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EDITORIAL COMMENT

Sweet and Sticky

Diabetic Platelets, Enhanced Reactivity, and Cardiovascular Risk*

Daniel I. Simon, MD, FACC,†
Alvin H. Schmaier, MD‡
Cleveland, Ohio

Platelets play a pivotal role in the pathogenesis of acute coronary syndromes and in the thrombotic complications after percutaneous coronary intervention. Oral antiplatelet agents are the cornerstone of pharmacological therapy for preventing major adverse cardiovascular events. Aspirin and thienopyridine derivatives (ticlopidine, clopidogrel), by blocking thromboxane A_2 formation and the adenosine diphosphate (ADP) receptor $P2Y_{12}$ platelet activation pathways, respectively, are the major oral antiplatelet drugs in clinical practice. The Swedish angina pectoris aspirin trial, in which 2,035 patients were allocated to receive 75 mg of aspirin daily or placebo (1), showed that aspirin therapy led to significant reductions in death and myocardial infarction among patients with unstable angina (46% reduction), those undergoing coronary angioplasty (53% reduction), and those with stable angina (33% reduction). When given in combination with aspirin, thienopyridines inhibit platelet aggregation to a greater extent than either agent alone (2).

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Several studies have demonstrated a dramatic, nearly 5-fold reduction in acute and subacute stent thrombosis when aspirin in combination with a thienopyridine was used after stenting compared with either aspirin alone or aspirin plus warfarin (3,4). In the setting of acute coronary syndromes, combination therapy with aspirin and clopidogrel significantly reduce ischemic complications compared to aspirin alone (9.3% vs. 11.4%, relative risk reduction 20%, $p < 0.001$) (5). The benefit with clopidogrel was noted early (within 24 h of treatment), was sustained at 1 year, and observed in all patients with acute coronary syndromes regardless of their level of risk or treatment strategy (i.e., medical therapy, percutaneous coronary intervention, or coronary artery bypass grafting).

Variability in the response to antiplatelet drugs has been recognized for decades. Antiplatelet drug resistance or "nonresponsiveness" is used to describe the clinical observation of the inability of the antiplatelet agent to prevent thrombotic vascular events or the laboratory phenomenon of reduced effect of the antiplatelet agent on one or more tests of platelet function. The mechanisms of aspirin and clopidogrel nonresponsiveness are incompletely defined. In the case of aspirin, multiple cellular, clinical, and genetic factors likely contribute to variable responses among individuals when given a fixed dose of aspirin (6). For clopidogrel, the metabolic activity of hepatic P450 3A4 is largely responsible for converting clopidogrel to its active thiol metabolite that binds to and inhibits the $P2Y_{12}$ ADP receptor. Increasing clopidogrel dose or hepatic P450 3A4 activity enhances the platelet inhibitory response of clopidogrel (7).

There is a growing amount of evidence demonstrating that hypo- or nonresponsiveness to antiplatelet drugs in ex vivo testing is associated with adverse clinical events in diverse patient populations with atherosclerotic disease in both stable and unstable phases as well as in the postpercutaneous coronary and peripheral intervention settings (8). For example, clopidogrel nonresponsiveness is associated with adverse cardiovascular outcomes after elective (9) and emergency (10) percutaneous coronary intervention. Enhanced platelet reactivity also has been observed in patients with postpercutaneous coronary intervention treated with aspirin and clopidogrel who go on to develop stent thrombosis compared with those who remain free of stent thrombosis ($63.28 \pm 9.56\%$ vs. $39.80 \pm 10.9\%$, $p < 0.001$) (11).

In this issue of the *Journal*, Angiolillo et al. (12) provide additional evidence for the potential importance of variable antiplatelet responsiveness by showing that residual high platelet reactivity (HPR) in a subset of diabetic patients with coronary artery disease on dual antiplatelet therapy is associated with increased risk of major adverse cardiovascular events. Platelet light transmission aggregometry (LTA) and flow cytometry for expression of epitopes indicating previous thrombocyte activation were assessed in type 2 diabetes mellitus patients ($n = 173$) who had previously undergone percutaneous coronary intervention and were on chronic treatment with aspirin (100 mg daily) and clopidogrel (75 mg daily). Platelet reactivity was determined using 20 μM ADP-induced platelet aggregation and divided into quartiles. The upper quartile (Agg_{max} >62%) defined HPR. The strongest independent predictor of major adverse cardiovascular events was HPR with a hazard ratio of 3.35 (95% confidence interval 1.68 to 6.66, $p = 0.001$). Interestingly, patients with HPR to ADP tended to have HPR to multiple additional agonists, including collagen,

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From the †Division of Cardiovascular Medicine and ‡Division of Hematology-Oncology, University Hospitals Case Medical Center, Case Western Reserve University School of Medicine, Cleveland, Ohio. This work was supported by the National Institutes of Health (HL57506 and HL60942 to Dr. Simon and HL52779 and HL55907 to Dr. Schmaier). Dr. Simon receives research funding and Speakers' Bureau and scientific advisory board honoraria from Schering-Plough. Dr. Simon also receives research funding from Accumetrics.

epinephrine, and thrombin receptor agonist peptide. This study contrasts with others in diabetics where no significant differences in platelet aggregation responses were noted (13,14). The distinguishing feature of the Angiolillo et al. (12) study is that these investigators stratified their diabetic patient population into quartiles of ADP-induced platelet responsiveness to recognize that the subgroup with the least inhibited platelets have higher risk for adverse events.

