

Platelet Function Tests: Why They Fail to Guide Personalized Antithrombotic Medication

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General Considerations

Despite the essential role of platelets in arterial thrombogenesis, personalized antiplatelet therapy based on platelet function tests (PFTs) did not improve clinical outcome after coronary revascularization¹ and did not predict recurrent ischemic or bleeding events in individual patients.^{2,3} To understand why these point-of-care PFTs have yielded so little clinical benefit, we scrutinized their mechanism of thrombus assessment for its relevance to the pathomechanism of arterial thrombogenesis.

Fundamental differences exist between thrombus generation at high shear in native blood in vivo and agonist-induced platelet aggregation at low shear in anticoagulated blood ex vivo. Most PFTs in current use reproduce the second scenario. These tests, which are variants of the classical platelet aggregometer, are based on the assumption that the secretion of agonists from activated platelets is the major determinant of thrombus growth. Accordingly, various soluble agonists are used (adenosine diphosphate [ADP], thromboxane A₂ [TXA₂], or α -thrombin) to activate platelets in citrate-anticoagulated whole blood at very low shear, and the formation of small platelet aggregates (attached to fibrinogen-coated beads to obtain better optical signals) are measured. Under physiological conditions, at arterial shear rates ($\approx 420 \text{ s}^{-1}$), platelet aggregation occurs only in response to activation by agonists; however, in vivo, at sites of turbulent flow near the site of a severe stenosis and thus high shear (above $\approx 10\,000 \text{ s}^{-1}$), long-lasting adhesion and

aggregation occur without any requirement for platelet activation. Consequently, the main soluble agonists are not involved in the initial shear-induced platelet aggregation but rather contribute to stabilization of the unstable platelet aggregate. There are also essential differences between platelet aggregation in citrated blood and native blood. In citrated blood, in response to activation of platelets by ADP, collagen, or arachidonic acid, soluble agonists are released from platelet storage granules or generated by platelets (release reaction), which substantially enhances the initial platelet reaction (secondary aggregation). In contrast, in native blood, whether in vivo or in vitro, only activation by α -thrombin binding to platelet surface glycoprotein Ib α receptors can induce platelet adhesion, dense granule secretion, and aggregation. Under pathological conditions, thrombin is essential not only for initiation and propagation of the initial platelet aggregation but importantly for stabilization of the platelet thrombus through enzymatic and structural effects. By releasing plasminogen activator inhibitor 1, the main inhibitor of the fibrinolytic system, from the storage granules of platelets and activating thrombin-activatable fibrinolysis inhibitor, thrombin confers resistance on the arterial thrombus against endogenous fibrinolysis. Thrombin also anchors unstable platelet aggregates, together with fibrin, to the site of vascular injury, imparting structural stability to the thrombus, thereby preventing downstream embolization due to the effects of arterial flow (Figure 1).

Importance of High Shear Forces

Evidence shows that shear gradient-dependent platelet aggregation is the primary mechanism initiating thrombus formation under conditions of pathological high shear, such as those that exist in a severely stenosed artery. Soluble agonists play only a secondary role, serving to stabilize the initial platelet aggregates.

High wall shear rates are considered pathological in the range 2500 to $200\,000 \text{ s}^{-1}$, and the maximum shear rate across a severe short stenosis can exceed $250\,000 \text{ s}^{-1}$.⁴ An average 10% decrease in vessel diameter at the stenosis site can increase shear rates by 40% to 90% and initiate platelet

