Impact of adjunctive cilostazol therapy on platelet function profiles in patients with and without diabetes mellitus on aspirin and clopidogrel therapy

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Summary
Cilostazol is a platelet inhibitor which when added to aspirin and clopidogrel has shown to reduce the risk of recurrent ischaemic events without an increase in bleeding. These clinical benefits have shown to be more pronounced in patients with diabetes mellitus (DM). However, it remains unknown whether cilostazol exerts different pharmacodynamic effects in patients with and without DM. This was a randomised, double-blind, placebo-controlled, cross-over pharmacodynamic study comparing platelet function in patients with and without DM on aspirin and clopidogrel therapy. Patients (n=111) were randomly assigned to either cilostazol 100 mg or placebo twice daily for 14 days and afterwards crossed-over treatment for another 14 days. Platelet function was performed at baseline, 14 days post-randomisation, and 14 days post-cross-over. Functional testing to assess P2Y12 signalling included flow cytometric analysis of phosphorylation status of vasodilator-stimulated phosphoprotein measured by P2Y12 reactivity index (PRI), light transmittance aggregometry and VerifyNow. Thrombin generation processes were also studied using thrombelastography. Significantly lower PRI values were observed following treatment with cilostazol compared with placebo both in DM and non-DM groups (p < 0.0001). The absolute between-treatment differences of PRI between groups was a 35.1% lower in patients with DM (p=0.039). Similar results were obtained using all other functional measures assessing P2Y12 signalling. Thrombin generation was not affected by cilostazol. Cilostazol reduces platelet reactivity both in patients with and without DM, although these pharmacodynamic effects are enhanced in patients with DM. Despite the marked platelet inhibition, cilostazol does not alter thrombin-mediated haemostatic processes, which may explain its ischaemic benefit without the increased risk of bleeding.

Keywords
Diabetes mellitus, coronary artery disease, platelets

Introduction
Cilostazol is a selective and reversible inhibitor of phosphodiesterase 3 (PDEIII) which exerts platelet inhibitory effects by increasing intraplatelet cyclic adenosine monophosphate (cAMP) levels (1, 2). Vascular smooth muscle cells and endothelial cells also are targets of cilostazol effects (1, 2). In patients undergoing percutaneous coronary interventions (PCI), both with bare metal and drug-eluting stents (DES), numerous studies have shown that treatment with cilostazol in adjunct to aspirin and thienopyridine therapy (“triple antiplatelet therapy”) is associated with a reduced risk of recurrent ischaemic events, including stent thrombosis, compared with standard dual antiplatelet therapy (3–11). Importantly, despite the more aggressive platelet inhibiting strategy, triple antiplatelet therapy is not associated with increased bleeding compared to standard dual antiplatelet therapy (3–11).

The clinical benefit of cilostazol has shown to be more prominent in high risk settings, particularly in patients with diabetes mellitus (DM) (3–11). This may be explained by the fact that cilostazol strongly impacts intraplatelet cAMP levels, which are grossly abnormal in patients with DM, making them particularly susceptible to cilostazol effects (12–15). Pilot observations from our group showing a marked increase in CAMP-mediated phosphorylation of vasodilator-stimulated phosphoprotein (VASP-P) and P2Y12 inhibitory effects are in line with this hypothesis (15). However, if cilostazol is more effective in inhibiting platelet function in patients with DM compared with patients without DM, thus contribute to the different degree of clinical benefit observed with ci-
lostazol in these patient populations, remains unknown. The primary aim of the present study was to compare cilostazol-induced effects on platelet P2Y12 inhibition in patients with and without DM on treatment with standard dual antiplatelet therapy. The study hypothesis is that cilostazol achieves greater platelet inhibition in patients with DM compared with patients without DM.

Methods

Study design and patient population

This was a prospective, randomised, double-blind, double-dummy, placebo-controlled platelet function study with a crossover design. Patients with and without DM were eligible for the study if they were between 18 and 75 years of age, had previously undergone elective coronary stenting and were on aspirin (81 mg/daily) and clopidogrel (75 mg/daily) therapy for at least 30 days. Patients with DM needed to be on hypoglycaemic treatment (oral medications or insulin) for at least 30 days. General exclusion criteria included: known allergies to aspirin, clopidogrel or cilostazol; left ventricular ejection fraction <35%; blood dyscrasia; gastrointestinal bleed within last six months; any malignancy; concomitant use of other antithrombotic drugs (oral anticoagulants, dipyridamole, ticlopidine) or non-steroid anti-inflammatory drugs; recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist; platelet count <100 x10^11/ml; haematuria; diastolic blood pressure >90 mmHg; liver disease (bilirubin level > 2 mg/dl). Patients were recruited from the outpatient cardiology clinic of the University of Florida College of Medicine Shands Jacksonville Hospital.

