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Impact of Pentoxifylline on Platelet Function Profiles in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease on Dual Antiplatelet Therapy With Aspirin and Clopidogrel

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Objectives The aim of this study was to evaluate the impact of the phosphodiesterase (PDE) inhibitor pentoxifylline on platelet function profiles in patients receiving dual antiplatelet therapy (DAPT).

Background Previous studies have shown that, in patients receiving DAPT, the adjunctive use of a PDE inhibitor enhances platelet inhibition, particularly in those presenting with diabetes mellitus (DM). However, the pharmacodynamic (PD) effects of the PDE inhibitor pentoxifylline on platelet function profiles in DM patients receiving DAPT are unknown.

Methods This was a prospective, randomized, double-blind, parallel design study conducted in DM patients with stable coronary artery disease receiving DAPT. Patients were randomly assigned to either pentoxifylline 400 mg or placebo 3 times daily for 14 days. The PD effects were assessed by vasodilator-stimulated phosphoprotein phosphorylation assay, light transmittance aggregometry, VerifyNow P2Y12 assay (Accumetric, Inc., San Diego, California), and multiple electrode aggregometry at baseline and 14 days. The PD effects were also assessed according the presence or absence of high on-treatment platelet reactivity status.

Results A total of 40 patients were available for analysis. At 14 days, there were no differences in the $P2Y_{12}$ reactivity index as assessed by vasodilator-stimulated phosphoprotein phosphorylation between treatment groups (primary endpoint; $p = 0.93$). Intra-group comparisons also failed to show any differences between baseline and 14-day $P2Y_{12}$ reactivity index assessment in the placebo and pentoxifylline arms ($p = 0.61$). There were no significant inter- and intra-group differences in all other PD measures. The PD effects did not vary according the presence or absence of high on-treatment platelet reactivity.

Conclusions Adjunctive treatment with pentoxifylline is not associated with increased platelet inhibitory effects in DM patients with coronary artery disease receiving DAPT. (J Am Coll Cardiol Intv 2011;4:905–12) © 2011 by the American College of Cardiology Foundation

From the *University of Florida College of Medicine-Jacksonville, Jacksonville, Florida; and the †Jacksonville Transplant Center–Shands Jacksonville, Jacksonville, Florida. Dr. Ferreiro reports receiving honoraria for lectures from Daiichi Sankyo, Inc., and Eli Lilly Company. Dr. Bass reports receiving honoraria for lectures from Eli Lilly Company and Daiichi Sankyo, Inc.; consulting fees from Eli Lilly Company and Daiichi Sankyo, Inc.; and research grants from Baxter. Dr. Angiolillo reports receiving honoraria/lectures from Bristol-Myers Squibb/Sanofi-Aventis, Eli Lilly Co., and Daiichi Sankyo, Inc.; honoraria/advisory board for Bristol-Myers Squibb/Sanofi-Aventis, Eli Lilly Co., Daiichi Sankyo, Inc., The Medicines Company, Portola, Novartis, Medicure, Accumetrics, Arena Pharmaceuticals, AstraZeneca, Merck, and Evolva; and research grants from Bristol-Myers Squibb/Sanofi-Aventis, GlaxoSmithKline, Otsuka, Boston Scientific, Eli Lilly Co., Daiichi Sankyo, Inc., The Medicines Company, Portola, Accumetrics, Schering-Plough, AstraZeneca, Eisai, and Johnson & Johnson. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 4, NO. 8, 2011
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Dual antiplatelet therapy (DAPT) with aspirin and clopidogrel improves clinical outcomes in high-risk patients, such as those with acute coronary syndrome and undergoing percutaneous coronary intervention (1). However, despite the clinical benefit associated with this treatment regimen, there are still a considerable number of patients who continue to have recurrent ischemic events (2,3). Numerous investigations have demonstrated that the presence of high on-treatment platelet reactivity (HPR) among patients receiving DAPT to be an independent predictor of adverse outcomes, including stent thrombosis (3). This phenomenon occurs more frequently among certain populations, such as those with diabetes mellitus (DM), underscoring the need for achieving more optimal platelet inhibitory effects particularly in these high-risk patients (4–10).