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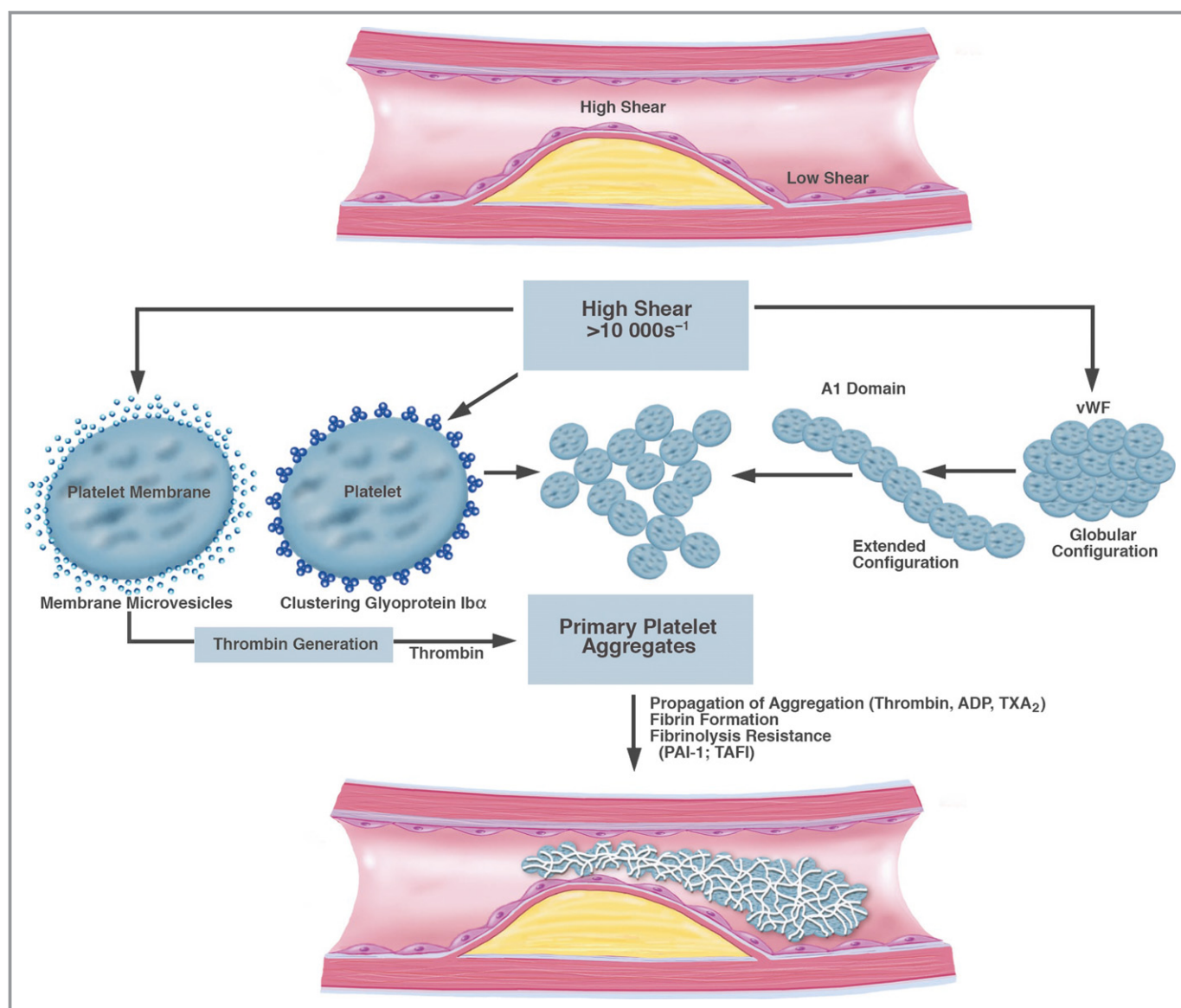


Figure 1. Contribution of high shear forces and thrombin to arterial thrombogenesis. Exposed to high shear, platelets with clustered membrane glycoprotein Iba interact with the exposed A1 domains of vWF, and loose platelet aggregates are formed. High shear also induces formation of platelet-derived microparticles, which generate thrombin. Acting on the loose primary platelet aggregates, thrombin (1) propagates aggregation by releasing ADP from platelet granules and allowing the formation of thromboxane A₂, (2) provides structural stability to the thrombus by enmeshing the tight platelet aggregate with a fibrin network, and (3) makes the platelet-rich thrombus resistant to endogenous fibrinolysis by releasing the main fibrinolysis inhibitor PAI-1 from platelets and inducing the formation of TAFI. ADP indicates adenosine diphosphate; PAI-1, plasminogen activator inhibitor 1; TAFI, thrombin-activatable fibrinolysis inhibitor; TXA₂, thromboxane A₂; vWF, von Willebrand factor.