Patients within DM and non-DM groups were randomised to receive cilostazol 100 mg or matching placebo tablets twice daily for 14 days and afterwards crossed-over treatment assignments for another 14 days. Placebo and treatment tablets were provided by Otsuka Pharmaceuticals (Tokyo, Japan). Investigators, laboratory personnel, and patients were blinded to treatment assignments. Unblinding was done at the end of the study. Platelet function testing was performed at three time points: (i) at baseline (before randomisation); (ii) 14 days after randomisation; (iii) 14 days after cross-over. Only patients who completed both treatment periods were included in the analysis. Patient compliance to antiplatelet treatment was assessed by interview and pill counting.

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 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.

Endpoints and sample size calculation

The primary endpoint of this study was the comparison of the mean differences in P2Y12 reactivity index (PRI) following treatment with cilostazol versus placebo achieved in patients with and without DM. We assumed an absolute between-treatment difference in PRI within the DM group of 23.6, which was that previously demonstrated in our pilot study (15). Considering a standard deviation of 16, a sample size of 38 patients per group would be required to provide a 90% power to detect a 50% lower absolute between-treatment difference within the non-DM cohort, with a two-sided α-level of 0.05. Other endpoints included comparisons in platelet function profiles by means of other assays assessing P2Y12 receptor signalling including light transmittance aggregometry (LTA) and VerifyNow P2Y12 testing. Ultimately, an analysis to evaluate if cilostazol affects thrombin generation assessed by thrombelastography was performed.

Blood sampling and laboratory assessments

Blood samples were collected 2–4 hours (h) after intake of antiplatelet medications (aspirin, clopidogrel, and cilostazol/placebo) from an antecubital vein. The first 2–4 ml of blood were discarded to avoid spontaneous platelet activation and samples were processed for functional assessments within 1 h after blood drawing.

P2Y12 Reactivity index (PRI)

The PRI was determined through assessment of VASP-P according to standard protocols (15, 16). VASP-P was measured by quantitative flow cytometry (Beckman Coulter FC500, Miami, FL, USA) 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 E1 (PGE1) and PGE1 plus adenosine diphosphate (ADP). PGE1 increases VASP-P levels through stimulation of adenylyl cyclase; ADP binding to purinergic receptors leads to inhibition of adenylyl cyclase; thus, the addition of ADP to PGE1-stimulated platelets reduces levels of PGE1-induced VASP-P. The PRI was calculated as follows: ([MFI PGE1]-[MFI PGE1+ADP])/[MFI PGE1]×100%. A reduced PRI is indicative of greater inhibition of the P2Y12 signalling pathway (15, 16).

VerifyNow assay

The VerifyNow assay (Accumetrics, Inc., San Diego, CA, USA) is a rapid whole-blood point-of-care and was utilised according to the instructions of the manufacturer (15, 17). The VerifyNow P2Y12 assay reports the results as P2Y12 reaction units (PRU). This assay mimics turbidometric aggregation and utilises disposable cartridges containing 20 mM ADP and 22 nM PGE1; Aggregation testing using ADP as a sole agonist activates P2Y1 and P2Y12 puriner-
Elastic signalling, while adding PGE\textsubscript{1} increases the specificity of the test for P2Y\textsubscript{12} signalling (18). 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 (AA). Results are expressed as aspirin reaction units (ARU; value $\geq$550 indicates aspirin responsiveness) (15, 17).

Light transmittance aggregometry (LTA)
Platelet aggregation was performed using LTA according to standard protocols (15, 16). 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, USA). PRP was obtained as a supernatant after centrifugation of citrated blood at 80 rpm for 10 minutes (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 2,500 rpm 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 $\mu$M) in the presence of PGE\textsubscript{1} (5 nM) in order to make the test more reflective of P2Y\textsubscript{12} signalling (15, 18). The final extent of platelet aggregation, measured at 5 min, was recorded (15). AA-induced platelet aggregation was also performed in order to assess compliance and responsiveness to aspirin, defined as platelet aggregation $\geq$20% following 1 mM AA stimuli (15, 16).