Abbreviations and Acronyms

ADP = adenosine diphosphate

CAD = coronary artery disease

cAMP = cyclic adenosine monophosphate

DAPT = dual antiplatelet platelet therapy

DM = diabetes mellitus

HPR = high on-treatment platelet reactivity

LTA = light transmittance aggregometry

MEA = multiple electrode aggregometry

PGE1 = prostaglandin E_1

PRP = platelet-rich plasma

PRI = $P2Y_{12}$ reactivity index

VASP-P = phosphorylation of vasodilator-stimulated phosphoprotein

Phosphodiesterase (PDE) inhibitors such as cilostazol, a PDE3 inhibitor, and pentoxifylline, a nonselective PDE inhibitor commonly used for treatment of intermittent claudication, have antiplatelet properties that some studies have suggested to be more pronounced in patients with DM (11,12). Several reports have shown that, in patients receiving DAPT, the adjunctive use of cilostazol ("triple antiplatelet therapy") enhances platelet inhibition (7,11,13–15). Although controversies exist on the clinical benefit of adjunctive cilostazol therapy (16), most studies have shown improved outcomes in high-risk settings—particularly in DM patients—without any increase in bleeding (17–22). However, cilostazol therapy is associated with a high incidence of nonbleeding side effects, such as headaches, palpitations, and tachycardia, which frequently lead to treatment discontinuation. Compared with cilostazol, pentoxifylline is less expensive and associated with fewer side effects, explaining its broader use in clinical practice for the treatment of relief of peripheral vascular disease symptoms (23). However, whether adjunctive pentoxifylline therapy in patients receiving DAPT is associated with enhanced platelet inhibition is unknown. The aim of the present pharmacodynamic (PD) investigation was to evaluate the impact on platelet function profiles associated with adjunctive pentoxifylline treatment in DM patients with coronary artery disease (CAD) receiving DAPT with aspirin and clopidogrel.

Methods

Patient population. This was a prospective, randomized, double-blind, parallel design PD study. Patients with type 2 DM were eligible for the study if they were between 18 and 80 years of age, had stable CAD, and were receiving standard DAPT with aspirin (81 mg/day) and clopidogrel (75 mg/day) for at least 30 days. All patients had previously undergone coronary stenting and were clinically stable. Patients needed to be taking hypoglycemic treatment (oral medications or insulin) for at least 30 days. General major exclusion criteria included: known allergies to aspirin, clopidogrel, or pentoxifylline; left ventricular ejection fraction <30%; blood dyscrasia; serum creatinine level >2 mg/dl; active bleeding or bleeding diathesis; gastrointestinal bleed, hemodynamic instability, or acute coronary event within 6 months; cerebrovascular accident within 3 months; any malignancy; concomitant use of other antithrombotic drugs (oral anticoagulants, dypiridamole, ticlopidine, or cilostazol); recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist; platelet count $<100 \times 10^3/\mu l$; and liver disease (baseline alanine transaminase >2.5 times the upper limit of normal). Patients were recruited from the outpatient cardiology clinic of the University of Florida College of Medicine–Shands Jacksonville Hospital.

Patients were randomized to receive pentoxifylline 400 mg or matching placebo capsules 3 times daily for 14 days. Pentoxifylline 400 mg 3 times daily was chosen because this is the Food and Drug Administration recommended dose for the treatment of patients with intermittent claudication on the basis of chronic arterial disease of the limbs. The randomization and consecutive preparation of the study medication were performed by the pharmacy of our University Hospital. Pentoxifylline and matching placebo were identical-appearing white capsules. Investigators, laboratory personnel, and patients were blinded to treatment assignments. Unblinding was performed only at the end of the study for data analysis purposes. Platelet function was performed at 2 time points: 1) at baseline (before randomization); and 2) 14 days (after randomization). Patient compliance with 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. An independent data safety monitoring committee was instituted for adjudication of adverse events.

