Current State and Novel Approaches of Antiplatelet Therapy

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Abstract—An unresolved problem with clinical use of antiplatelet therapy is that a significant number of individuals either still get thrombosis or run the risk of life-threatening bleeding. Antiplatelet drugs are widely used clinically, either chronically for people at risk of athero/thrombotic disease or to prevent thrombus formation during surgery. However, a subpopulation may be resistant to standard doses, while the platelet targets of these drugs are also critical for the normal hemostatic function of platelets. In this review, we will briefly examine current antiplatelet therapy and existing targets while focusing on new potential approaches for antiplatelet therapy and improved monitoring of effects on platelet reactivity in individuals, ultimately to improve antithrombosis with minimal bleeding. Primary platelet adhesion-signaling receptors, glycoprotein (GP)IIb-IX-V and GPVI, that bind von Willebrand factor/collagen and other prothrombotic factors are not targeted by drugs in clinical use, but they are of particular interest because of their key role in thrombus formation at pathological shear. (Arterioscler Thromb Vasc Biol. 2015;35:1327-1338. DOI: 10.1161/ATVBAHA.114.303413.)

Key Words: anticoagulants ■ blood coagulation ■ blood platelets ■ drug therapy ■ platelet adhesion inhibitor

Antiplatelet and anticoagulant drugs, used alone or in combination, are a cornerstone of clinical treatment for human diseases associated with increased athero/thrombotic risks, such as heart attack and stroke, and in preventing thrombosis during surgery or other medical procedures involving vascular disruption or perturbed blood flow.1-4 The confounding issue is that rapid platelet activation and aggregation leading to thrombus formation in response to vascular injury is also a critical physiological mechanism,2 and blocking platelet function can therefore elevate bleeding risk. Individuals within a population or a disease cohort will also vary in their relative platelet reactivity and thrombotic propensity because of variations in platelet phenotype or genotype coincident with other thrombotic risk factors, thereby complicating the development of practical clinical guidelines for antiplatelet drugs, as well as dosing requirements and monitoring. For the latter, there is currently a lack of reliable platelet analytic, genotyping or functional testing that confidently predicts either thrombotic/bleeding risk or efficacy of antiplatelet treatment in individuals. Of further importance, the availability of drugs (oral or intravenous) and their pharmacological half-life in blood can potentially affect user compliance or reversibility, respectively. Thus, the problem of controlling bleeding versus thrombosis by antiplatelet therapy is likely to exist for the foreseeable future, emphasizing the clear need for improved approaches for therapy and monitoring.

There are several classes of antiplatelet drugs, either currently in clinical use (Table 1) or in preclinical or early clinical stages of development (Table 2), which act at a variety of targets. These include platelet receptors, their binding partners, signaling proteins, or soluble mediators of platelet function (Figure 1). In this review, we will discuss these therapeutic targets and their known pathological and physiological roles in healthy or diseased vasculature, the scientific basis for their selection and inhibition by existing agents, and new approaches for modulating their pathological function. Importantly, mechanisms of platelet activation and thrombus formation at the vessel wall need to be considered in the context of shear stress and normal or abnormal blood flow.

Platelet Activation and Thrombus Formation at the Vessel Wall

The capacity for human platelets circulating in the bloodstream to attach to a specific region of the vasculature and activate and aggregate within seconds to minutes is a sophisticated biological accomplishment, in particular at arterial shear rates where fluid shear forces act powerfully against cell adhesion. Adherent activated platelets promote localized coagulation, release proinflammatory mediators, and recruit leukocytes to initiate wound healing.2 At arterial shear rates, this is enabled principally by a specialized platelet-specific
adhesion-signaling system, made up of glycoprotein (GP) Ib-IX-V that binds von Willebrand factor (vWF; via the GPIbα subunit) and collagen (via GPV) and GPVI that binds collagen and the collagen-associated matrix protein, laminin. Thus, on exposure of collagenous subendothelial matrix in the vasculature, platelet GPIb-IX-V/GPVI can rapidly induce platelet adhesion and activation. Furthermore, in human platelets, these receptor–ligand systems are highly evolved to mediate thrombus formation as the shear rate increases to high physiological or pathological, as found in occluded coronary arteries with atherosclerotic plaque or where normal blood flows are disrupted, for example, in ventricular assist devices.

