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A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study

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Aims Patients with type 2 diabetes mellitus (T2DM) have reduced platelet inhibition compared with non-diabetics following $P2Y_{12}$ receptor blockade. Whether inhibition of $P2Y_{12}$ signalling can be enhanced by adjunctive treatment with cilostazol in T2DM patients is unknown. The aim of this pilot study was to assess the functional impact of cilostazol in T2DM patients on standard aspirin and clopidogrel treatment.

Methods and results This was a prospective, double-blind, double-dummy, placebo-controlled, randomized, cross-over platelet function study. T2DM patients on dual antiplatelet therapy were assigned to receive cilostazol 100 mg or placebo twice daily for 14 days and afterwards crossed-over treatment assignments for another 14 days. Platelet function was performed at three time points: at baseline, 14 days after randomization, and 14 days after treatment cross-over. The $P2Y_{12}$ reactivity index, determined through flow cytometric assessment of the phosphorylation status of the vasodilator-stimulated phosphoprotein, was the primary endpoint measure. In addition to this flow cytometric evaluation, light transmittance aggregometry and VerifyNow testing were performed. A total of 25 T2DM patients were randomized; five patients discontinued treatment due to side effects. The $P2Y_{12}$ reactivity index was significantly lower following cilostazol treatment compared with placebo (36.3 ± 20 vs. $59.9 \pm 16\%$; $P = 0.0002$). All other $P2Y_{12}$-specific functional assessments showed enhanced inhibition of this signalling pathway following treatment with cilostazol.

Conclusion Adjunctive treatment with cilostazol in T2DM patients on standard dual antiplatelet therapy enhances inhibition of platelet $P2Y_{12}$ signalling.

Keywords Diabetes mellitus • Platelets • Thrombosis • Clopidogrel • Cilostazol

Patients with type 2 diabetes mellitus (T2DM) have reduced responsiveness to $P2Y_{12}$ receptor antagonists, including clopidogrel, compared with non-diabetics as assessed by *in vitro* and *ex vivo* studies.[1–4] Normally, cyclic adenosine monophosphate (cAMP) levels increase following $P2Y_{12}$ receptor blockade which in turn augments the phosphorylated status of vasodilator-stimulated phosphoprotein (VASP-P), a key intraplatelet mediator of $P2Y_{12}$ signalling.[5,6] However, platelets from T2DM patients are characterized by

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reduced cAMP levels thus contributing to their lower degree of platelet inhibition compared with non-diabetics following treatment with $P2Y_{12}$ receptor antagonists.[1–4,7–9] These laboratory findings may explain why diabetic patients have an enhanced risk of ischaemic events, including stent thrombosis, despite the use of clopidogrel[10–14] and suggest the need for more aggressive and/or tailored antiplatelet treatment regimens in these high-risk patients.[8] Given the site of dysregulation of intraplatelet signalling, we hypothesized that intervening on pathways that increase cAMP levels can augment VASP-P and enhance inhibition of $P2Y_{12}$ signalling in T2DM patients.

Cilostazol is an inhibitor of phosphodiesterase 3 (PDEIII) and leads to an increase in intraplatelet cAMP levels.[15] In patients undergoing coronary stenting, treatment with cilostazol in adjunct to aspirin and thienopyridine therapy ('triple antiplatelet therapy') has been associated with a reduced risk of stent thrombosis and major adverse cardiac events compared with standard dual antiplatelet therapy.[16–19] Importantly, cilostazol has been shown to be particularly effective in diabetic patients.[20–23] The ever raising concerns of stent thrombosis in the current era of drug-eluting stents (DESs) has led to a more diffuse use of cilostazol in adjunct to standard aspirin and clopidogrel therapy, particularly in high-risk patients. However, whether treatment with cilostazol enhances inhibition of $P2Y_{12}$ receptor signalling remains unknown.

The aim of this pilot study was to evaluate the functional impact of adjunctive treatment with cilostazol in T2DM patients with coronary artery disease (CAD) on standard dual antiplatelet therapy. Our hypothesis was that the adjunctive use of cilostazol will lead to enhanced $P2Y_{12}$ inhibition compared with standard dual antiplatelet therapy in these high-risk patients.

Methods

Patient population and study design

Patients were eligible for the study if they had T2DM, either on insulin or oral hypoglycaemic medication, and were between the age of 25 and 80 years. T2DM was defined according to the World Health Organization Report.[7,24] All patients included had documented CAD, as all patients had previously undergone PCI, and were in a steady-state phase of clopidogrel treatment (75 mg/daily). Since pharmacokinetic and pharmacodynamic profiles vary markedly in the initial days or weeks after initiation of dual antiplatelet therapy,[6] patients had to be in their maintenance phase of treatment for at least 30 days to be included. All patients were also treated with low-dose aspirin (81 mg/daily). This dose of aspirin was chosen in order to reduce bleeding risk in patients on dual antiplatelet therapy.[25] Major exclusion criteria included: known allergies to aspirin, clopidogrel or cilostazol; impaired glucose tolerance or T2DM without pharmacological treatment, gestational diabetes, or transient hyperglycaemia; left ventricular ejection fraction $<35\%$; blood dyscrasia; serum creatinine level >2 mg/dL; active bleeding or bleeding diathesis; gastrointestinal bleed within last 6 months haemodynamic instability; cerebrovascular accident within 3 months; any malignancy; concomitant use of other antithrombotic drugs (oral anticoagulants, dypiridamole, ticlopidine) or non-steroid anti-inflammatory drugs; recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist; platelet count $<100 \times 10^6/\mu$L; haematocrit $<25\%$; liver disease (bilirubin level >2 mg/dL).