Endpoints and sample size calculation. The primary endpoint of this study was the comparison of the $P2Y_{12}$ reactivity index (PRI) determined by whole blood vasodilator-stimulated phosphoprotein between groups after 2 weeks of treatment with pentoxifylline or placebo. Assuming that the PRI SD is 10, we would be able to detect a difference between groups

of 15% with 20 patients/group with a 90% power and a 2-tailed p value <0.05. A difference in PRI of 15% was chosen on the basis of the results obtained with cilostazol in our previous study in DM (7). This assumes that the data are analyzed as a parallel design. Other endpoints included comparisons in platelet function profiles with other assays, including light transmittance aggregometry (LTA), multiple electrode aggregometry (MEA), and VerifyNow P2Y12 assay (Accumetrics, Inc., San Diego, California). Overall, these assays enable a comprehensive assessment of platelet function profiles, because they measure purinergic and non-purinergic mediated signaling.

Blood sampling and functional assessments. Peripheral venous blood samples were drawn with a loose tourniquet to avoid artifacts through a short venous catheter inserted into a forearm vein. The first 2 to 4 ml of blood was discarded to avoid spontaneous platelet activation. Samples were processed within 1 h after blood-drawing.

PRI. The PRI was calculated as a measure of the functional status of the $P2Y_{12}$ signaling pathway. The PRI was determined through assessment of phosphorylation status of vasodilator-stimulated phosphoprotein (VASP-P), a key and specific intra-platelet mediator of $P2Y_{12}$ signaling, according to standard protocols (6–8). In brief, VASP-P was measured by quantitative flow cytometry (Beckman Coulter FC500, Miami, Florida) with commercially available labeled monoclonal antibodies (Biocytex, Inc., Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels after challenge with prostaglandin E_1 (PGE_1) and PGE_1 plus adenosine diphosphate (ADP). The PGE_1 increases VASP-P levels through stimulation of adenylate cyclase, whereas ADP binding to purinergic receptors leads to inhibition of adenylate cyclase. Therefore, the addition of ADP to PGE_1-stimulated platelets reduces levels of PGE_1-induced VASP-P. The PRI was calculated as follows: ([MFI PGE_1] − [MFI PGE_1 + ADP] ÷ [MFI PGE_1]) × 100. Elevated PRI values indicate upregulation of the $P2Y_{12}$ signaling pathway (6,7,8).

LTA. Platelet aggregation was performed with LTA according to standard protocols (6,7,8). In brief, platelet aggregation was assessed with platelet-rich plasma (PRP) by the turbidimetric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp., Havertown, Pennsylvania). The PRP was obtained as a supernatant after centrifugation of citrated blood at 800 rpm for 10 min. The isolated PRP was kept at 37°C before use. Platelet-poor plasma 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 platelet-poor plasma for each measurement and assessed after challenge with ADP (5 and 20 μmol/l) (6–8). The results were reported as maximal percentage platelet aggregation (6–8).

MEA. Blood was collected in hirudin treated tubes. The MEA was assessed in whole blood with the Multiplate analyzer (Dynabyte Medical, Munich, Germany) as previously described (24). This instrument can perform up to 5 parallel aggregometry measurements, assessing the change in impedance caused by the adhesion of platelets onto sensor units formed by silver-covered electrodes. The following agonists were used: 0.5 mmol/l arachidonic acid, 6.4 μmol/l ADP with and without 9.4 nmol/l PGE_1, 32 μmol/l thrombin receptor activating peptide, and 3.2 μg/ml collagen. Curves were recorded for 6 min, and platelet aggrega-