GPIbα, disulfide-linked to GPIbβ, GPIX, and GPV are all members of the transmembrane leucine-rich repeat family. Analogous leucine-rich repeat proteins are involved in innate immunity in premammalian species. GPVI is a member of the immunoreceptor family, with 2 extracellular immunoglobulin domains, and forms a noncovalent complex with the Fc receptor γ-chain required for GPVI surface expression and ligand-induced signaling on human platelets. Signaling immediately downstream of GPIb-IX-V/GPVI involves activation of Src and Syk kinase–dependent tyrosine phosphorylation pathways and Src/Syk-dependent and Src/Syk-independent generation of intracellular reactive oxygen species (ROS). The cytoplasmic domains of GPIb-IX-V and GPVI directly bind to signaling or adaptor proteins, including phosphatidylinositol 3-kinase (via the p85 subunit) and constitutively phosphorylated Lyn (via a proline-rich sequence of the p85 subunit) respectively. In the case of GPVI, ligand-induced receptor cross-linking enables active Lyn to phosphorylate Syk associated with the immunoreceptor tyrosine–based activation motif (ITAM) in the cytoplasmic domain of Fc receptor γ-chain, initiating canonical Syk–dependent signaling pathways. Other ITAM-bearing receptors in platelets include the low-affinity IgG receptor, FcγRIIa, and the C-type lectin family receptor-2. Positively charged conserved sequences of both GPIbβ and GPVI also directly bind to tumor necrosis factor receptor–associated factor 4, a receptor-localizing subunit of the nicotinamide adenine dinucleotide phosphate-oxidase complex (Nox-1/2), which generates intracellular ROS in platelets. Rapid elevation of intracellular ROS promotes platelet activation and supports platelet adhesion to collagen, by a mechanism that may involve ROS-dependent inactivation of protein tyrosine phosphatases that inhibit signaling involving Lyn and associated phosphorylated proteins.

Engagement of GPIb-IX-V/GPVI by vWF/collagen or other ligands leads to rapid platelet activation, morphological changes, in platelet shape and rearrangement of the cytoskeletal actin filaments, and changes in platelet plasma membrane phosphatidylserine expression that accelerate coagulation leading to activation of factor Xa and thrombin. GPIbα binds coagulation factors, XII, XI, high-molecular weight kinogen (of the intrinsic coagulation pathway), and thrombin, localizing coagulation at the platelet surface, whereas increased phosphatidylserine facilitates formation of procoagulant complexes of factor Xa to increase thrombin generation. Active thrombin bound to GPIbα activates platelets and facilitates stimulation of the G-protein–coupled 7-transmembrane receptor, protease-activated receptor-1; thrombin also activates human platelets via protease-activated receptor-4. In activated platelets, secretion of the agonist, ADP, and activation of the cyclooxygenase pathway leading to synthesis and secretion of thromboxane A2 (TxA2) reinforce platelet activation by autocrine stimulation of G-protein–coupled receptors for ADP (P2Y1 and P2Y12) or TxA2, respectively (Figure 1). In this way, activation via primary platelet receptors, GPIb-IX-V/GPVI, or secondary receptors for ADP, TxA2 or, thrombin leads to activation of the platelet integrin, αIIbβ3 (GPIIb/IIIa) that binds fibrinogen or vWF and mediates platelet aggregation. This receptor is expressed in a low-affinity form that does not bind ligand, but after intracellular signaling, it forms a high-affinity ligand-binding site, enabling platelet aggregation and outside-in-signaling to reinforce platelet activation. In addition to αIIbβ3, other platelet integrins, α2β1, α5β1, and α6β1 that bind collagen, fibronectin, or laminin, respectively, also become functional on activated platelets and promote firm platelet adhesion to collagenous subendothelial matrix. This amplification of platelet activation through release of secondary agonists is critical in stabilizing the thrombus and augmenting the thrombus volume. In cases of unrestrained growth, which can result in occlusion and ischemia, inhibition of secondary messengers, such as TxA2, by aspirin or platelet adhesion integrins assists in controlling thrombus size on the vessel wall. Figure 2 demonstrates how targeting of specific steps, using αIIbβ3 as an example, modulates thrombus formation.