activation and thrombus formation.⁵ Earlier investigators of fluid mechanics characterized the hemodynamics, specifically, the wall shear rates, in a severely stenosed coronary artery and proposed a cardinal role for high shear in initiating thrombus formation^{6–9}; however, these basic studies could not explain the interaction between shear and thrombus growth. Although confirming earlier findings, later studies provided strong evidence for the threshold and mechanism of high shear–induced platelet activation.^{4,10–12}

Variables affecting the actual shear rates in a severely stenosed artery are shown in Figure 2. From a detailed analysis, stenosis length, height, and roughness emerged as the main determinants of the local shear rates, whereas stenosis eccentricity or flow pulsatility were not significant contributors to local hemodynamics.¹² The extended length of stenosis reduced the shear rate by a factor of 4. Compared with a smooth surface, roughness of the plaque or thrombus surface greatly increased shear rate. Even at an early stage of

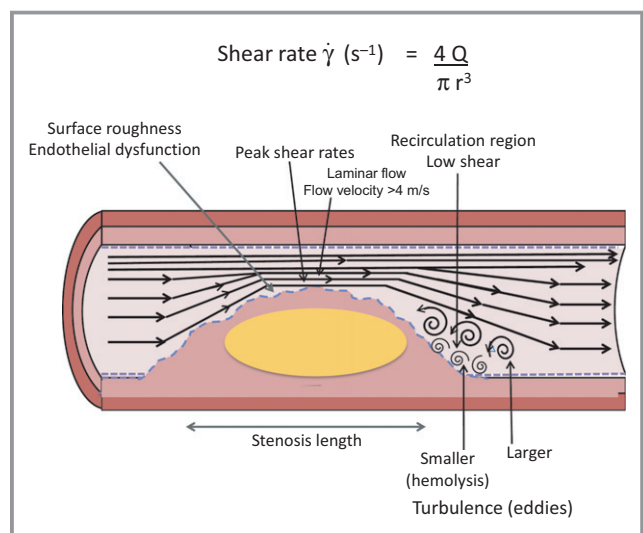


Figure 2. Variables affecting local shear rates in a severely stenosed artery. The definition of shear rate ($\dot{\gamma}$) is shown, in which Q is the flow rate and r is the radius of the vessel. Streamline pattern of blood flow through a stenotic vessel is shown. The peak shear in the apex of the stenosis is reduced with increasing length of the stenosis, whereas surface roughness of the stenosis increases shear rates. In the poststenotic recirculation zone, shear rate is low and flow of turbulent; small eddies cause hemolysis by dissipating shear energy to the membrane of red cells.

atherosclerosis, the luminal surface of the arterial wall becomes rough because of accumulation of lipid-filled macrophages within the intima and detachment of endothelial cells.

Under normal flow conditions, von Willebrand factor (vWF) and platelets circulate together without interacting, and their interaction to form thrombi is regulated by changes in shear stress. At physiological arterial shear rates ($\approx 420 \text{ s}^{-1}$), platelet aggregation occurs only after activation by agonists; however, if shear rates exceed $10\,000 \text{ s}^{-1}$, glycoprotein $\text{Ib}\alpha$ receptors on the platelet surface form clusters, and plasma vWF undergoes conformational transformation exposing its A_1 domain. As a result of these structural changes, platelets interact with vWF, and aggregates are formed just downstream in the poststenotic segment, at which there is low shear (shear deceleration zone) and turbulent flow (Figure 1). At these high shear rates, platelet aggregation occurs independently of ADP, TXA_2 , and thrombin and is mediated exclusively by vWF–glycoprotein $\text{Ib}\alpha$ adhesive bonds.^{6–8} In addition, above threshold shear rates of $>10\,000 \text{ s}^{-1}$, microvesicles (microparticles) are formed on the platelet surface and are responsible for abundant thrombin generation.¹³ Furthermore, in the poststenotic region at low shear and turbulent flow, small eddies can induce hemolysis (Figure 2), and the disrupted red cell membrane fragments

also contribute to thrombin generation. The significance of shear-induced thrombus formation in a stenotic artery is supported by increased platelet reactivity due to vWF release after coronary angioplasty.¹⁴ Because of this key role in arterial thrombogenesis, vWF is regarded as a risk factor, mediator, and pharmacological target in antithrombotic strategy.¹⁵