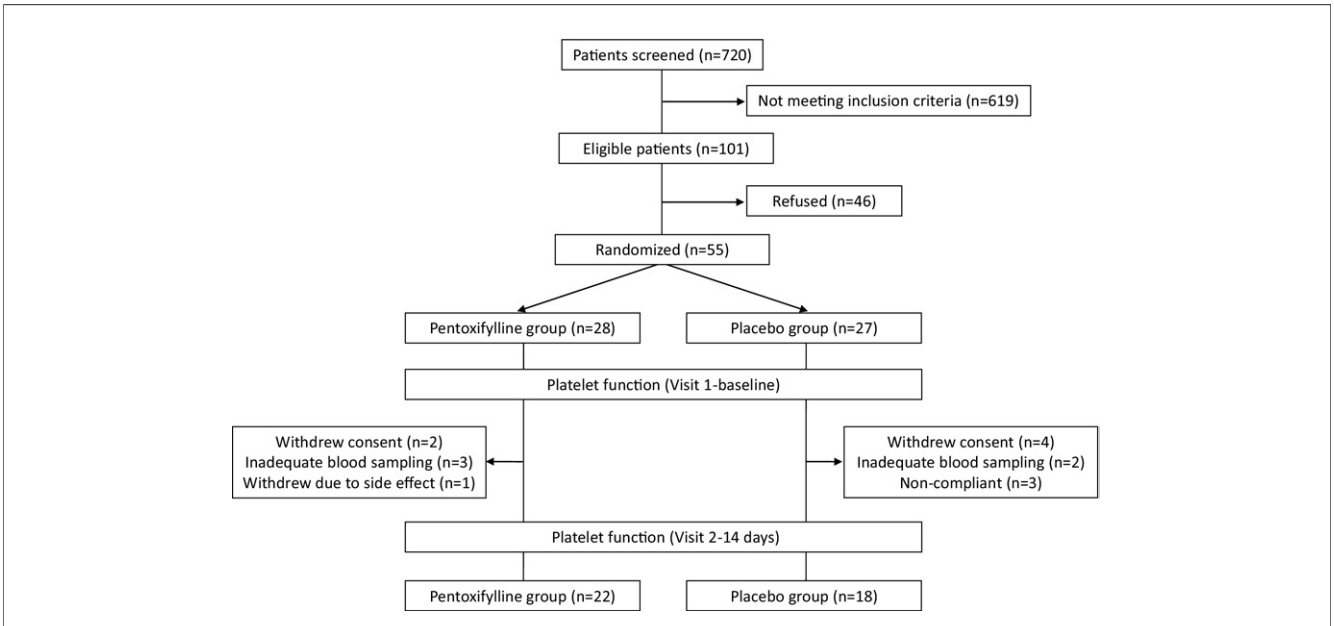

Figure 1. Flow Diagram and Subject Disposition

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 4, NO. 8, 2011
AUGUST 2011:905–12

tion was determined as arbitrary aggregation units and area under the curve.

VERIFYNOW P2Y12 ASSAY. The VerifyNow P2Y12 assay (Accumetrics, Inc.) is a rapid whole-blood point-of-care assay and was used according to the instructions of the manufacturer (7,8). The VerifyNow P2Y12 assay reports the results as $P2Y_{12}$ reaction units. This assay mimics turbidometric aggregation and uses disposable cartridges containing 20 mmol/l ADP and 22 nmol/l PGE_1. Aggregation testing with ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signaling, whereas adding PGE_1 increases the specificity of the test for $P2Y_{12}$ signaling. In a separate channel of the cartridge in which iso-thrombin receptor activating peptide 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.

Definition of HPR. The effects of pentoxifylline were also assessed according to the presence or absence of HPR at time of randomization (baseline sample). Patients were defined as having HPR with various cutoff levels that have been associated with increased risk of recurrent ischemic events previously defined in the published data and/or agreed upon in a consensus statement (2,3,8). These included the following cutoff values: PRI >50%, $P2Y_{12}$ reaction units >230, LTA-ADP (20 μmol/l) >50%, LTA-ADP (5 μmol/l) >46%, and MEA-ADP >462 area under the curve (AUC) of arbitrary units (AU*min). The absolute changes (Delta) from baseline value in platelet reactivity according to the presence or absence of HPR were calculated.

Statistical analysis. Continuous variables were expressed as a mean ± SD or median (interquartile range) when appropriate. Categorical variables were expressed as frequencies and percentages. Paired Student *t* test was used to compare continuous variables. Comparisons between categorical variables were performed with McNemar test or binomial exact test. Only patients who completed both treatment periods were included in the analysis. A p value <0.05 was considered statistically significant. Statistical analysis was performed with the SPSS software (version 15.0, SPSS, Inc., Chicago, Illinois).

