Inhibited and Uninhibited Platelet Deposition Within a Thrombus
Does It Depend on the Antiplatelet Drug?

Paul A. Gurbel, Udaya S. Tantry

Platelet-rich thrombus generation at the vascular injury site is a primary underlying mechanism of ischemic event occurrence in patients with coronary artery disease. Thromboxane (Tx) A₂ and ADP act synergistically during platelet aggregation, and the ADP–P2Y₁₂ receptor interaction plays a central role in sustaining the activation of glycoprotein (GP) IIb/IIIa receptors by amplifying the response to agonists. On the basis of large-scale clinical trial data, therapy with clopidogrel (a P2Y₁₂ receptor blocker) plus aspirin (a cyclo-oxygenase [COX]-1 inhibitor)—dual antiplatelet therapy—has revolutionized the treatment of patients with high-risk coronary artery disease. However, multiple pharmacodynamic studies primarily based on light transmittance aggregometry in patients undergoing stenting, uniformly revealed wide response variability and high platelet reactivity to ADP during clopidogrel therapy. The latter observation subsequently was linked to single nucleotide polymorphisms in a major gene involved in the generation of clopidogrel active metabolite. Additional studies demonstrated an independent relation among high platelet reactivity, single nucleotide polymorphism carriage, and poststenting ischemic event occurrence. Persistent treatment failure in large-scale clinical trials despite the ability of more potent and pharmacodynamically predictable P2Y₁₂ inhibitors, such as prasugrel and ticagrelor, to overcome aspirin-induced platelet inhibition. However, it is in contrast to an earlier observation of near absence of aspirin resistance assessed by light transmittance aggregometry in patients with high-risk coronary artery disease, a group expected to have high platelet turnover.

In the accompanying article, Hoefer et al used confocal microscopy and flow cytometric imaging techniques in addition to light transmittance aggregometry to elucidate the potential role of uninhibited and inhibited (by aspirin or prasugrel active metabolite [PAM]) platelet subpopulations during in vitro platelet aggregation in static samples. The authors should be congratulated for their extensive and innovative investigations. First, they demonstrated that ≈30% uninhibited platelets of the total platelet population can overcome aspirin-induced platelet inhibition. This observation is, in part, in line with earlier demonstrations of a nonlinear relationship between in vivo platelet COX-1 inhibition marked by urinary 2,3-dinor-TxB₂ excretion and ex vivo TxA₂ biosynthesis marked by serum TxB₂; the latter suggesting the role of TxA₂ produced by a small proportion of uninhibited platelets in sustaining arachidonic acid–induced aggregation. After absorption, aspirin rapidly acetylates the platelet COX-1 enzyme in the prehepatic circulation. Aspirin is only transiently present in the plasma (up to 6 hours post administration). Thereafter, newly formed platelets that enter the circulation are uninhibited and have the capacity to generate TxA₂. The latter TxA₂ can bind to the TxA₂ receptor in aspirin-inhibited and aspirin-uninhibited platelets resulting in GP IIb/IIIa receptor activation and platelet aggregation. The latter mechanism may explain the homogeneous distribution of both inhibited and uninhibited platelets observed after arachidonic acid–induced platelet aggregation in the current study (Figure [A]). In addition, the latter phenomenon may also explain the ability of uninhibited platelets to overcome aspirin-induced platelet inhibition. However, it is in contrast to an earlier observation of near absence of aspirin resistance assessed by light transmittance aggregometry in patients with high-risk coronary artery disease, a group expected to have high platelet turnover. Nevertheless, in conditions of high platelet turnover, twice daily aspirin dosing or administration of extended release aspirin may improve 24-hour inhibition of platelet COX-1 and translate into positive antithrombotic effects.

Second, the authors report that ≈80% of uninhibited platelets of the total platelet population are required to fully overcome the inhibition of the P2Y₁₂ receptor by PAM with a linear relationship between the proportion of uninhibited platelets and the recovery of platelet response to ADP. Again, these observations are in line with an earlier definition of ≤10% inhibition of ADP-induced aggregation as clopidogrel resistance—an absence of a drug effect. After stimulation by ADP, uninhibited platelets were observed to form a core of the platelet aggregate. ADP released by uninhibited platelets can activate only uninhibited platelets in a paracrine manner resulting in the amplification of platelet aggregation. Unlike the activation of aspirin-treated platelets by released TxA₂, PAM-inhibited platelets cannot be activated by released ADP. Therefore, inhibited platelets were excluded from the core of the platelet aggregate (Figure [B]). The mechanism(s) responsible for the recruitment of PAM-inhibited platelets to the uninhibited core deserves further studies that may help to unravel the mysteries behind antiplatelet resistance.

Some limitations should be acknowledged when we consider the relevance of the in vitro study results to the in vivo situation. The in vitro experiments were conducted in static conditions that ignore the influence of arterial shear, which influences platelet function. Repeated platelet washings are
known to induce artifacts. Studies conducted in the presence of an anticoagulant ignore the important contributions of thrombin and fibrin during in vivo thrombus generation. Finally, the observations of the current study seem based on the assumption of complete inhibition of COX-1 by aspirin and P2Y12 receptors by PAM. Despite these limitations, these elegant in vitro experiments by Hoefer et al5 further our understanding of the mechanisms of antiplatelet response, the potential importance of uninhibited platelets in treatment failure, and the differences in platelet aggregate formation in the setting of thienopyridine and COX-1 inhibition.

Disclosures
Dr Gurbel reports serving as a consultant for Daiichi Sankyo, Bayer, AstraZeneca, Boehringer, Merck, CSL, and Haemonetics; receiving grants from the National Institutes of Health, Daiichi Sankyo, CSL, AstraZeneca, Harvard Clinical Research Institute, Haemonetics, Coramed and Duke Clinical Research Institute; receiving payment for lectures, including service on speakers’ bureaus, from Daiichi Sankyo, AstraZeneca, and Merck; Dr Gurbel is holding stock or stock options in Merck, Medtronic, and Pfizer and holding patents in the area of personalized antiplatelet therapy and interventional cardiology. Dr Tantry reports no conflicts of interest.

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