Point-of-Care Platelet Function Tests That Use High Shear

Presently, 3 commercially available point-of-care PFTs claim to test platelet reactivity under high shear stress: the PFA-100 (Siemens Inc), the Global Thrombosis Test (GTT; Thromboquest Ltd), and the PlaCor Platelet Reactivity Test (PlaCor Inc).

In the PFA-100, shear rates of 5000 s^{-1} are described in the $147\text{-}\mu\text{m}$ aperture of the collagen-coated membrane impregnated with specific platelet agonists; however, histology of the occluded aperture revealed that adhesion and aggregation of platelets take place on the proximal (and not distal) surface of the membrane around the aperture, and the arrest of flow was caused by the extension of those proximally formed platelet aggregates into the aperture. This is important because the platelets, which react with the collagen-coated membrane and form occlusive aggregates, were exposed to shear only in the capillary conduit ($200\text{-}\mu\text{m}$ diameter) on their way to the membrane^{16,17} and had not passed through the $147\text{-}\mu\text{m}$ aperture. Because shear rate is inversely proportional to the cube of the arterial radius, the true initial shear rate in the PFA-100 is likely to be $<2500 \text{ s}^{-1}$.

The manufacturer of the PlaCor Platelet Reactivity Test claims that a shear rate of 1500 s^{-1} exists in the restricted segment of the capillary conduit and that platelet-rich thrombus formation on the steel coil, which forms the restriction, is a reflection of high shear-induced thrombosis. In fact, deposition of platelets and leukocytes on stainless steel surfaces at low shear and low flow rates is a recognized phenomenon on cardiac catheters¹⁸ and has little relevance to the thrombotic occlusion of a stenotic artery at high flow and shear rates.

The GTT uses native blood and an initial shear rate of $\approx 16\,000 \text{ s}^{-1}$ as the sole agonist to activate platelets and initiate the thrombotic occlusion of narrow channels. When platelets are exposed to such high shear, they become activated, aggregate, form microparticles, and generate thrombin. Because the shear rate in the GTT is higher than the threshold of $>10\,000 \text{ s}^{-1}$, there is no requirement for the use of any chemical agonist. Because only fibrin-stabilized thrombi can cause the measured occlusion and arrest of flow, the GTT measurement reflects both platelet reactivity to pathologically relevant high shear stress and thrombin

generation. At elevated shear stress, platelet thrombus formation depends entirely on the binding of vWF to platelet glycoprotein Ib α and glycoprotein IIb/IIIa receptors. This is reflected in the GTT, in which occlusion time was inversely correlated with plasma vWF antigen and vWF ristocetin cofactor activity,¹⁹ and antagonism of vWF with the glycoprotein Ib inhibitor aurin tricarboxylic acid significantly inhibited occlusion,²⁰ showing that vWF is essential for thrombosis formation in this system.

It has to be emphasized that despite the vast theoretical knowledge accumulated in this field, the many variables make it extremely difficult to simulate hemodynamics that occur in vivo in a stenosed atherosclerotic coronary artery. Based on evidence from theoretical studies, however, a PFT should claim relevance to the coronary situation in vivo only if it exerts high shear stress ($>10\,000\text{ s}^{-1}$) on platelets and a shear gradient (flow deceleration zone with turbulence) is created in which the propagation and stabilization of the initial shear-induced platelet aggregates could take place.