Results

From September 2009 to October 2010, a total of 720 patients were screened. Of these, 101 patients met study inclusion criteria, and 55 patients were randomized in the study. A total of 15 patients did not complete the study. Overall, 40 patients with available PD data were studied to test for the study hypothesis. Disposition of the study population is illustrated in Figure 1. There were no differences in baseline demographic data between patients who did and those who did not complete the study (data not shown). Baseline demographic data and clinical characteristics of randomized patients are provided in Table 1. Patients were similar for all baseline characteristics, except for a higher prevalence of men in the placebo group. During the study period, there were no changes in medical therapy. There were no bleeding complications during the study. Two patients randomized to the placebo arm developed headache and nausea, which subsided after 2 days. However, these patients remained compliant to treatment and completed the study. One patient randomized to pentoxifylline developed nausea leading to treatment discontinuation and incompletion of study protocol procedures.

At baseline, there were no differences in PD measures between patients randomized to pentoxifylline and those randomized to placebo (Table 2). At 14 days, there were no differences in PRI between treatment groups (primary endpoint; p = 0.93) (Fig. 2). Intra-group comparisons also failed to show any differences between baseline and 14-day PRI assessment in the placebo and pentoxifylline arms (p = 0.61). Furthermore, there were no significant differences in the absolute changes in PRI at baseline and

Table 1. Baseline Demographic Data and Clinical Characteristics

Variable	Pentoxifylline (n = 22)	Placebo (n = 18)	p Value
Age (yrs)	62.7 ± 8.6	61.8 ± 8.5	0.76
Male	7 (32)	15 (83)	0.02
Race			
Caucasian	13 (59)	11 (61)	1.00
African-American	7 (32)	6 (33)	1.00
Hispanic	1 (4.5)	0 (0)	1.00
Asian	1 (4.5)	1 (6)	1.00
Risk factors/past medical history			
Smoking	5 (23)	6 (33)	0.49
Hyperlipidemia	21 (96)	18 (100)	1.00
Hypertension	21 (96)	18 (100)	0.26
Body mass index	36.0 ± 10.2	32.9 ± 4.6	0.21
Prior myocardial infarction	14 (64)	12 (67)	1.00
Prior CABG	4 (18)	5 (28)	0.71
Prior stroke	4 (18)	1 (6)	0.36
Treatment			
Beta-blockers	18 (82)	15 (83)	0.27
Calcium antagonist	10 (48)	7 (39)	0.75
Nitrates	9 (41)	9 (50)	0.75
ACE inhibitors/ARB	20 (91)	16 (89)	1.00
PPI	3 (14)	7 (39)	0.14
CYP3A4 metabolizing statin	13 (59)	14 (78)	0.31
Non-CYP3A4 metabolizing statin	5 (23)	3 (17)	0.71
Oral hypoglycemic agents	17 (77)	15 (83)	0.71
Insulin therapy	7 (32)	5 (28)	1.00

Values are mean ± SD or n (%).

ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blockers; CABG = coronary artery bypass grafting; CYP3A4 = cytochrome P450 3A4 isoenzyme; PPI = proton pump inhibitors.

Table 2. Pharmacodynamic Effects of PTX Versus Placebo

	Baseline			14 Days			p Value for Intra-Group	
	PTX	Placebo	p Value	PTX	Placebo	p Value	PTX	Placebo
LTA								
ADP 20 μmol/l (%)	56.6 ± 13.2	53.8 ± 12.6	0.51	57.1 ± 12.5	50.9 ± 11.9	0.12	0.79	0.26
ADP 5 μmol/l (%)	45.8 ± 13.5	41.6 ± 13.7	0.34	47.5 ± 13.8	40.0 ± 13.9	0.11	0.48	0.17
MEA								
AA (AU*min)	226 ± 248	212 ± 145	0.84	203 ± 184	178 ± 72	0.59	0.45	0.28
ADP (AU*min)	526 ± 300	493 ± 305	0.74	447 ± 304	412 ± 184	0.68	0.11	0.07
ADP + PGE_1 (AU*min)	312 ± 258	300 ± 161	0.87	305 ± 269	282 ± 171	0.77	0.66	0.47
TRAP (AU*min)	1,161 ± 268	1,000 ± 337	0.11	1,124 ± 266	974 ± 301	0.12	0.49	0.69
Collagen (AU*min)	549 ± 237	551 ± 286	0.98	463 ± 177	492 ± 189	0.65	0.04	0.18
VerifyNow P2Y12 assay								
$P2Y_{12}$ inhibition (%)	26.4 ± 20.9	27.7 ± 13.2	0.95	28.2 ± 22.6	28.4 ± 17.3	0.98	0.85	0.62
$P2Y_{12}$ reactivity units	284 ± 96	256 ± 83	0.34	270 ± 98	245 ± 72	0.39	0.23	0.79