The study complied with the Declaration of Helsinki, was approved by the Institutional Review Board of the University of Florida College of Medicine-Jacksonville, and all patients gave their informed written consent. Since in the present study cilostazol was used for an indication non-approved by the Food and Drug Administration (FDA), an independent data safety monitoring committee was instituted for adjudication of adverse clinical events. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

The OPTIMUS-2 (Optimizing anti-Platelet Therapy In diabetes MellitUS) study was a prospective, double-blind, double-dummy, placebo-controlled, randomized, cross-over platelet function study of patients with T2DM and CAD. Patients were recruited from the outpatient cardiology clinic of our hospital. Patients were assigned to receive cilostazol 100 mg or placebo tablets twice daily for 14 days and afterwards crossed-over treatment assignments for another 14 days. There was no wash-out phase between the two treatment periods. Placebo and treatment tablets were provided by Otsuka pharmaceuticals (Tokyo, Japan). Randomization and blinding were carried out by our division's pharmacy that computer-generated the randomization sequence and blindly dispensed the study medication and placebo. After determining eligibility and obtaining consent, the patient's study number was sent to the division's pharmacy and then the patient was randomized to one of two different sequence groups: placebo/cilostazol or cilostazol/placebo. Investigators and patients were blinded to treatment assignments. Placebo and treatment tablets were identical in size, colour, shape, and weight, and placed in standard medicine bottles. Each bottle was dispensed individually by the research coordinator at the appropriate patient visit. Unblinding was done at the end of the study. Platelet function was performed at three time points: (i) at baseline (before randomization); (ii) 14 days after randomization; (iii) 14 days after cross-over. Patient compliance to antiplatelet treatment was assessed by interview and pill counting. The flow-diagram of the study is shown in *Figure 1*. The mechanism of action of cilostazol and how this may modulate $P2Y_{12}$ signalling, thus supporting our study hypothesis, is illustrated in *Figure 2*.

Primary endpoint and sample size calculation

The primary endpoint of this study was the $P2Y_{12}$ reactivity index (PRI) achieved following treatment with cilostazol. Using a power analysis for paired testing, we hypothesized a 15% mean reduction in the primary endpoint following treatment with cilostazol compared with placebo with a standard deviation of the differences between the two groups to be 16%. Thus, 17 patients per group would be required to provide a 90% power to detect a statistical difference between groups with a two-sided α-level of 0.05. Estimation of platelet function values was based on our previous data in T2DM patients;[7] in particular, more aggressive antiplatelet therapy showed a $\sim$15% reduction in PRI.[7]

Blood sampling

Blood sampling for platelet function analyses were collected from an antecubital vein using a 21-gauge needle 2–4 h after intake of aspirin, clopidogrel, and cilostazol/placebo. The first 2–4 mL of blood were discarded to avoid spontaneous platelet activation. Samples were processed within 1 h after blood drawing by operators blind to patient's treatment assignment. Laboratory personnel were also blinded to treatment assignments.

