of both serum TXB₂ and platelet aggregation and this effect lasts for at least 24 hours. This is, however, not necessarily the case in certain conditions where aspirin-induced inhibition of platelet function at 24 hours is incomplete (see figure).³,⁴ The concept of such “aspirin resistance” is not new and has been attributed primarily to increased platelet turnover with reappearance of new platelets with intact COX-1.⁵ Increased platelet turnover might result from either abnormal megakaryopoiesis (eg, ET) or increased peripheral consumption by injured blood vessels, as might be the case with diabetes mellitus (DM), coronary artery disease, or tobacco use.³,⁶ Either way, suboptimal 24-hour durability of aspirin effect has been associated with increased risk of arterial events⁷ and calls for new treatment approaches to overcome the particular phenomenon.

In a carefully selected group of non-diabetic ET patients not receiving other antiplatelet drugs, Pascale et al report that twice-daily enteric-coated aspirin at 100 mg dose was superior to once-daily enteric-coated aspirin at 200 mg dose, which was in turn superior to once-daily plain or enteric-coated aspirin at 100 mg dose, in favorably modifying residual thromboxane activity in “aspirin resistant” cases; the latter were defined as displaying ≥ 4 ng/mL of serum TXB₂ at 24 hours after aspirin intake and constituted 78% of screened patients.¹ There was no difference in the antiplatelet effect of enteric-coated versus plain aspirin at equal doses, which is consistent with previous observations.⁸ The biochemical evidence of improved aspirin effect by dose and schedule modification was also apparent by some but not other platelet function assays. The authors also showed that immature (ie, reticulated) platelet count was the sole predictor of adequate 24-hour suppression of thromboxane synthesis.

Although the potential merit of twice-daily aspirin dosing has also been suggested in patients with DM,⁹ it is particularly appealing in myeloproliferative neoplasms (MPN) because (1) abnormal megakaryopoiesis and increased platelet production are integral components of the disease phenotype, (2) some of the risk factors for arterial thrombosis in MPN (eg, advanced age, angiopathy, leukocytosis, JAK2V617F, DM, smoking) have also been associated with aspirin resistance or increased immature platelet count, and (3) aspirin is a key component of treatment in MPN with level “A” evidence of value, at least in polycythemia vera.¹⁰ Accordingly, it is reasonable to consider the possibility that aspirin resistance contributes to treatment resistance in some MPN patients with microvascular symp-toms and to the residual risk of thrombosis in otherwise adequately treated cases. The concept of twice-daily aspirin therapy is potentially practice-changing but requires additional controlled studies to determine the clinical relevance of reversing biochemical resistance to aspirin and safety of twice-daily dosing, especially in terms of long-term bleeding risk. In the context of MPN, one has to also dissect the confounding effect of cytoreductive therapy, which is known to markedly suppress platelet turnover and therefore possibly attenuate aspirin resistance. For now, it would be premature to indiscriminately order platelet function tests looking for aspirin resistance and place patients on a twice-daily aspirin regimen wishing for a better clinical outcome. Such measures should be reserved for those patients with clear evidence of treatment failure.

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THROMBOSIS & HEMOSTASIS

Comment on Fung et al, page 3613

New approaches for measurement of platelet reactivity

Aaron J. Marcus  WEILL CORNELL MEDICAL COLLEGE

In a highly interesting, intricate, and novel paper in this issue of Blood, Fung and colleagues have extended their previous pioneering studies and now reveal that molecules such as ATP can promote platelet activation through the P2X1 receptor.¹ This adds to the known mechanisms of how the P2X1 receptor differs from other mechanisms that elevate the major second messenger, calcium. This occurs during stimulation by different agonists. They characterized steps in the evolving thrombotic process. In this system prostacyclin and nitric oxide cannot prevent ATP release and P2X1-dependent elevation of calcium. In addition, the role of inflammation via Toll-like receptors is not inhibitable by nitric oxide and prostacyclin. This generated the idea that because Toll-like receptors are stimulated by agonists of the innate immune system, they represent one of the mechanisms whereby platelets play a role in the inflammatory process.² These results may help explain the recent results using the thrombin–receptor antagonist vorapaxar in the treatment of myocardial infarction.³
Fung et al have provided new and important information concerning biochemical evolution of a platelet thrombus. New early interactions between cell components and the microenvironment have been defined and may help provide additional information concerning evolution of platelet thrombus so that thrombus formation can be approached in a precise, therapeutic manner. This may explain the disappointing results from what was thought to be the correct anti-thrombotic mechanisms. Many diagrams showing the pathogenesis of atherosclerosis have a small collection of platelets on top of the atherosclerotic plaque. The thrombus narration ends where it should begin if we are to discern more pathologic and therapeutic information to reduce the occurrence of 1 vascular event every 39 seconds in the United States. Fung and colleagues have demonstrated that activation of platelets (Ca\(^{2+}\) responses) is coupled to glycoprotein VI and Toll-like receptors that are not inhibitable by known compounds from endothelial cells. This is due to secondary activation of P2X1 receptors and release from intracellular Ca\(^{2+}\) stores. It therefore can now be concluded that platelets contain inhibitor-resistant Ca\(^{2+}\) mobilization pathways involving P2X1 receptors. This means that the results may have particular importance for our understanding of thrombotic or immune-dependent platelet activation. The research presented here investigates how endogenous platelet inhibitors regulate calcium responses to a broad range of agonists and inhibitors in the presence of P2X1 receptors. The agonists for studying the reactions described included collagen, thrombin, and a thromboxane A2 analog. The data obtained also support the concept that ATP released from platelets locally and efficiently activates P2X1 receptors. Therefore, this ion channel can rapidly generate calcium influx.