Thrombelastography
The Thrombelastograph® (TEG®) Hemostasis System (Haemoscope Corporation, Niles, IL, USA) equipped with automated software for the determination of the first derivative was used according to the manufacturer’s instructions (19, 20). With this technology, several parameters related to the rate of development of the tensile strength of the developing clot are produced from the first derivative of the waveform generated by the TEG system. In brief, TEG is a viscoelastic monitor that measures platelet-fibrin-mediated clot strength through a rotating sample cup with a stationary pin suspended by a torsion wire. The torque of the rotating cup is transmitted to the pin immersed in the blood sample and the movement of the pin, which depends on the contribution of platelets to the clot strength through platelet-fibrin binding, is transformed into an electrical signal generating a tracing. The reaction time ($R$), expressed in minutes, is a measure of time to initial thrombin induced platelet-fibrin clot formation and has been correlated with the velocity of thrombin generation (21). The analytical software of the TEG system also allows use of the first derivative of the waveform generated by the system to determine the time to maximum rate of thrombin generation (TMRTG). About 1 ml of heparinised blood was transferred to a vial containing kaolin and mixed by inversion. Afterwards, 500 $\mu$l of the activated blood was transferred to a vial containing heparinase and mixed to neutralise the heparin effect. The neutralised blood (360 $\mu$l) was immediately added to a heparinase-coated cup and assayed in the TEG analyser.

Statistical analysis
Categorical variables are expressed as frequencies and percentages and were compared with the use of chi-square test or Fisher’s exact test, as appropriate. Continuous variables are presented as mean ± standard deviation. Continuous variables were analysed for a normal distribution with the Shapiro-Wilk goodness-of-fit test (using a p-value $<0.1$ as threshold). Paired and non-paired t-tests were used for comparison of normally distributed continuous variables in the same and different groups, respectively. Wilcoxon and Mann-Whitney’s U tests were used for paired and non-paired comparisons of continuous variables not following a normal distribution, respectively.

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 within the DM and non-DM groups. The absolute between-treatment mean differences and 95% confidence intervals (CI) for the functional endpoints specific to P2Y\textsubscript{12} receptor signalling were estimated and then compared in the diabetic and non-diabetic groups. Generalised linear models were computed to correct for differences between DM and non-DM in the PRI values after treatment with placebo and before treatment with cilostazol, in the comparison between placebo versus cilostazol and in the one between cilostazol versus PRI value pre-cilostazol, respectively. In addition, to allow for an unbiased estimation of the treatment effect, period and sequence effects were evaluated both within the DM and non-DM groups. To test for sequence treatment effects, we evaluated the primary endpoint 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 randomised to placebo first and then cilostazol, and within the other sequence in which patients were randomised to cilostazol first and then placebo. Then, the absolute mean differences between the pre-crossover and the post-crossover values achieved in each sequence were compared within and among the DM and non-DM groups. 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 SPSSv 15.0 software (SPSS Inc., Chicago, IL, USA).
Results

Patient disposition is illustrated in Figure 1. A total of 111 patients were randomised. Of these, 58 and 53 patients with and without DM were randomized, respectively. Following randomisation, a total of 26 (23.4%) patients withdrew from the study within 48–72 h because of side effects (headaches, gastrointestinal symptoms, and tachycardia). These were more common with cilostazol compared with placebo (18% vs. 5.4%; p=0.007). Side effects leading to withdrawal occurred in 14 (12.6%) and six (5.4%) patients treated with cilostazol and in three (2.7%) and three (2.7%) treated with placebo, in the DM and non-DM groups, respectively. Similar side effects, but of lower severity which did not lead to withdrawal of study medication, occurred in three (2.7%) and five (4.5%) patients treated with cilostazol and in one (0.9%) and two (1.8%) patients treated with placebo, in the DM and non-DM groups, respectively. Four patients decided to withdraw consent after randomisation (2 allocated to cilostazol and 2 to placebo). Finally, two samples were invalidated due to haemolysis after allocation to placebo. Therefore, a total of 79 patients were available to test the study hypothesis: 40 and 39 patients with and without DM, respectively (Fig. 1).

Baseline demographics and clinical characteristics of patients with and without DM are shown in Table 1. Among patients with DM, 40% (n=16) were on insulin therapy. The only statistically significant difference between groups was body mass index, which was higher in the DM group (Table 1). There were no differences in baseline demographics within groups of patients with and without DM who did and did not complete the study protocol (data not shown). During the study period, there were no changes in medical therapy. There were no bleeding complications or other serious adverse events during the study.