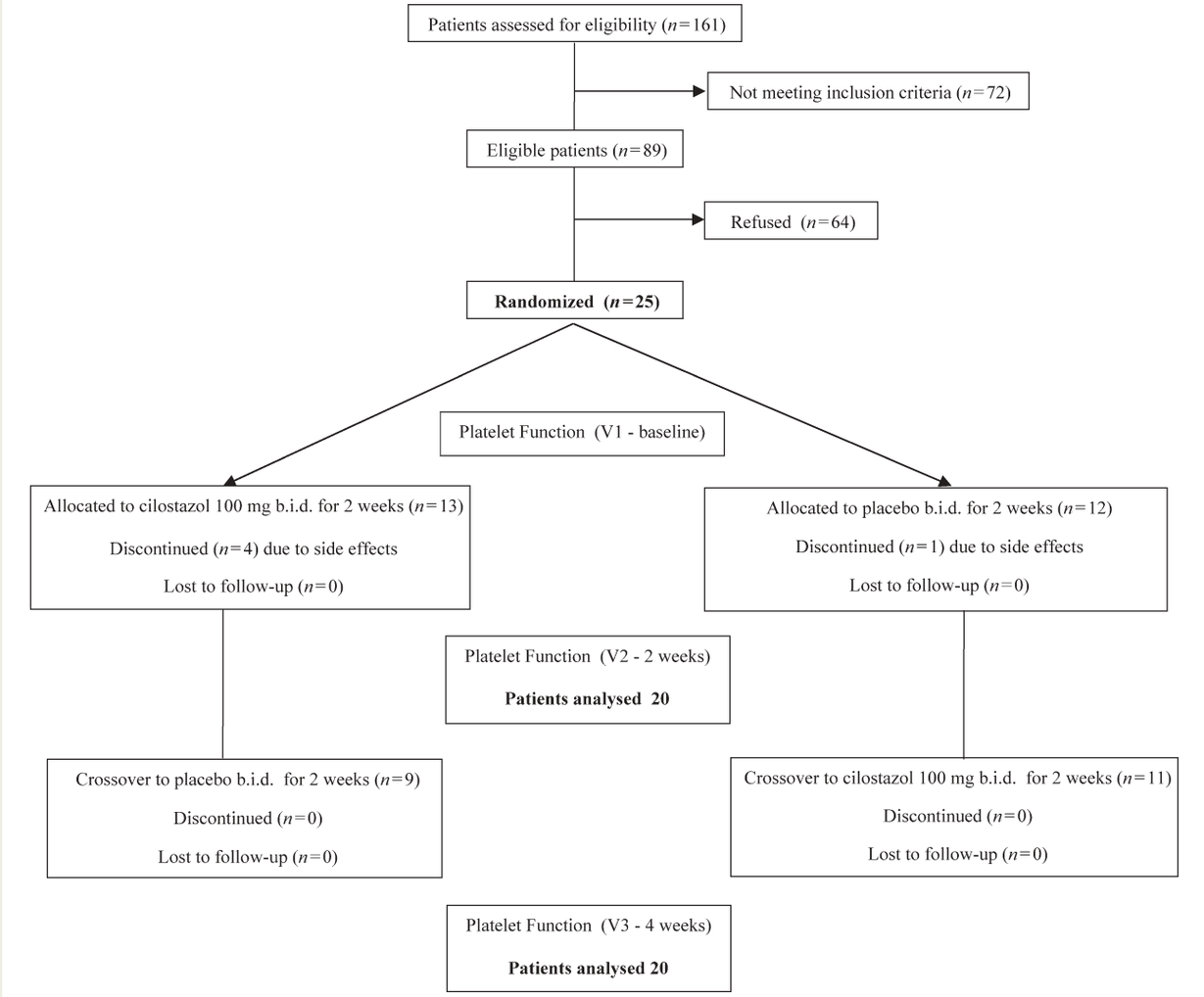

Figure 1 Flow diagram of the study.

$P2Y_{12}$ reactivity index and phosphorylated status of vasodilator-stimulated phosphoprotein

PRI was determined through assessment of VASP-P according to standard protocols.[7,26] VASP-P was measured by quantitative flow cytometry (Beckman Coulter FC500, Miami, FL) using commercially available labelled monoclonal antibodies (Biocytex Inc., Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels following challenge with prostaglandin E_1 (PGE_1) and PGE_1+adenosine diphosphate (ADP). PGE_1 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 PGE_1-stimulated platelets reduces levels of PGE_1-induced VASP-P.[7,26] The PRI was calculated as follows: ([MFI PGE_1] − [MFI PGE_1+ADP]/[MFI PGE_1]) × 100%.[7,26] A reduced PRI is indicative of greater inhibition of the $P2Y_{12}$ signalling pathway.[7,26]

VerifyNow assay

The VerifyNow™ assay (Accumetrics, Inc., San Diego, CA, USA) is a rapid whole-blood point-of-care and was utilized according to the instructions of the manufacturer.[27,28] The VerifyNow™ $P2Y_{12}$ assay reports the results as $P2Y_{12}$ reaction units (PRU). This assay mimics turbidometric aggregation and utilizes disposable cartridges containing 20 μM ADP and 22 nM PGE_1. Aggregation testing using ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signalling, while adding PGE_1 increases the specificity of the test for $P2Y_{12}$ signalling.[29] In a separate channel of the cartridge in which iso-TRAP is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of platelet inhibition without having to wean the patient off antiplatelet treatment. Sensitivity to aspirin was evaluated using the VerifyNow system by means of dedicated cartridges containing arachidonic acid. Results are expressed as aspirin reaction units (value ≥550 indicates aspirin responsiveness).[27]