Importance of Thrombin Generation

The formation of procoagulant phospholipids (microparticles) on the membranes of platelets and subsequent thrombin generation are cardinal features of arterial thrombogenesis (Figure 1). The crucial contribution of thrombin to arterial thrombosis is thought to be 2-fold. First, thrombin, the most powerful platelet agonist, accelerates the initial aggregation both by direct action and by releasing potent agonists (ADP, TXA₂, platelet activating factor 4) from platelets. Second, by forming fibrin, thrombin imparts structural stability to the initially unstable thrombus mass.

As stated earlier, in vivo, only thrombin activation can induce a platelet release reaction, namely, the release of ADP and formation of TXA₂ (ie, the agonists often used in PFT assays), and generate microparticles on the platelet surface.²¹ It has been shown that patients with stable ischemic heart disease on dual antiplatelet therapy express high levels of the thrombin receptor protease-activated receptor 1 (PAR-1) that are associated with profound thrombin-inducible platelet activation. Nonetheless, neither ex vivo PAR-1 expression nor in vitro thrombin-inducible PAR-1 activation was correlated with ADP-inducible platelet aggregation,²² and no interaction was observed between PAR-1 or P2Y₁₂ antagonist treatment after acute coronary syndromes on efficacy or safety of outcomes.²³ Measurement of platelet thrombus formation on collagen fibrils in flowing blood did not reveal a correlation between inhibition of thrombus formation and the level of P2Y₁₂ inhibition.²⁴ Furthermore, the relative importance of agonist-induced

platelet activation, release reaction, and contribution to thrombus growth at pathological high shear rates has been challenged.^{25,26}

Evidence now points to thrombin as the main player at an early stage by providing firm attachment of platelet aggregates to the vessel wall and later by stabilizing the loose platelet aggregates with fibrin and rendering the thrombus resistant to fibrinolysis. Activation of thrombin receptors PAR-1/PAR-4 by thrombin results in the synthesis and secretion of plasminogen activator inhibitor 1 from aggregated platelets²⁷ and activation of thrombin-activatable fibrinolysis inhibitor, conferring resistance on the arterial thrombus against endogenous fibrinolysis (Figure 1). Such fibrin-stabilized thrombus can resist high arterial flow and, protected from endogenous thrombolysis, can cause lasting occlusion of the artery.

Effect of Anticoagulation on Thrombin

Thrombin formation takes place only above a critical plasma calcium concentration. For convenience, so that samples can be stored and analyzed when convenient, most PFTs are performed on citrate-anticoagulated blood, in which the calcium concentration is well below the necessary threshold for thrombin generation.² In citrated blood, many common platelet agonists can cause secondary aggregation due to secretion of ADP and formation of TXA₂ (release reaction). In contrast, in native blood, only thrombin can induce significant platelet granule secretion (ADP release, TXA₂ formation).²⁸ In the absence of thrombin generation due to citrate anticoagulation, all common point-of-care PFTs (PFA-100, VerifyNow assay [Accumetrics], Plateletworks [Helena Laboratories]) that use citrated blood, in which there is an absence of thrombin generation, use specific agonists to induce platelet aggregation. Only the GTT and the PlaCor Platelet Reactivity Test use native blood, the former using venous blood and the latter using fingerstick blood; however, PFTs using fingerstick blood have little relevance to platelet reactivity in vivo. First, the most reactive platelets react and remain inside the wound and are not present in the collected sample. This is confirmed by findings showing fingerstick platelet counts to be systematically lower and to correlate poorly with venous counts.²⁹ Furthermore, release of tissue factor from the wound accelerates coagulation; therefore, such a test at low shear rates more closely reflects coagulation than pure platelet reactivity. Thrombin generated from activated platelets (procoagulant activity) plays a pivotal role in GTT measurement. Microparticles derived from red cells or platelets exert a common mechanism of triggering thrombin generation. To assess endogenous thrombin potential, a technique of hemolysis-induced microparticle formation was used with

the GTT and shown to cause enhanced thrombotic occlusion, a convenient model to assess total endogenous thrombin potential.³⁰

Does the Specific Inhibition of TXA₂ or ADP-Induced Platelet Aggregation Reflect the Sole Mechanism of the Antithrombotic Effect of Aspirin and P2Y₁₂ Inhibitors?