AA = arachidonic acid; ADP = adenosine diphosphate; AU*min = area under the curve of arbitrary units; LTA = light transmittance aggregometry; MEA = multiple electrode platelet aggregometry; PGE = prostaglandin E_1; PTX = pentoxifylline; TRAP = thrombin receptor agonist peptide.

after treatment between the pentoxifylline and placebo arms (1.8 ± 15.9 vs. 1.7 ± 12.4; p = 0.99). In addition, there were no differences at 14 days in absolute content of VASP-P assessed with MFI PGE_1 (25.6 ± 6.3 vs. 24.0 ± 8.3, p = 0.51) and MFI PGE_1 + ADP (9.1 ± 4.6 vs. 8.7 ± 5.2, p = 0.82) between pentoxifylline and placebo arms. There were no significant inter- and intra-group differences in all other PD measures (Table 2). The SD values of PD measures were higher than anticipated in our statistical assumptions. However, the post hoc power to detect the pre-specified difference of 15% in PRI was 84% with the SD obtained in our study.

Rates of HPR in the study population ranged from 40% to 85%, depending on the definition used (Table 3). The absolute changes (Delta) from baseline value in platelet reactivity according to baseline response status assessed by different platelet function tests are shown in Figure 3.

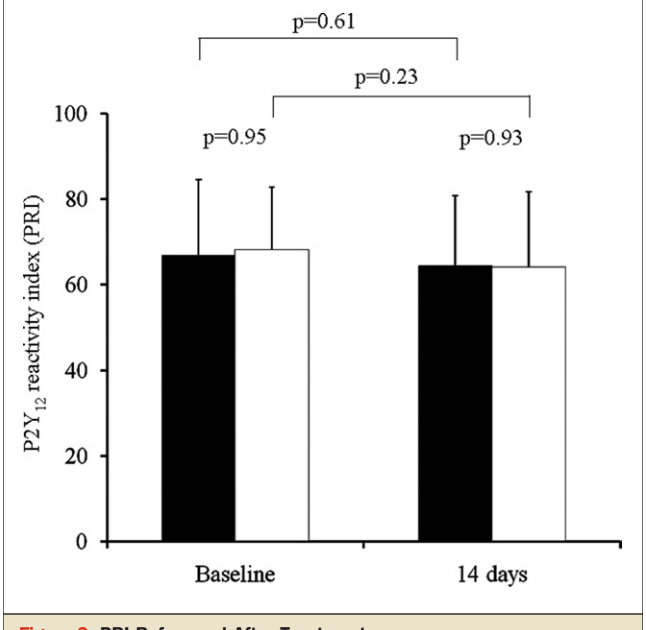

Figure 2. PRI Before and After Treatment

Solid bars = pentoxifylline. **Open bars** = placebo. Values are expressed as percentage of PRI. **Error bars** indicate SDs of the mean.

Discussion

Pentoxifylline is a PDE inhibitor currently recommended for the treatment of relief of peripheral vascular disease symptoms (23). Ex vivo and in vitro investigations have also demonstrated the antiplatelet properties of this compound (12,25–30). However, at difference of the PDE inhibitor cilostazol, to date there are no studies that have assessed the impact of pentoxifylline on platelet function profiles in patients receiving DAPT. The need

Table 3. Rate of HPR Assessed by Different Platelet Function Tests in the Study Population

	HPR (%)
VASP	
PRI	85
LTA	
ADP 20 μmol/l	80
ADP 5 μmol/l	40
MEA	
ADP 6.4 μmol/l	50
VerifyNow P2Y12 assay	
$P2Y_{12}$ reactivity units	60

VASP = vasodilator-stimulated phosphoprotein; other abbreviations as in Table 2.