Cilostazol effects on platelet function in patients with and without DM

Significantly lower PRI values were observed following treatment with cilostazol versus placebo both within the DM (p < 0.0001)
Table 1: Baseline demographics and clinical characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DM (N=40)</th>
<th>Non-DM (N=39)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.5 ± 8.5</td>
<td>61.2 ± 8.5</td>
<td>0.73</td>
</tr>
<tr>
<td>Gender (male), n (%)</td>
<td>25 (63)</td>
<td>28 (72)</td>
<td>0.52</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>30 (77)</td>
<td>30 (75)</td>
<td>1.00</td>
</tr>
<tr>
<td>African-American</td>
<td>9 (23)</td>
<td>7 (18)</td>
<td>0.82</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (3)</td>
<td>2 (5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Risk factors/past medical history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>11 (28)</td>
<td>19 (49)</td>
<td>0.09</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>40 (100)</td>
<td>37 (95)</td>
<td>0.24</td>
</tr>
<tr>
<td>Hypertension</td>
<td>38 (95)</td>
<td>34 (87)</td>
<td>0.26</td>
</tr>
<tr>
<td>Body mass index</td>
<td>31.7±5.4</td>
<td>29.0±6.0</td>
<td>0.04</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>24 (60)</td>
<td>25 (64)</td>
<td>0.89</td>
</tr>
<tr>
<td>Prior CABG</td>
<td>13 (33)</td>
<td>8 (21)</td>
<td>0.34</td>
</tr>
<tr>
<td>Multivessel CAD</td>
<td>21 (53)</td>
<td>24 (62)</td>
<td>0.56</td>
</tr>
<tr>
<td>Treatment, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>30 (75)</td>
<td>34 (87)</td>
<td>0.27</td>
</tr>
<tr>
<td>Nitrates</td>
<td>8 (20)</td>
<td>15 (38)</td>
<td>0.12</td>
</tr>
<tr>
<td>ACE inhibitors/ARB</td>
<td>32 (80)</td>
<td>28 (72)</td>
<td>0.55</td>
</tr>
<tr>
<td>PPI-2C19 specific</td>
<td>13 (33)</td>
<td>9 (23)</td>
<td>0.50</td>
</tr>
<tr>
<td>PPI-Non 2C19 specific</td>
<td>4 (10)</td>
<td>0</td>
<td>0.12</td>
</tr>
<tr>
<td>CYP3A4 metabolising statin</td>
<td>26 (65)</td>
<td>30 (77)</td>
<td>0.36</td>
</tr>
<tr>
<td>Non-CYP3A4 metabolising statin</td>
<td>9 (23)</td>
<td>7 (18)</td>
<td>0.82</td>
</tr>
</tbody>
</table>
| DM, diabetes mellitus; CAD, coronary artery disease; CABG, coronary artery bypass grafting; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blockers; PPI, proton pump inhibitors; CYP3A4, cytochrome P450 3A4 isoenzyme.

Figure 2: P2Y12 reactivity index (PRI) in patients with and without diabetes mellitus. White boxes: cilostazol. Black boxes: placebo. Values are expressed as percentage (%) of P2Y12 reactivity index. Error bars indicate standard deviations of the mean. DM, diabetes mellitus.

Cilostazol compared to placebo was also associated with enhanced platelet inhibitory effects with regards to the other P2Y12 specific functional parameters both within the DM and non-DM cohorts (Fig. 3). However, the absolute between-treatment mean differences for all these functional parameters were higher among patients with DM as summarised in Table 2. In addition, at all three study visits, randomised patients were compliant and sensitive to aspirin based on LTA assessments (AA-induced aggregation < 20%). At all three study visits, using the VerifyNow aspirin assay, all randomised patients had ARU values <550 except for one patient who had an ARU above this value at only one time point.