Dual antiplatelet therapy coupling aspirin with a P2Y₁₂ inhibitor is currently the therapy of choice for the prevention of recurrent thrombotic events following coronary stent implantation. In such a combination, aspirin inhibits platelet TXA₂ generation, and the P2Y₁₂ receptor inhibitor prevents ADP-induced platelet activation. Aspirin and P2Y₁₂ antagonists are regarded as “agonist-specific” drugs, selectively inhibiting platelet activation by TXA₂ or ADP, respectively. Nonetheless, several studies indicate that these drugs exert antithrombotic effects that are unrelated to inhibition of ADP- or TXA₂-induced platelet aggregation.^{31–33} Recent findings suggest that 12-hydroxyleicosatetraenoic acid, a procoagulant (tissue factor release) and proaggregatory eicosanoid that is generated alongside TXA₂ from arachidonic acid–stimulated platelets, may play a significant role in variable sensitivity to aspirin.³⁴ Intake of aspirin significantly delayed and inhibited thrombin generation in native thromboplastin-activated blood, whereas it had no such effect in citrated plasma.³⁵ Aspirin appears to have a significantly reduced effect on collagen-induced thrombus formation at high wall shear rates. In a canine model of coronary artery stenosis, aspirin was effective only at low shear rates of 500 s^{−1}, whereas at shear rates of 2000 s^{−1}, aspirin failed to prevent cyclic flow reduction, even at high doses.^{5,36} Aspirin did not reduce platelet deposition and thrombus formation with collagen in a model replicating an 80% stenosis at a shear rate of 10 500 s^{−1}.³⁷ In contrast to its effects at low shear rates of 500 and 1500 s^{−1}, aspirin was unable to fully prevent occlusive thrombus formation at high shear rates of 4000 and 10 000 s^{−1}, even at doses 20 times the recommended daily oral dose of 100 mg.³⁸ In patients with peripheral arterial disease on aspirin, platelet responsiveness to various agonists was normal when tested under high shear–flow conditions, except for inhibited platelet reactivity to PAR-1 stimulation.³⁹

A marked reduction in thrombus formation with frequent thrombus detachment from collagen surfaces after aspirin use^{38–40} indicates that at high flow and shear conditions, it is the inhibitory effect of aspirin on thrombus stabilization, rather than inhibition of platelet cyclooxygenase and TXA₂ synthesis, that is responsible for much of its therapeutic effect.

Because the potent antithrombotic effects of platelet P2Y₁₂ receptor blockers are attributed solely to antagonism of ADP-induced platelet aggregation, tests of ADP-induced aggregation are used to monitor their therapeutic effectiveness; however, ADP-induced platelet aggregation is also affected by aspirin and is not specific for P2Y₁₂ receptor inhibition. Furthermore, ADP release from dense granules after platelet stimulation with ADP in citrated blood results in significantly increased peak aggregation compared with the same stimulation with ADP in hirudin-anticoagulated blood, in which ADP activation does not cause release and secondary aggregation. As such, ADP-induced platelet aggregation tests underestimate the in vivo effect of P2Y₁₂ inhibitors.⁴¹ The relationship between circulating levels of P2Y₁₂ inhibitors and inhibition of ADP-induced platelet aggregation is nonlinear. The most specific method for assessing platelet P2Y₁₂ receptor activity is the flow cytometric analysis of the phosphorylation state of vasodilator-stimulated phosphoprotein. Although ≈50% of patients were found to be clopidogrel resistant as measured by ADP-induced platelet aggregation (VerifyNow), the vasodilator-stimulated phosphoprotein assay identified only 11.7% of patients as nonresponders.⁴² Most PFTs measuring ADP-mediated platelet aggregation use prostaglandin E1, which was shown to overestimate the therapeutic response to platelet P2Y₁₂ inhibitors.⁴³ On these grounds, the suitability of ADP-induced platelet aggregation for monitoring P2Y₁₂ antagonist medication is questioned.^{44–46}

