitations

The present study was neither sized adequately nor of sufficient duration to examine the relation of clinical outcomes to platelet function. The patient population had stable CAD, and similar findings may not occur in the analysis of platelet function in patients with unstable CAD or patients undergoing coronary stent implantation.

Conclusions

Ticagrelor achieved more rapid and greater platelet inhibition than high-loading-dose clopidogrel in patients with stable CAD. This inhibition was sustained during the maintenance phase and was faster in offset than clopidogrel. These effects may explain why ticagrelor treatment in the PLATO trial was associated with a lower occurrence of the primary end point than seen with clopidogrel therapy, whereas no difference in coronary artery bypass graft–related bleeding occurred between the 2 groups.

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CLINICAL PERSPECTIVE

In the present study, ticagrelor compared with high-loading-dose clopidogrel achieved more rapid and greater platelet inhibition in patients with stable coronary artery disease. Greater inhibition was also sustained during the maintenance phase, and the offset of action was faster with ticagrelor therapy than with clopidogrel. These pharmacodynamic effects may explain why ticagrelor treatment was associated with a lower occurrence of the primary end point (myocardial infarction, stroke, or cardiovascular death), similar coronary artery bypass graft–related bleeding, and no overall difference in major bleeding compared with clopidogrel therapy in the PLATO (PLATelet inhibition and patient Outcomes) trial.

SUPPLEMENTAL MATERIAL

Supplemental Tables

Table S1. Patient Enrollment in Each Center

Region	Center	Randomized Patients
United States	1	43
United Kingdom	2	40
United States	3	7
United States	5	4
United States	7	3
United States	8	12
United States	9	4
United States	10	10

Table S2. Platelet Function Testing Schedule

Measurement	Time in Onset and Offset Phases
2 mM arachidonic acid- induced platelet aggregation by light transmittance aggregometry	<ul style="list-style-type: none"> 0 h (pre-dose), on visit 2 and visit 4.
5 and 20 μ M ADP-, and 2 μ g/ml collagen- induced platelet aggregation by light transmittance aggregometry	<ul style="list-style-type: none"> Onset: 0 (pre-dose), 0.5, 1, 2, 4, 8 and 24 h after first dose Offset: 0 (pre-dose), 2, 4, 8, 24, 48, 72, 120, 168 and 240 h after last dose
VASP-Phosphorylation and Platelet receptors (GPIIb/IIIa and P-selectin) VerifyNow™ P2Y ₁₂ assay	<ul style="list-style-type: none"> Onset: 0 (pre-dose), 2, 8 and 24 h after first dose Offset: 0 (pre-dose), 8, 24, 48, 120, and 240 h after last dose

**Table S3. Correlation of Inhibition of Platelet Aggregation
(final extent, 20μM ADP) Versus Other Pharmacodynamic Measurements**

	Ticagrelor (n=54)		Clopidogrel (n=50)		Placebo (n=12)	
	Correlation coefficient	<i>P</i> -value	Correlation coefficient	<i>P</i> -value	Correlation coefficient	<i>P</i> -value
IPA (%)						
5μM ADP (maximum)	0.9099	<0.0001	0.8805	<0.0001	0.6149	<0.0001
5μM ADP (final)	0.9257	<0.0001	0.9067	<0.0001	0.5698	<0.0001
20μM ADP (maximum)	0.9290	<0.0001	0.9396	<0.0001	0.9400	<0.0001
2ug/mL Collagen (maximum)	0.6249	<0.0001	0.4471	<0.0001	0.3909	<0.0001
2ug/mL Collagen (final)	0.6640	<0.0001	0.4298	<0.0001	0.3689	<0.0001
Flow Cytometry						
PRI (%)	0.7463	<0.0001	0.3973	<0.0001	-0.1310	0.1707
Inhibition of Stimulated P- Selectin Expression	0.4731	<0.0001	0.3586	<0.0001	-0.3561	0.0002
Inhibition of Stimulated GPIIb/IIIa Expression	0.3584	<0.0001	0.2934	<0.0001	-0.2343	0.0129
VerifyNow P2Y12 Assay						
Inhibition (%)	0.8483	<0.0001	0.7408	<0.0001	0.0054	0.9567
PRU	-0.8631	<0.0001	-0.5921	<0.0001	-0.1264	0.2640

ADP indicates adenosine diphosphate; IPA, inhibition of platelet aggregation; PRI, platelet reactivity index; PRU, Platelet Reactivity Units

Supplemental Legends

Figure S1. Adenosine diphosphate- stimulated p-selectin expression by protocol time and treatment. Data expressed as mean \pm SE.