High post-treatment platelet reactivity is also an important predictor of 30-day clinical outcomes in patients with acute coronary syndromes undergoing coronary stenting (15). Cuisset et al. (15) randomized patients to 300- or 600-mg loading dose of clopidogrel at least 12 h before percutaneous coronary intervention. Immediately before stenting, ADP-induced platelet aggregation was performed on a single post-treatment blood sample, and HPR was defined as Agg_{max} >70%. They found that HPR was less common in patients receiving 600 mg rather than 300 mg of clopidogrel (15% vs. 25%, p = 0.03). In a multivariable logistic regression analysis that included both clopidogrel loading doses, HPR, and several cardiovascular risk factors and inflammatory parameters, only HPR remained independently associated with cardiovascular events (hazard ratio = 13.82; 95% confidence interval 5.30 to 36.04, p < 0.0001).

Although LTA is used widely to detect classic platelet function disorders, its usefulness in identifying HPR in patient populations is not universally accepted. Healthy individuals (n = 359) exhibit considerable interindividual variability in aggregation responses (16). For example, the "threshold" concentration of ADP- or epinephrine-induced second wave aggregation and granule secretion in normal platelets varies from 1 to 7.5 μM and 0.5 to 10 μM, respectively (17). Further, for each agonist tested at submaximal concentrations, a small proportion of individuals demonstrate unusually robust aggregation responses (16). In Yee et al. (16), epinephrine and collagen related-peptide agonists were the most reliable and efficient in detecting hyper-reactivity, and in an important substudy, the authors note that epinephrine hyper-reactivity persists for up to 3 years. These authors proposed that classification of HPR be restricted to individuals who demonstrate >60% aggregation to 0.4 μM epinephrine or >50% aggregation to 0.005 μg/ml collagen-related peptide on at least 2 occasions (18). It is important to note that only now standard approaches to perform platelet aggregation studies are being proposed; thus, it may be necessary to develop a broader definition of what constitutes HPR (17,19). Presently, each institution needs to develop its own measure of normal and determine who in a patient population is characterized in the most reactive quartile.

Marked differences in platelet reactivity between healthy individuals and between patients with cardiovascular disease raise important questions about their molecular determinants. Both Angiolillo et al. (12) and Yee et al. (16) demonstrated that individuals who demonstrate hyperreactivity to one agonist tended to show a similar response to other agonists, indicative of a global hyperactive platelet status. This observation suggests that HPR result from common downstream signaling events involving g-proteins, calcium transients, kinases/phosphatases, and phosphodiesterases that can be influenced by genetic and/or other conditions.

The present study of Angiolillo et al. (12) also has important implications for the understanding and prevention of recurrent cardiovascular events in diabetic patients. Diabetes mellitus markedly increases the risk of myocardial infarction, stoke, amputation, and death (20). The abnormal metabolic state that accompanies diabetes alters the function of multiple cell types, including endothelial cells and platelets. Chronic hyperglycemia, insulin resistance, and hyperlipidemia promote endothelial dysfunction and also enhance platelet reactivity and thrombosis. The mechanism by which diabetes enhances platelet function is multifactoral (21). Platelets from diabetics have activation of protein kinase C (22), elevated adenine nucleotides (23), increased platelet Fc receptor expression (24), greater levels of phosphorylated Syk (24), and more sensitivity to ADP (25). Diabetic platelets also have decreased production of platelet-derived, as well as endothelial-derived nitric oxide, which inhibits platelet adhesion and aggregation, and increased formation of O_2^- (22), which further diminishes the bioavailability of nitric oxide by forming peroxynitrite ($ONOO^-$). Production of peroxynitrite also impairs the production of endothelial-derived prostacyclin, which has important antiadhesive and antiaggregatory actions on platelets (26). Finally, obesity is central to the pathogenesis of type 2 diabetes mellitus, and its influence on leptin modulates platelet function. Platelets express the leptin receptor, and leptin potentates the aggregation of platelets in response to multiple agonists, including ADP (27). Leptin-deficient and leptin receptor-deficient mice have impaired thrombus formation in vivo, indicating that leptin has an essential role in hemostasis (27,28). Diabetics also have elevated adiponectin levels in association with increased basal platelet reactivity (29).

Management of diabetic patients with platelet hyperactivity is uncertain. Increasing the clopidogrel dose may be insufficient to eliminate hyperactivity because suboptimal clopidogrel response was still present in 60% of diabetic patients on the 150-mg rather than 75-mg clopidogrel regimen in the randomized OPTIMUS study (Optimizing antiplatelet therapy in Diabetes Mellitus) (30). The observations of Angiolillo et al. (12) and Yee et al, (16) indicate that there is a subset of individuals with and without diabetes with HPR who may require combinations of new antiplatelet agents (e.g., second-generation $P2Y_{12}$ inhibitors, thrombin receptor antagonists, glycoprotein VI inhibitors) in addition to aspirin and clopidogrel to optimize platelet "anesthesia" and ameliorate risk for cardiovascular events.

Reprint requests and correspondence: Dr. Daniel I. Simon, University Hospitals Case Medical Center, 11100 Euclid Avenue LKSD 3001, Cleveland, Ohio 44022. E-mail: daniel.simon@uhhospitals.org.

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