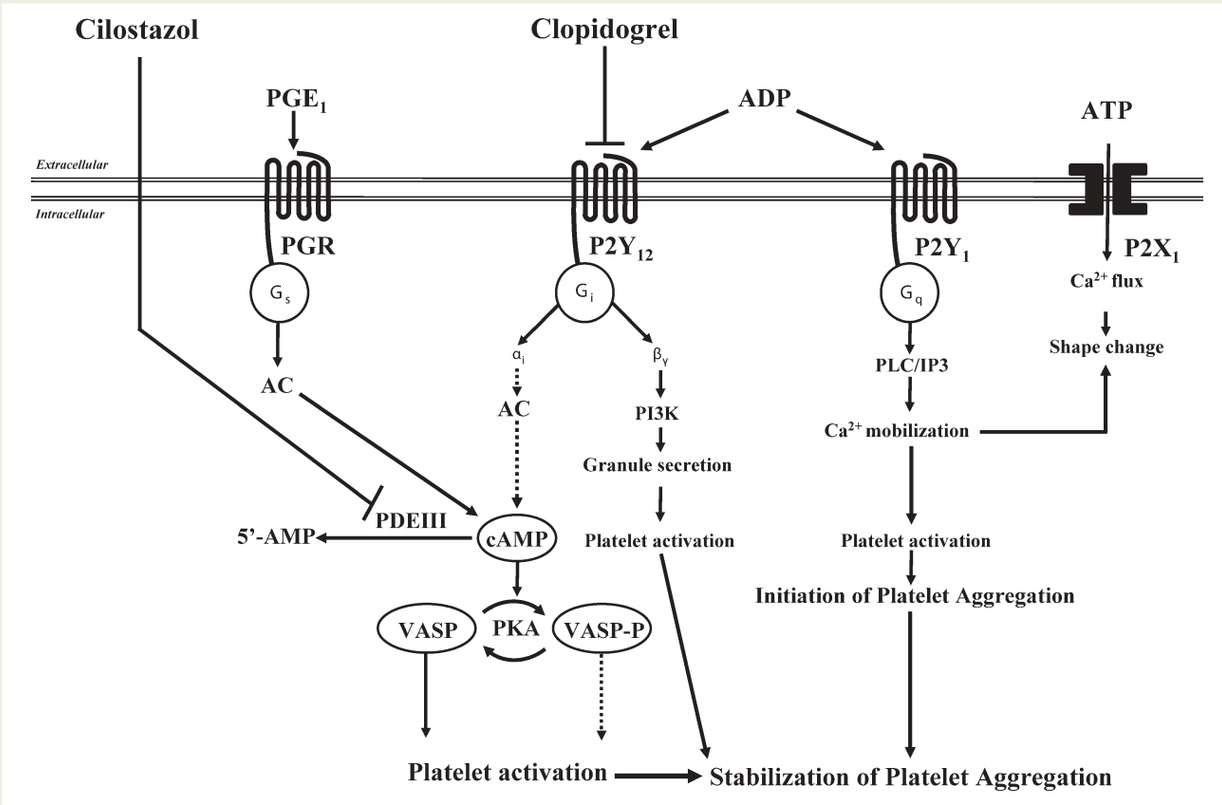

Figure 2 Mechanism of action of cilostazol and postulated modulation of $P2Y_{12}$ receptor signalling. Three P2 receptors are present on human platelets: $P2X_1$, $P2Y_1$, and $P2Y_{12}$. $P2X_1$ mediates extracellular calcium influx and utilizes adenosine triphosphate (ATP) as an agonist. $P2Y_1$ and $P2Y_{12}$ are G-coupled proteins which utilize adenosine diphosphate (ADP) as an agonist. The binding of ADP to the G_q-coupled $P2Y_1$ receptor leads to the activation of phospholipase C (PLC) which generates inositol triphosphate (IP3) leading to mobilization of intracellular calcium inducing alteration in shape and initiate a weak and transient phase of platelet aggregation. The binding of adenosine diphosphate to the G_i-coupled $P2Y_{12}$ receptor liberates the G_i protein subunits α_i and β_γ and leads to the stabilization of platelet aggregation. The α_i subunit leads to inhibition of adenylyl cyclase (AC), which reduces cyclic adenosine monophosphate (cAMP) levels. The decrease in cyclic adenosine monophosphate production reduces the activation of specific protein kinases (PKA), which in turn decreases phosphorylation (P) of vasodilator-stimulated phosphoprotein (VASP) leading to increased platelet activation and aggregation; the subunit β_γ activates kinases which induce granule secretion. Prostaglandin E_1 (PGE_1) activates adenylyl cyclase which increases cyclic adenosine monophosphate levels and VASP-P. Following hepatic metabolism, the active metabolite of clopidogrel irreversibly inhibits the $P2Y_{12}$ receptor, increasing cyclic adenosine monophosphate levels and VASP-P. Cilostazol increases cyclic adenosine monophosphate levels by means of PDEIII inhibition, potentially modulating VASP-P and intraplatelet $P2Y_{12}$ signalling. Type 2 diabetes mellitus patients have upregulation of $P2Y_{12}$ signalling secondary to low cyclic adenosine monophosphate levels, therefore making platelets of these patients a target to test for the effects of cilostazol on modulating receptor signalling. Solid arrows indicate activation. Dotted arrows indicate inhibition.

Light transmittance aggregometry

Platelet aggregation was performed using light transmittance aggregometry (LTA) according to standard protocols.[1–2,7] In brief, platelet aggregation was assessed using platelet-rich plasma (PRP) by the turbidimetric method in a two-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp., Havertown, PA). PRP was obtained as a supernatant after centrifugation of citrated blood at 800 r.p.m. for 10 min. The isolated PRP was kept at 37°C before use. Platelet-poor plasma (PPP) was obtained by a second centrifugation of the blood fraction at 2500 r.p.m. for 10 min. Light transmission was adjusted to 0% with PRP and to 100% for PPP for each measurement and assessed following challenge with ADP (5 and 20 μmol/L) in the presence and absence of PGE_1 (5 nM). Similar to the VASP-P and VerifyNow $P2Y_{12}$ assays, PGE_1 was added to the LTA assay to make the test more reflective of $P2Y_{12}$ signalling.[29] The concentration of PGE_1 chosen was that used in correlation analysis between LTA and the VerifyNow $P2Y_{12}$ assay which served for FDA approval of the latter device. Curves were recorded for 6 min. Aggregation was measured at peak (Agg_{max}) and at 5 min (Agg_{late}).[7] Inhibition of platelet aggregation (IPA) was defined as the percent decrease in Agg_{max} and Agg_{late} values.[7] Percentage of platelet disaggregation (D) between Agg_{max} and Agg_{late} values was defined as: $D\ (\%) = 100 \times (1 - Agg_{late}/Agg_{max})$.[7] Arachidonic acid-induced platelet aggregation was also performed in order to assess compliance and responsiveness to aspirin, defined as Agg_{max} <20% following 1 mmol/L arachidonic acid stimuli.[30]

Statistical analysis

Categorical variables are expressed as frequencies and percentages. Continuous variables are presented as mean ± SD. Continuous variables were analysed for a normal distribution with the Shapiro–Wilks goodness-of-fit test (using *P*-value <0.1 as threshold). Only patients who successfully completed at least one treatment phase of the study were considered for comparisons. Paired *t*-tests were used for comparison of normally distributed continuous variables in the same group. Wilcoxon tests were used for paired comparisons of continuous variables not following a normal distribution. Treatment effects were evaluated comparing the functional parameters observed in the overall patient population after cilostazol treatment with those achieved after placebo regardless of the sequence in which patients received either cilostazol or placebo. The absolute between-treatment mean differences and 95% confidence intervals (CI) for the functional endpoints specific to $P2Y_{12}$ receptor signalling were then estimated in the two groups. In addition, in order to perform an unbiased estimation of the treatment effect, period and sequence effects, which may occur in crossover studies, were evaluated. To test for sequence treatment effects, we evaluated the functional endpoints within the two treatment sequences in which patients received either cilostazol or placebo. In particular, we compared the pre-crossover with the post-crossover functional values within the treatment sequence in which patients were randomized to placebo first and then cilostazol, and within the other sequence in which patients were randomized to cilostazol first and then placebo. Then, the absolute mean differences between the pre-crossover and the post-crossover values and comparisons of these between-treatment differences achieved in each sequence were computed. To test for period effects, for each sequence the average of the difference of the two periods and the sum of these two averages were calculated and then these two achieved averages of each sequence were compared. A *P*-value <0.05 was considered statistically significant. Statistical analysis was performed using a SPSSv14.0 software (SPSS Inc., Chicago, IL).