* $P < 0.0001$, $^{\dagger}P < 0.005$, $^{\ddagger}P < 0.05$, Ticagrelor vs Clopidogrel

Figure S2. Adenosine Diphosphate- Stimulated GPIIb/IIIa Expression by Protocol Time and Treatment. Data are expressed as mean \pm standard error.

* $P < 0.0001$, $^{\dagger}P < 0.05$, Ticagrelor vs Clopidogrel

Supplemental Figures

Figure S1.

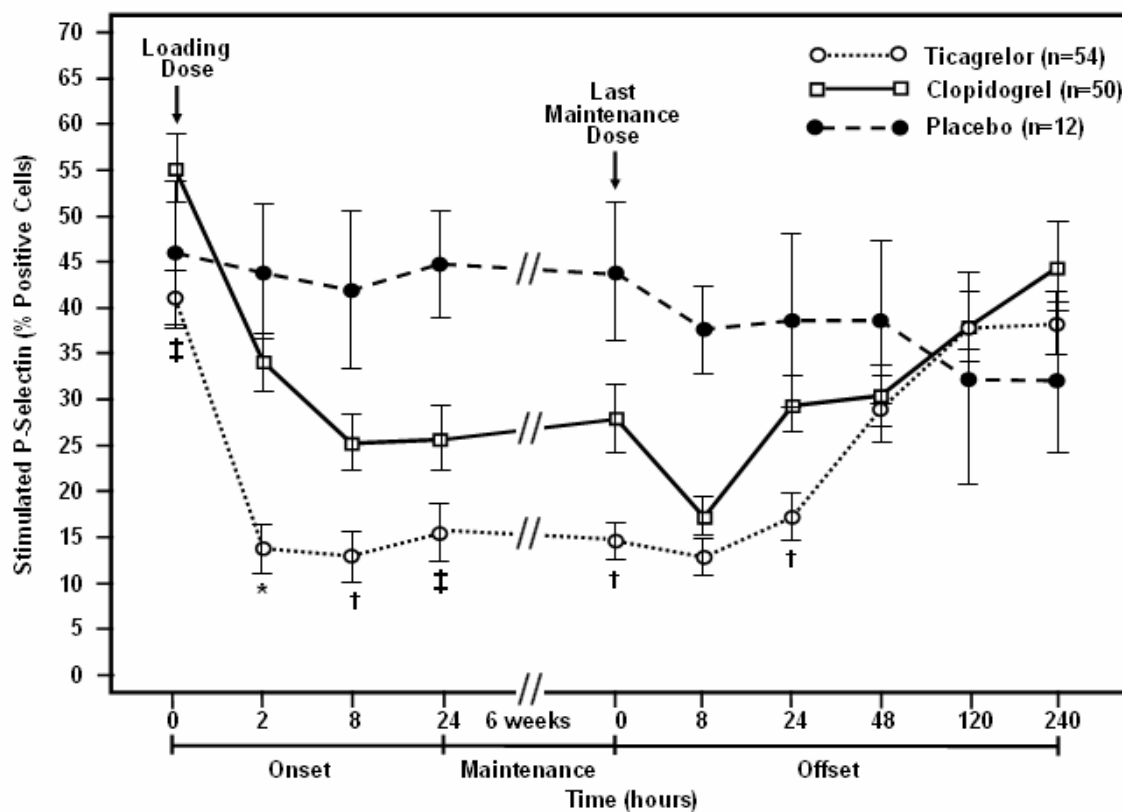


Figure S2.