Earlier claims that P2Y receptors on platelets could be activated only by nucleotides (ADP) have been challenged. Several P2Y receptors display agonist promiscuity and tissue specificity and can be activated not only by ADP but also by other agonists such as leukotrienes in nanomolar concentrations. As such, P2Y₁₂ inhibitors may exert important effects on thrombotic and inflammatory processes through mechanisms that are independent of inhibition of ADP-induced platelet aggregation or unrelated to P2Y₁₂ antagonism. Vessel wall (not platelet surface) P2Y₁₂ receptors were shown to be important in early atheroma formation, and the latter was not inhibited by platelet P2Y₁₂ inhibition.⁴⁷ Activation of neutrophils and the release of active tissue factor at the site of plaque rupture during acute myocardial infarction are important thrombogenic stimuli. The P2Y₁₂ receptor antagonist prasugrel metabolite has been shown to inhibit neutrophil activation through a mechanism that does not involve P2Y₁₂ receptors in neutrophils.⁴⁸

It has been shown recently that P2Y₁₂ antagonism disrupts the stability of newly formed platelet aggregates, promotes disaggregation, and reverses arterial thrombotic occlusion. Consequently, in addition to activating platelets, signaling via P2Y₁₂ receptors seems to be required for stabilizing platelet thrombi.^{49,50} Nitric oxide released from vascular endothelium is a powerful inhibitor of platelet aggregation. Blockade of P2Y₁₂ receptors dramatically enhanced the antiplatelet

potency of nitric oxide, resulting in a 1000- to 100 000-fold increase in inhibitory activity against platelet aggregation and release reaction in response to activation by thrombin or collagen.⁵¹

These findings support the hypothesis that mechanisms other than inhibiting ADP-induced platelet aggregation play the main role in the antithrombotic effect of P2Y₁₂ antagonists. Furthermore, inhibition of thrombin generation, which is unaffected by P2Y₁₂ inhibitors, is a common-denominator mechanism of effective antithrombotic therapies. The reevaluation of the thrombin hypothesis is reflected in the proposed advantages of adding novel oral anticoagulants that directly or indirectly inhibit thrombin to dual antiplatelet therapy to further reduce cardiovascular thrombotic events.^{52–54} Although this approach may reduce thrombotic events, it also increased bleeding risk and thus cannot be recommended as a panacea for all comers.

Anticoagulation of Blood Prevents the Use of High Shear Stress and Assessment of Thrombin Generation in Point-of-Care Platelet Function Tests

Thrombus stability, namely, its firm attachment to the vessel wall and resistance to embolization by the arterial flow, is a major determinant of the outcome of arterial thrombus formation. Thrombin formed at the site of thrombus growth plays a pivotal role in both thrombus growth and stability. In citrate-anticoagulated blood, thrombin is not formed from activated platelets due to very low concentrations of calcium; in addition, citrate exerts a direct effect on platelet reactivity that is unrelated to the effect on ionized calcium levels.^{2,55} In citrated blood at very low shear, such as that which occurs in platelet aggregometry, only small, loosely packed aggregates are formed in response to agonist stimulation. If higher, pathologically relevant shear rate is applied to citrated blood, the adhesion of platelets to the subendothelium is greatly reduced and, because of the weak attachment between platelets, aggregation is abolished.⁵⁶ In contrast to the effect in citrated blood, the effect of aspirin on platelet thrombus growth was markedly attenuated in native blood subjected to high shear rate.⁵⁷

In the GTT, native blood is tested at high shear rates, and the measured occlusion and arrest of flow is caused by fibrin-stabilized thrombi. As such, thrombin generation is an essential part of the test. It is claimed that in a single measurement, the GTT can detect major determinants of hemostasis including platelet reactivity, endogenous fibrinolytic, and thrombin-generating potential.³⁰ In the GTT, the occlusion time is a reflection of platelet reactivity. Short occlusion times may represent enhanced platelet reactivity

such as that occurring in a prothrombotic state, whereas prolonged occlusion times may reflect the effect of antiplatelet or anticoagulant medication. The second phase of the test assesses lysis time, a measurement that reflects the rapidity of lysis of the platelet thrombus formed in the first phase. The more prolonged the lysis time, the less effective the endogenous thrombolytic activity, and this appears to correlate with an increased risk of thrombotic events.