Results

A total of 25 patients were randomized. Baseline demographics and clinical characteristics of randomized patients are shown in *Table 1*. Following randomization, a total of five patients withdrew from the study within 48–72 h due to the development of side effects. Side effects leading to study drug discontinuation included headaches, gastrointestinal symptoms, and tachycardia. These occurred in four patients treated with cilostazol and in one treated with placebo. Similar side effects, but of lower severity not leading to withdrawal of study medication, occurred in three patients treated with cilostazol and in one patient treated with placebo. Therefore, a total of 20 patients were available to test for the study hypothesis (*Figure 1*). There were no differences in baseline demographics between patients who did and did not complete the study protocol (data not shown). During the study period, there were no changes in medical therapy, including T2DM medications or anti-anginal therapy. There were no bleeding complications or other serious adverse events during the study. At all three study visits, all randomized patients were compliant and sensitive to aspirin based on LTA assessments (arachidonic-acid-induced aggregation <20%). At all three study visits, using the VerifyNow aspirin assay, all randomized patients had ARU values <550 except for one patient who had an ARU above this value at only one time point.

Table 1 Baseline demographics and clinical characteristics

Variable	*n* = 20
Age (years)	64 ± 10
Gender (Male), *n* (%)	15 (60)
Race, *n* (%)	
Caucasian	19 (76)
African-American	5 (20)
Hispanic	1 (4)
Risk factors/past medical history, *n* (%)	
Insulin-dependent diabetes	9 (36)
Non-insulin-dependent diabetes	16 (64)
HbA1C	7.7 ± 1.8
Smoking	6 (24)
Hyperlipidaemia	23 (92)
TC (mg/dL)	163 ± 54
LDL-C (mg/dL)	86 ± 37
HDL-C (mg/dL)	38 ± 11
TG (mg/dL)	205 ± 213
Hypertension	24 (96)
Body mass index (kg/m^2)	31 ± 5
Prior myocardial infarction	16 (64)
Prior CABG	7 (28)
Multivessel CAD	19 (76)
Treatment, *n* (%)	
Beta-blockers	19 (76)
Nitrates	5 (20)
ACE inhibitors/ARB	21 (84)
CYP3A4 metabolizing statin	17 (68)
Non-CYP3A4 metabolizing statin	5 (20)

HbA1C, haemoglobin A1C; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; CAD, coronary artery disease; CABG, coronary artery bypass grafting; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; CYP3A4, cytochrome P450 3A4 isoenzyme.

Significantly lower PRI values were observed following treatment with cilostazol vs. placebo ($P = 0.0002$; primary endpoint; *Figure 3*). The absolute between-treatment difference of PRI was 23.6 (95% CI, 14.1–33.2). In the sequence in which patients ($n = 11$) were randomized to placebo first and then cilostazol, the pre-crossover PRI value was 59.6 ± 17.7 and the post-crossover value was 31.6 ± 17.7, resulting in a significant reduction in PRI crossing from placebo to cilostazol ($P = 0.001$). In the sequence in which patients ($n = 9$) received cilostazol first and then placebo, the pre-crossover PRI value was 42.4 ± 21.2 and the post-crossover value was 60.3 ± 14.7, resulting in a significant increase in PRI crossing from cilostazol to placebo ($P = 0.008$). The comparison of absolute between-treatment differences in PRI measurements for each treatment sequence was not significant ($P = 0.23$). Also, no period effect was observed ($P = 0.68$).

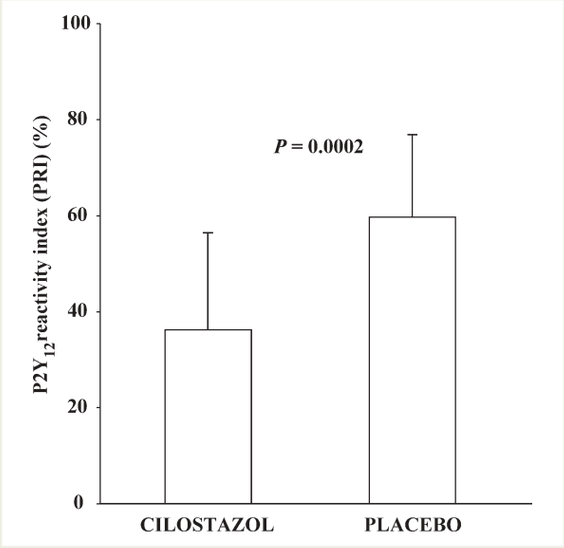

Figure 3 $P2Y_{12}$ reactivity index (PRI) following treatment with cilostazol vs. placebo (primary endpoint). Values are expressed as percentage (%) of $P2Y_{12}$ reactivity index. Error bars indicate standard deviations of the mean.