Analysis within treatment sequences

Within the DM cohort in the sequence in which patients (n = 19) were randomised to placebo first and then cilostazol, the pre-crossover PRI value was 51.7 ± 23.9 and the post-crossover value was 30.3 ± 17.2, resulting in a significant reduction in PRI crossing from placebo to cilostazol (p < 0.0001). In the sequence in which patients (n = 21) received cilostazol first and then placebo, the pre-crossover PRI value was 28.9 ± 18.2 and the post-crossover value was 53.4 ± 20.4, resulting in a significant increase in PRI crossing from cilostazol to placebo (p < 0.0001). The comparison of absolute between-treatment differences in PRI measurements for each treatment sequence was not significant (p=0.57). Within the non-DM group, in the sequence in which patients (n = 19) were randomised to placebo first and then cilostazol, the pre-crossover PRI value was 48.4 ± 24.2 and the post-crossover value was 34.5 ± 16.7, resulting in a significant reduction in PRI crossing from placebo to cilostazol (p = 0.001). In the sequence in which patients (n = 20) received cilostazol first and then placebo, the pre-crossover PRI value was 31.8 ± 21.1 and the post-crossover value was 47.9 ± 23.8,
resulting in a significant increase in PRI crossing from cilostazol to placebo (p = 0.002). The comparison of absolute between-treatment differences in PRI measurements for each treatment sequence was not significant (p=0.36). Overall, no period effect was observed (p=0.46). In both sequences, although the statistical significance was not reached as this study was not powered for the within sequence analysis, the absolute between-treatment difference of PRI tended to be higher in DM compared to non-DM, 21.5 ± 16.4 vs. 13.9 ± 15.3, p=0.15 in the placebo-cilostazol sequence, respectively, and 24.5 ± 17.2 vs. 16.1 ± 19.6, p=0.15 in the cilostazol-placebo sequence, respectively.

Cilostazol and thrombin generation

No significant differences in the R values were found between cilostazol versus placebo within the DM (7.65 ± 1.43 vs. 7.59 ± 1.67,
p=0.78) as well as non-DM (7.84 \pm 2.24 vs. 7.26 \pm 1.45, p=0.20) groups. Also, TMRTG values did not significantly differ between cilostazol and placebo in DM (9.45 \pm 1.85 vs. 9.11 \pm 2.11, p=0.24) and non-DM (9.44 \pm 2.45 vs. 8.98 \pm 1.73, p=0.41) patients. Individual R and TMRTG values after cilostazol and placebo are shown in Figure 4.

There were no significant changes from the pre-cilostazol R values to those following treatment with cilostazol both within the DM (7.65 \pm 1.43 vs. 7.62 \pm 1.71, p=0.91) and non-DM (7.84 \pm 2.24 vs. 7.32 \pm 1.78, p=0.21) groups, respectively. Similarly, no significant differences were observed with respect to the TMRTG values both within the DM (9.45 \pm 1.85 vs. 9.30 \pm 2.19, p=0.76) and non-DM (9.44 \pm 2.45 vs. 9.00 \pm 2.13, p=0.34) groups, respectively.

Discussion

The present study was designed to evaluate and to compare the functional implications of adjunctive cilostazol treatment in patients with and without DM on treatment with aspirin and clopidogrel. In a previously reported pilot study from our group conducted in a smaller cohort of patients with DM, adjunctive treatment with cilostazol increased inhibition of platelet P2Y12 receptor-mediated signalling (15). This led to the design of this larger and more comprehensive pharmacodynamic study which confirms these findings and extends these functional observations to patients without DM in whom cilostazol also induces marked platelet inhibition. However, patients with DM experience greater inhibitory effects with cilostazol than those without DM, confirming our primary study hypothesis. This is supported by the concordant findings observed from the variety of functional assays used in this study, which included flow cytometry, point-of-care testing, and LTA. Our study further expands on the understanding of the pharmacodynamic actions of cilostazol showing that despite the marked platelet inhibition achieved, this does not affect thrombin-mediated haemostatic processes.

Numerous clinical studies have shown that, in patients undergoing coronary stenting, treatment with cilostazol in adjunct to standard dual antiplatelet therapy with aspirin and clopidogrel is...
associated with reduced major adverse cardiac event rates compared to standard dual antiplatelet therapy (3–11). However, most of the studies assessing the pharmacodynamic and clinical effects of cilostazol have been limited to investigations performed in Asia where the drug is more broadly used. Limited information on the benefits of cilostazol derive from multicentre, international investigations. Most recently, the clinical benefit of adjunctive cilostazol therapy was not confirmed in a prospective randomised trial of DES-treated patients (22). In fact, although in this study cilostazol enhanced platelet inhibition compared with placebo, and high platelet reactivity was associated with worse outcomes, patients randomised to adjunctive cilostazol therapy did not have a significant reduction in the primary efficacy endpoint. The limited sample size of the study, as well as the risk-profile of the patient population may have contributed to these contrasting findings with prior studies (22). Clinical investigations have shown that although the benefit of adjunctive cilostazol therapy has been shown in both patients with and without DM, this is enhanced in patients with DM (3–11). The findings of our study showing that cilostazol affects significantly the functional activity of platelets from both patients with and without DM, but with a greater treatment effect in patients with DM, may support these findings. Indeed, the fact that cilostazol modulates cAMP levels, one of the key intraplatelet abnormalities in patients with DM, may explain this preferential effect (12–14).