The GTT has limitations, including the need for timely prompt assessment of blood immediately on withdrawal because native blood is used. Although providing a clear physiological advantage, this approach could be seen by some as an inconvenience in the present era of collecting anticoagulated blood samples and testing them later in specialized laboratories. Furthermore, the instrument should be situated close to the patient, and this may not be always possible or practicable. Delays in starting the measurement or prolonged or difficult blood draws could have important effects on the results of the occlusion and lysis times. Importantly, the GTT has not been assessed and validated in large clinical trials, so its value in guiding personalized antithrombotic therapy or predicting ischemic and bleeding events after coronary intervention is largely unknown.

Assessment of Endogenous Thrombolytic Status

The overall clinical outcome of a thrombotic stimulus will be determined not only by the strength of the stimulus for promoting thrombus formation and stabilization but also by the efficacy of the natural protective endogenous thrombolytic enzymes. The GTT is the only available PFT that measures thrombolytic status.⁵⁸ That the lysis time measured in the GTT is specific for thrombolysis is shown by the fact that plasminogen activator streptokinase and tissue-type plasminogen activator dose-dependently enhanced thrombolysis (lysis time) without affecting platelet function (occlusion time). The plasmin inhibitor tranexamic acid prevented plasminogen activator-induced thrombolysis, whereas inhibition of clot retraction by cytochalasin B did not affect the lysis time.²⁰ The potential clinical relevance of assessing lysis time has been shown in patients with recent acute coronary syndromes^{58,59} and with renal failure^{58,60} in whom impaired endogenous thrombolysis, as demonstrated by prolonged lysis time, was associated with an increased risk of myocardial infarction and cardiovascular death. In a small cohort of patients presenting with ST-elevation myocardial infarction, those with spontaneous ST resolution and normal coronary flow before intervention had very short lysis times (efficient endogenous lysis), and those with markedly impaired (prolonged) lysis times had higher incidence of no-flow and future adverse events.⁶¹

Conclusion

In the mechanism of coronary atherothrombosis, high shear stress and thrombin play the cardinal roles. Although some PFTs claim to subject blood to high shear, actual shear rates are well below the threshold required for direct platelet activation and inducing thrombin generation. Furthermore, for convenience, these tests are carried out on anticoagulated blood, in which thrombin is not generated. Current antiplatelet therapy and thus PFTs target thromboxane A₂ and the ADP–P2Y₁₂ platelet activation pathways. In vivo, however, only thrombin-induced platelet activation results in ADP release and TXA₂ formation, and even this makes little contribution to thrombus growth at pathological shear rates. The most important function of thrombin in atherothrombosis is to impart structural stability to the thrombus mass. Experimental evidence points to reduced thrombus stability at the sites of severe stenoses, as one of the most important therapeutic effects of antiplatelet drugs.

Disregarding the importance of both high shear forces, as primary activators of platelets in a stenosed artery, and thrombin generation, which is responsible for thrombus stability, has great negative impact on the clinical usefulness and physiological relevance of many currently available PFTs.

Disclosures

Gorog is related through family to a company director in Thromboquest Ltd, but neither she nor her spouse or children have any shares or financial interests or receive any benefits from this company. She has received no grants, honoraria or financial sponsorship from this company. There are no other conflicts of interest to disclose. Jeong has received honoraria for lectures from AstraZeneca, Sanofi-Aventis, Daiichi Sankyo/Lilly, Haemonetics, Otsuka and Yuhan Pharmaceuticals; and research grants or support from AstraZeneca, Korean Society of Interventional Cardiology, Han-mi Pharmaceuticals, and Haemonetics.

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