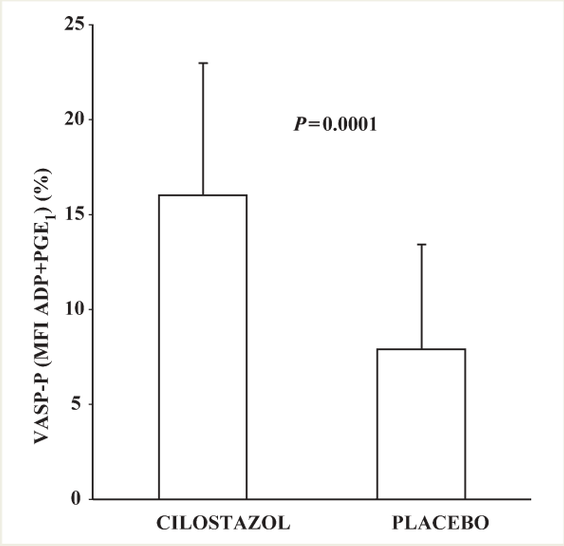

Figure 4 Content in phosphorylated vasodilator-stimulated phosphoprotein (VASP-P) following treatment with cilostazol vs. placebo. Values are expressed as mean fluorescence intensity (MFI) after coadministration of adenosine diphosphate (ADP)+ Prostaglandin E_1 (PGE_1). Error bars indicate standard deviations of the mean.

Flow cytometric assessment of VASP-P content was significantly higher following treatment with cilostazol vs. placebo ($P = 0.0001$; *Figure 4*). Using the VerifyNow $P2Y_{12}$ assay, $P2Y_{12}$ inhibition ($P = 0.0001$) and PRU values ($P = 0.002$) were significantly higher and lower, respectively, following treatment with cilostazol vs. placebo (*Figure 5*).

LTA parameters more specific to $P2Y_{12}$ signalling by using PGE_1 in combination with ADP showed significantly lower platelet reactivity values following treatment with cilostazol compared with placebo, while no differences were observed between treatment groups using ADP as a sole agonist (*Table 2*). Accordingly, treatment with cilostazol led to a significant increase in IPA for all LTA parameters using PGE_1 in combination with ADP ($P < 0.0001$). Platelet disaggregation was also increased with cilostazol therapy compared with placebo (ADP 20 μmol/L+PGE_1: 64 ± 21 vs. $35 \pm 23\%$, $P < 0.0001$; ADP 5 μmol/L+PGE_1: 72 ± 18 vs. $56 \pm 27\%$, $P = 0.02$). The absolute between-treatment mean differences and 95% CI for the endpoints specific to $P2Y_{12}$ signalling are summarized in *Table 3*. As observed with the primary endpoint (PRI), no significant sequence and period effects were found for all evaluated secondary endpoints (data not shown).

Discussion

This platelet function study confirms the hypothesis that adjunctive treatment with cilostazol in a high-risk group of patients with T2DM and CAD already on standard dual antiplatelet therapy enhances inhibition of $P2Y_{12}$ receptor-related signalling. This was corroborated by multiple functional assessments, including flow cytometry, LTA, and a point-of-care device, evaluating this platelet signalling pathway. These findings may explain the better clinical outcomes in patients undergoing coronary stenting treated with triple compared with dual oral antiplatelet therapy[16–19] as well as why cilostazol is particularly efficacious in diabetic patients.[20–23]

Inhibition of platelet $P2Y_{12}$ receptor signalling with clopidogrel is pivotal for the prevention of thrombotic complications in patients with acute coronary syndromes and/or undergoing PCI, also in patients with diabetes mellitus.[31–33] However, despite the clinical benefit associated with clopidogrel treatment, diabetic patients continue to have a higher risk of ischaemic events, as well as stent thrombosis, compared with non-diabetics.[8–14,31,32] This may be attributed, at least in part, to the greater prevalence of inadequate $P2Y_{12}$ inhibition achieved with recommended regimens of clopidogrel in diabetic patients.[1–2,4] Numerous investigations have shown inadequate platelet inhibition in clopidogrel-treated patients to be associated with a higher risk of atherothrombotic complications, also in T2DM patients.[6,34] These findings have led to hypothesize that more potent $P2Y_{12}$ inhibition may be more beneficial, particularly in high-risk patients. The prognostic implications of achieving more potent $P2Y_{12}$ inhibition in high-risk patients has been recently demonstrated in the TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction) trial in which treatment with prasugrel led to better clinical outcomes, including reduced stent thrombosis rates, compared with clopidogrel;[35] the magnitude of this benefit was greatest in diabetic patients, the subgroup in which the clinical efficacy of prasugrel was not offset by increased bleeding.[35]

Numerous factors are responsible for suboptimal clopidogrel-induced antiplatelet effects in T2DM patients, among which abnormalities of intracellular cAMP levels play a contributing