Accumulating evidence has shown that in patients on dual antiplatelet therapy with aspirin and clopidogrel, recurrent atherothrombotic events, including stent thrombosis, may be attributed to inadequate platelet inhibition, also known as “resistance” or “suboptimal response” (23, 24). These observations urge for more potent antiplatelet treatments. This is supported by recent findings from large scale clinical trials using either double-dose clopidogrel or more potent novel generation P2Y12 inhibitors which have shown to reduce ischaemic event rates, including stent thrombosis, to a greater extent than standard dual antiplatelet treatment regimens in patients undergoing PCI (25–27). However, these more potent P2Y12 receptor inhibiting strategies are accompanied by an increase in spontaneous bleeding rates (25–27). The detrimental impact of bleeding on outcomes, including mortality, has raised concern on the use of these potent platelet inhibiting strategies, particularly in patients at high risk for bleeding (28–30). On the contrary to the aforementioned platelet inhibiting strategies, clinical studies with adjunctive cilostazol use have not been associated with any increase in bleeding (3–11, 22). Of note, cilostazol is associated with more potent antiplatelet effects than double-dose clopidogrel, which has shown to enhance only modestly platelet inhibition compared to standard dosing, particularly in high-risk settings, including patients with DM (16, 31, 32). This may be in part attributed to the dysfunctional status of the P2Y12 receptor signalling pathway in patients with DM (33). Therefore, adjunctive treatment with cilostazol may be an attractive treatment option particularly in patients who are at increased risk for both recurrent atherothrombotic events as well as bleeding.

Previous data to explain reduced bleeding rates were based on observations demonstrating that cilostazol-mediated elevation of basal cAMP levels results in the inhibition of activated platelets at the site of vascular injury, a phenomenon not encountered with other antiplatelet agents (34–36). In addition, bleeding times are less affected by cilostazol compared with other platelet inhibitors even when in adjunct to other agents (37, 38). Our study extends these explanations on the lack of increased bleeding risk showing that cilostazol does not affect thrombin generation. Activated platelets induce thrombin generation by providing a procoagulant surface through the rearrangement of their membrane phospholipids to expose phosphatidylserine and by releasing coagulation factors stored in their α-granules (39). P2Y12-mediated signalling plays an important role in thrombin generation as shown in a variety of studies, including using TEG technology as in our study (19, 20, 40, 41). The reasons for P2Y12 receptor inhibitors to be more potent P2Y12 receptor inhibitors, but not cilostazol, affect phosphoinositide 3-kinase-induced granule secretion, which is involved in thrombin generation (36, 42). Identifying platelet inhibiting strategies that do not impact plasmatically mediated haemostatic processes, such as that also suggested to occur with protease-activated receptor-1 (PAR-1) antagonists, is indeed an attractive pharmacological property in order to reduce ischaemic events without an increase in bleeding (43).

Despite these beneficial properties, cilostazol therapy has other types of undesired effects which may limit its use in clinical practice. Treatment with cilostazol in fact is characterised by a high prevalence of non-bleeding side effects (e.g. headache, gastrointestinal disturbances, cardiac palpitations) (22). This explains the overall high rates of treatment discontinuation with this drug as also confirmed in this study. However, when tolerated, the use of cilostazol in adjunct to standard dual antiplatelet therapy may represent a treatment option particularly in high-risk settings, such as patients with a prior cerebrovascular event in whom more potent platelet inhibition may be desired but a bleeding complication may be a major concern. Of note, studies have shown that cilostazol reduces stroke in patients after an ischaemic stroke with fewer haemorrhagic events compared with aspirin (44, 45). In addition, cilostazol potently inhibits progression of carotid intima-media thickness, an established surrogate marker of cardiovascular events, particularly in patients with type 2 DM (46). Further multicentre and international investigations are indeed warranted to support these findings.

**Study limitations**

In the study, the assumed 50% lower absolute between-treatment difference within the non-DM cohort compared to DM cohort was not detected, most likely because such a difference was too optimistic. However, a significant difference, albeit of lower magnitude than supposed, was still found between the two patients cohorts, confirming the study hypothesis that cilostazol achieves greater platelet inhibition in patients with DM compared with non-DM.
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Disclosures
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References

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