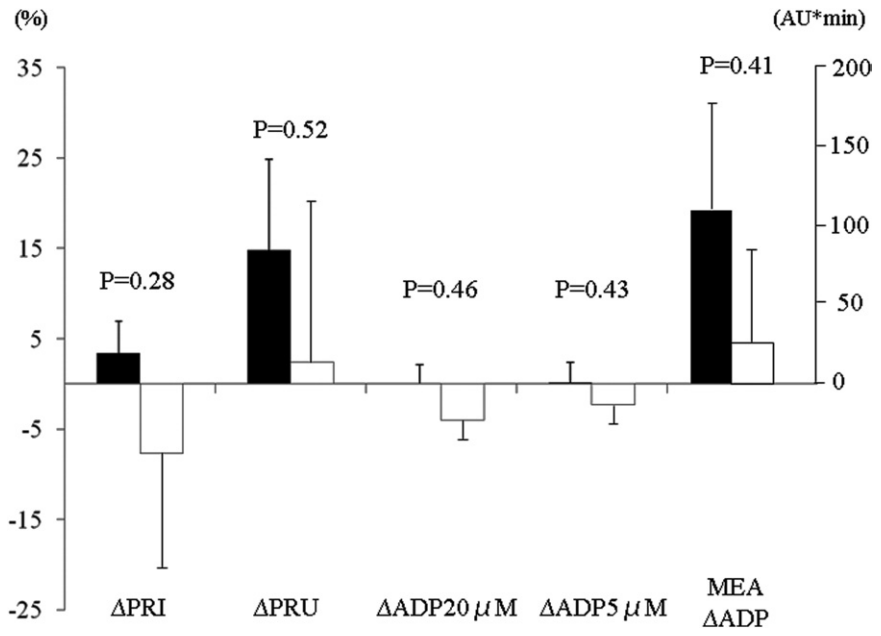

Figure 3. Delta From Baseline Value in Platelet Reactivity According to Presence or Absence of High On-Treatment Platelet Reactivity

Assessed by different platelet function tests in patients treated with pentoxifylline. The values for absolute change (Δ)-$P2Y_{12}$ reactivity index (PRI), Δadenosine diphosphate (ADP)20, and ΔADP5 are expressed as percentage. The values for Δ$P2Y_{12}$ reaction units (PRU) and Δmultiple electrode aggregometry (MEA) ADP are expressed as unit and area under the curve (AU*min), respectively. **Solid bars** = patients with high on-treatment platelet reactivity. **Open bars** = patients without high on-treatment platelet reactivity. **Error bars** indicate SEM.

for understanding how adjunctive PDE inhibitory therapies impact platelet reactivity emerges because a considerable number of patients receiving DAPT persist with HPR, which translates into an increased risk of recurrent ischemic events (2,3). This underscores the importance of achieving more optimal platelet inhibition in patients at risk of having HPR despite DAPT, such as patients with DM (31). The results of the present investigation confirm that patients with DM have an elevated prevalence of HPR as demonstrated in prior PD investigations (6–8). However, the results of the present investigation demonstrate that pentoxifylline is not associated with any significant changes in platelet reactivity, irrespective of HPR status, as assessed by multiple PD measures evaluating purinergic and non-purinergic mediated platelet signaling. Overall, these findings do not support the use of pentoxifylline as an adjunctive treatment in patients receiving DAPT to obtain more potent platelet inhibition.

Pentoxifylline acts primarily by increasing erythrocyte flexibility, reducing blood viscosity, and inhibiting platelet aggregation (12,25–30). An in vitro study showed that pentoxifylline inhibits platelet aggregation more in DM patients in comparison with non-DM subjects in a dose-dependent manner (12). Although these effects were confirmed with in vitro experiments, ex vivo experiments have been controversial (12,26). Several mechanisms might explain the antiplatelet properties of pentoxifylline. These might be related to a direct effect on the platelet or mediated by indirect actions of the drug. Pentoxifylline has been shown to strongly directly inhibit spontaneous and induced platelet aggregation in vitro via inhibition of membrane-bound phosphodiesterase, leading to raised cyclic adenosine monophosphate (cAMP) levels, thromboxane synthesis, and increased prostacyclin (prostaglandin I_2) synthesis (32). Other studies have shown pentoxifylline to have antiplatelet effects that are secondary to its rheological effects. These include reducing whole blood viscosity and plasma viscosity by decreasing plasma fibrinogen concentrations (33) and red cell deformability (25,27,32,34).