In studies involving large numbers of patients with stroke, myocardial infarction, and diabetes there are subjects whose aggregation responses to a wide variety of agonists are maximal and stay maximal despite dilution of the agonists, especially collagen. One can also speculate that in the case of patients who overcame inhibition by Celecoxib and Rofecoxib may also have had subgroups with low threshold platelets that overcame the inhibition and advanced to a prothrombotic state. This should be tested in surviving patients.

Another group of events that may give rise to increased or decreased platelet reactivity are the known cell-cell interactions that take place with different cell types. It is known that platelet endoperoxides can enter aspirin-treated endothelial cells and produce prostacyclin there from. A relationship between platelet thrombosis and the inflammatory process has also been demonstrated. Arachidonic acid escaping from activated platelets is capable of penetrating activated neutrophils with the production of leukotriene B4. There are now several known instances where metabolic interchange between cells of the circulation results in different end products. This is known as transcellular metabolism.

Fung and colleagues demonstrated Ca\(^{2+}\) mobilization pathways in platelets that represent events immediately after platelet activation. These pathways are resistant to inhibitors of platelet reactivity such as prostacyclin and nitric oxide. Ordinarily in the microenvironment of an evolving platelet thrombus, prostacyclin and nitric oxide in the milieu are active along with the action of the main control system for blood fluidity, the endothelial enzyme known as CD39 (NTPDase-1). Even in the complete absence of prostacyclin and nitric oxide, CD39 continues to control blood fluidity by converting ADP to AMP. The authors of this interesting paper have demonstrated that activation (Ca\(^{2+}\) responses) of activated platelets are coupled to glycoprotein VI and Toll-like receptors and are not inhibitable by known compounds derived from endothelial cells. This is due to secondary activation of P2X1 receptors and release from intracellular Ca\(^{2+}\) stores. P2X1-dependent amplification of threshold signaling through GPV1 would enhance the likelihood of individual platelet tethering and formation of an initial layer of platelets on damaged endothelium. This would be relevant for conditions involving high shear. These conditions provide time available for restriction of activation and an explanation for the greater contribution of P2X1 receptors to arterial compared with venous thrombosis.

This research provides evidence indicating that platelets possess at least 2 calcium mobilization mechanisms that are resistant to major endothelium-derived inhibitors of platelet functions. Influx through the ATP-gated P2X1 receptor is largely responsible for the inhibitor-resistant pathway at low levels of GPVI and TLR/1 stimulation.

This amplifying role of P2X1 receptors could be important at sites of atherosclerotic plaques where stenosis increases at a shear level several fold higher than in the normal circulation. Given this unique contribution to platelet activation of P2X1 antagonists, it should be considered as possible antithrombotic therapy possibly in combination with soluble CD39 to co-target P2Y receptor activation. Fung et al have also observed significant variation between donors of in the magnitude of robustness of P2X1 responses ex vivo. The extent to which this variation occurs in vivo is unknown. This raises the possibility that the effectiveness of anti-P2X1 therapies could be greater in a proportion of patients.

The aim of the research presented by Fung et al was to investigate how endogenous platelet inhibitors regulate calcium responses to a range of stimuli under conditions where P2X1 receptors are operative. Strong evidence was provided that there are at least 2 Ca\(^{2+}\) mechanisms, both resistant to the major endothelium-derived inhibitors of platelet function (prostacyclin and nitric oxide). Entry through the ATP-gated P2X1 receptor is the major component of this inhibitor-resistant pathway in the presence of low levels of GPV1 and TLR 2/1 stimulation. From the therapeutic standpoint P2X1 alone or in combination with ecto-ATPase/CD39 should be considered as interesting possibilities.

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New approaches for measurement of platelet reactivity

Aaron J. Marcus