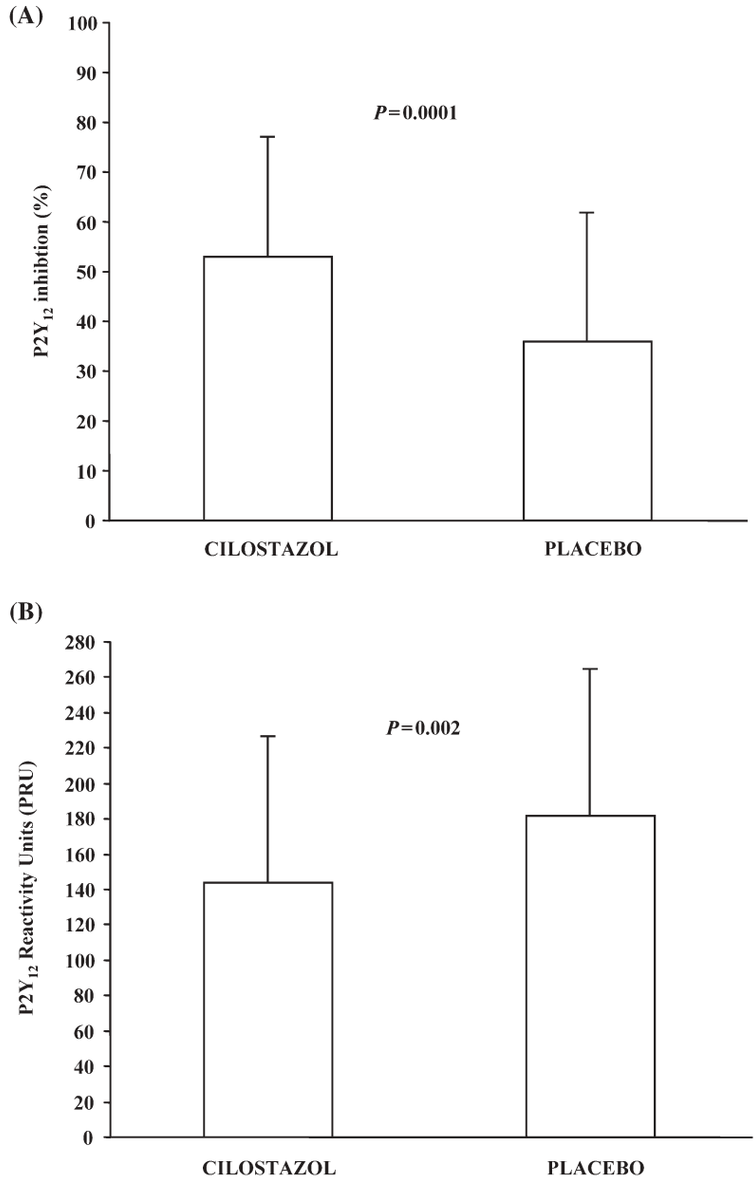

Figure 5 $P2Y_{12}$ inhibition (*A*) and $P2Y_{12}$ reactivity unit (PRU) (*B*) determined by the VerifyNow $P2Y_{12}$ assay. $P2Y_{12}$ inhibition values are expressed as percentage (%). Error bars indicate standard deviations of the mean.

role.[1–4,8–10] *In vitro* studies have shown that insulin reduces platelet aggregation by inhibiting the $P2Y_{12}$ pathway.[36] In fact, human platelets are targets of insulin, which interacts with its own receptor on the platelet surface and leads to loss of G_i activity.[9,36] This reduces cAMP suppression leading to an increase in cAMP levels, thus inhibiting $P2Y_{12}$ signalling and reducing platelet reactivity. However, platelets of T2DM patients have decreased sensitivity to insulin leading to lower cAMP levels and reduced $P2Y_{12}$ inhibition, which overall leads to increased platelet reactivity.[1–4] Since enhanced platelet reactivity is pivotal to the recurrence of ischaemic events, more aggressive and tailored antiplatelet treatment regimens may be warranted in T2DM patients.[37] The present study demonstrates that treatment with cilostazol, which exerts its antiplatelet effects by increasing cAMP, enhances $P2Y_{12}$ inhibition in these high-risk patients as determined by assays which specifically assess this signalling pathway. On the contrary, there were no effects induced by cilostazol when assessments less specific to evaluate this signalling pathway were used (e.g. LTA using ADP as a sole agonist). The fact that in our study cilostazol did not inhibit ADP-induced aggregation may be attributed to the fact that this is less sensitive than VASP-P to assess $P2Y_{12}$ signalling.[6] In addition, diabetic platelets have increased exposure to ADP, which stimulate all purinergic receptors, and the mechanism through which cilostazol exerts its inhibitory effects is not on the platelet surface but downstream[8,9] (*Figure 2*).

Table 2 Platelet aggregation assessed by light transmittance aggregometry

Variable	Placebo	Cilostazol	*P*-value (placebo vs. Cilostazol)
ADP+PGE_1 (%)			
Maximum ADP 20 μmol/L+PGE_1	40.2 ± 17.4	22.7 ± 11	0.0001
Late ADP 20 μmol/L+PGE_1	29.1 ± 19.1	9.6 ± 10	0.0001
Maximum ADP 5 μmol/L+PGE_1	25.0 ± 14.2	10.0 ± 5.5	0.0002
Late ADP 5 μmol/L+PGE_1	12.7 ± 14.6	2.4 ± 2.3	0.001
ADP (%)			
Maximum ADP 20 μmol/L	48.8 ± 12.1	49.1 ± 18.1	0.93
Late ADP 20 μmol/L	41.5 ± 16.5	38.5 ± 21.7	0.51
Maximum ADP 5 μmol/L	34.4 ± 10.4	33.1 ± 14.3	0.82
Late ADP 5 μmol/L	20.7 ± 14.9	18.7 ± 15.7	1.0

Table 3 Absolute between-treatment differences and 95% CI of platelet $P2Y_{12}$-specific functional measures

Variable	Between-treatment difference	95% CI
Flow cytometry		
PRI	23.6	14.1–33.2
VASP-P	8.2	4.3–12.0
VerifyNow $P2Y_{12}$ assay		
$P2Y_{12}$ inhibition	16.4	9.0–23.8
PRU	38.6	15.1–62.2
Light transmittance aggregometry		
Maximum ADP 20 μmol/L+PGE_1	17.5	10.5–24.4
Maximum ADP 5 μmol/L+PGE_1	15.0	8.5–21.6
Late ADP 20 μmol/L+PGE_1	19.5	11.2–27.8
Late ADP 5 μmol/L+PGE_1	10.3	4.0–16.7

PRI, $P2Y_{12}$ reactivity index; VASP-P, phosphorylated status of vasodilator-stimulated phosphoprotein; PRU, $P2Y_{12}$ reactivity units; ADP, adenosine diphosphate; PGE_1, prostaglandin E_1.