The reasons underlying the differences between cilostazol and pentoxifylline observed in PD studies might be related to multiple factors. It is known that PDE 3, 4, and 5 inhibitors induce significant increases in intra-cellular cAMP levels (35). Pentoxifylline is a nonselective PDE inhibitor (12,36). Although in our investigation intra-platelet cAMP levels were not assessed and would have been of additive value to interpret our study findings, prior studies have shown pentoxifylline to have only a weak effect on intra-platelet cAMP levels (27). This is in contrast to cilostazol, a selective and potent inhibitor of PDE 3, which has shown to be associated with marked increase in cAMP levels (37). Because cAMP is a pivotal modulator of

VASP-P, a key mediator of $P2Y_{12}$ receptor signaling, this might explain why studies have shown that adjunctive treatment with cilostazol are associated with even greater $P2Y_{12}$ mediated effects, compared with double-dose clopidogrel regimens (13,14). These pharmacological properties could also explain why pentoxifylline does not have enough evidence to improve objective (walking distance) and subjective (quality of life) outcomes in patients with intermittent claudication, whereas cilostazol has demonstrated benefit in both, albeit at the expense of more nonbleeding side effects (23,38). Indeed, it might be argued that novel and more potent $P2Y_{12}$ inhibiting strategies (e.g., prasugrel, ticagrelor) might overcome the limitations of clopidogrel, thus limiting the need to resorting to a third agent such as PDE inhibitors to obtain more potent platelet inhibition (39). However, these novel therapies are associated with an increased risk of spontaneous bleeding (40,41), which has not been observed with triple antiplatelet therapy (17–22), making the latter an attractive treatment option in patients who have an increased ischemic risk but where more potent $P2Y_{12}$ inhibitors might be contraindicated (31).

Another reason for the lack of additional platelet inhibition might be explained by the pharmacokinetic profile of pentoxifylline. Pentoxifylline, in fact, is metabolized in humans to at least 7 metabolites (27). The major metabolites are hydroxy metabolite 3,7-dimetyl-1-(5′hydroxyhexyl) xanthine), the 2 carboxylic acid metabolites 3,7-dimetyl-1-(4-carboxybutyl) xanthine, 3,7-dimetyl-1-(3-carboxypropyl) xanthine, and pentoxifylline (27,29). The main in vivo effect on platelet aggregation is due to pentoxifylline and hydroxy metabolite 3,7-dimetyl-1-(5′hydroxyhexyl) xanthine), whereas the remaining metabolites contribute by <10% each. Pharmacokinetic studies have shown that the peak plasma concentration of pentoxifylline is approximately 0.3 mg/l at approximately 3 h after administration of 400 mg pentoxifylline (27,42). However, a previous in vitro PD study showed that 0.25 mg/l and 0.5 mg/l of pentoxifylline were not sufficient to inhibit platelet aggregation, for which higher levels (0.75 mg/l and 1.0 mg/l) were needed (12). Therefore, it might be hypothesized that oral administration of 400 mg pentoxifylline could not be sufficient to reach plasma concentrations of its metabolites that can enhance platelet inhibitory effects.

Despite the neutral findings of our study, the lack of impact on the PD measures in CAD patients receiving DAPT also provides some reassurance. In fact, the addition of an agent with antiplatelet properties such as pentoxifylline, albeit with the objective of relief of peripheral vascular disease symptoms, can still raise concerns about the bleeding potential in these patients, which is per se increased given the use of clopidogrel on a background of aspirin therapy (43). The ever-raising concerns on the detrimental impact of bleeding on outcomes underscore identification of patients and strategies associated with increased risk (44). Indeed, patients with DM, as studied in our current investigation, have an increased risk of bleeding (45). In addition, they also frequently have concomitant CAD and peripheral vascular disease, thus commonly treated with DAPT as well as a PDE inhibitor, making the results of our PD investigation of potential clinical value. Indeed, our PD study was not powered to make any conclusions on the safety of pentoxifylline. However, the lack of enhanced platelet inhibitory effects might hamper the concerns over bleeding when pentoxifylline is used at recommended doses for its approved indication for relief of peripheral vascular disease symptoms in patients also treated with DAPT.

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