Cilostazol is currently approved by the FDA only for symptomatic relief of claudication pain in patients with peripheral arterial disease and for stroke prevention in Japan. In addition to its effects on platelets, cilostazol increases cAMP levels by means of PDEIII inhibition in endothelial and smooth muscular cells.[15] In Asian countries, cilostazol is used as an antiplatelet agent for secondary prevention of ischaemic events and is often preferred over aspirin or thienopyridines due to its comparable efficacy and more favourable safety profile.[15,38] Treatment with cilostazol, in fact, has shown to be associated with reduced bleeding compared with other antiplatelet agents.[15,38] Lower bleeding complications associated with cilostazol have been attributed to its protective effects on endothelial cells.[15,38,39] Functional studies have shown cilostazol to have antiplatelet effects similar to that achieved with thienopyridines.[40] In Asian countries, cilostazol has been broadly used in combination with aspirin for the prevention of stent thrombosis in patients undergoing coronary stenting, showing similar efficacy to thienopyridines.[41,42] Cilostazol also reduces neointimal proliferative reactions following coronary stent implantation, including DESs.[17–23] In clinical studies, triple antiplatelet therapy has shown to reduce major adverse cardiac events and stent thrombosis compared with dual antiplatelet therapy without increasing the risk of bleeding.[16–19,23] Of note, cilostazol has shown to be more efficacious in inhibiting neointimal proliferative reactions and reducing adverse clinical events in patients with diabetes.[17–21,23] These findings underscore the importance of the intraplatelet signalling pathway targeted by cilostazol in these patients.

Despite the functional implications of adjunctive treatment with cilostazol, as shown in this study, the use of this antiplatelet agent is accompanied by an elevated prevalence of side effects which often lead to discontinuation of treatment. The rate of treatment discontinuation in this study (~15%) was similar to that previously reported.[17] Indeed, it may be argued that initiation of cilostazol therapy with lower doses could have reduced the prevalence of side effects. However, the dose chosen in this study was that used in clinical studies testing the efficacy of triple antiplatelet therapy.[16–23] Overall, the elevated prevalence of side effects associated with cilostazol support the need for alternative treatment strategies to achieve greater $P2Y_{12}$ inhibition. Novel oral $P2Y_{12}$ receptor antagonists currently under advanced clinical investigation, which have more potent antiplatelet effects compared with clopidogrel, may represent potential future alternatives to treat these high-risk patients.[6] However, the increased risk of bleeding observed with more potent $P2Y_{12}$ inhibition, particularly in specific subgroups, remains of concern.[35,37,43] To this extent, the fact that clinical studies have not shown any increase in bleeding risk in patients treated with triple compared with dual antiplatelet therapy is noteworthy.[15–23] Thus, when tolerated, the use of cilostazol in adjunct to standard dual antiplatelet therapy may represent a treatment option particularly in high-risk patients who warrant

more potent $P2Y_{12}$ inhibition, but in whom bleeding may be of concern. The ever raising apprehension for stent thrombosis in the DES era has currently induced many interventionalists to use triple antiplatelet therapy in their high-risk patients. However, to date, the functional implications of this therapeutic approach has been poorly explored and studies have been limited to Asian populations, which are known to have different sensitivity to antiplatelet agents compared with non-Asian populations.[15] The present study is the first to specifically address the impact of cilostazol on $P2Y_{12}$ inhibition in a high-risk clinical scenario composed of patients with T2DM.

Study limitations

The present study did not account for conducting multiple significance tests or for potential correlation among various measures of platelet function. Also, our study was not powered to evaluate the safety and efficacy associated with the use of cilostazol in addition to aspirin and clopidogrel. Since our study did not include a wash-out phase between treatment periods, it may be questioned that carry over effects could have occurred and affected the study results. However, cilostazol is a reversible platelet inhibitor with a short half-life (cilostazol and its active metabolites have apparent elimination half-lives of ~11–13 h).[15] Since platelet function was performed 14 days after crossover, it is improbable that the treatment effects carried over throughout the following phase. In addition, our study hypothesis was confirmed in the within sequence analyses. Ultimately, our study findings cannot be extrapolated to non-diabetic patients who do not have the same dysfunctional status of platelet signalling pathways as diabetics.

Conflict of interest: D.J.A. is a consultant and on the speaker's bureau for Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly, Daiichi Sankyo, and Portola; S.G. has received honoraria and consulting fees from Eisai, Sanofi-Aventis, Daiichi-Sankyo, GlaxoSmithKline, Bristol-Myers Squibb, Otsuka, Bayer, Schering-Plough, Takeda, Astellas, AstraZeneca, Novartis. and Kowa. S.G. has also received research grants from Pfizer, Ono, Eisai, Otsuka, Daiichi-Sankyo, Sanofi-Aventis, Takeda, and Astellas within the last 3 years; M.M.Z. is a consultant and on the speaker's bureau for Bristol Myers Squibb and Sanofi-Aventis.

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