In addition to activation pathways, there are at least 2 inhibitory pathways that can control platelet reactivity. First, inhibitory receptors bearing an immunoreceptor tyrosine–based inhibitory motif domain, such as platelet/endothelial cell adhesion molecule 1 or carcinoembryonic antigen-related cell adhesion molecule-1, can attenuate ITAM-mediated signaling by GPVI/Fc receptor γ-chain or other ITAM-bearing receptors, via recruitment of phosphatases or other mechanisms, although how immunoreceptor tyrosine–based inhibitory motif–containing receptors on circulating human platelets are activated under physiological or pathological conditions is uncertain. Second, elevation of intracellular cAMP/cGMP inhibits platelet activation by activating cAMP/cGMP-dependent protein kinases, protein kinase A/protein kinase G, although the role of cGMP effects on platelet function remains controversial with several studies demonstrating that cGMP is stimulatory. Protein kinase A substrates include cytoplasmic serine residues within conserved sequences of GPIbβ, regulating the interaction with intracellular binding partners and attenuating vWF-dependent platelet activation. Inhibitory prostacyclin (also known as prostaglandin) leads to activation of adenyl cyclase to increase...
intracellular levels of cAMP/cGMP. Stimulation of the ADP receptor, P2Y1, leads to inhibition of adenylyl cyclase and reduced production of cAMP to facilitate platelet activation.27,32 Alternatively, phosphodiesterases can directly reduce the levels of cAMP to increase platelet reactivity, and phosphodiesterase inhibitors can thereby be used to inhibit platelet activation.36 These types of inhibitors are among a range of promising new antithrombotic strategies and may have advantages compared with current modes of antiplatelet therapy.36

Platelet Targets of Current Antithrombotics

Current antiplatelet agents used clinically (Table 1) target several key components of platelet activation and aggregation (Figure 1). The 3 major targets are cyclooxygenase of the TxA2

Table 1.  Antiplatelet Therapy: Current Drugs

<table>
<thead>
<tr>
<th>Type/Name (Drug Name)</th>
<th>Marketing Status</th>
<th>Route of Administration</th>
<th>Indication</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclooxygenase inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA and aspirin</td>
<td>Over-the-counter</td>
<td>Oral</td>
<td>To reduce the risk of nonfatal thrombotic events</td>
<td>41, 101–103</td>
</tr>
<tr>
<td>Triflusal</td>
<td>Available in some European countries</td>
<td>Oral</td>
<td>Prevention of cardiovascular events and acute treatment of infarction</td>
<td>104–106</td>
</tr>
<tr>
<td>ADP P2Y12 inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clopidogrel (Plavix)</td>
<td>Prescription</td>
<td>Oral</td>
<td>For ACS NSTEMI, STEMI, and reduction and prevent of ischemic events</td>
<td>107–109</td>
</tr>
<tr>
<td>Prasugrel (Effient)</td>
<td>Prescription</td>
<td>Oral</td>
<td>Use in combination with aspirin in patients with ACS undergoing PCI, also NSTEMI and STEMI patients undergoing PCI</td>
<td>107, 109–111</td>
</tr>
<tr>
<td>Ticagrelor (Brilinta)</td>
<td>Prescription</td>
<td>Oral</td>
<td>For prevention of thrombotic events. Recommend in addition to ASA for ACS patients</td>
<td>111–113</td>
</tr>
<tr>
<td>αIIbβ3 (GPIIb/IIIa) inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abciximab (Reopro)</td>
<td>Prescription</td>
<td>Injection</td>
<td>As an adjunct to PCI for prevention of ischemic complications</td>
<td>42, 114–116</td>
</tr>
<tr>
<td>Eptifibatide (Integrilin)</td>
<td>Prescription</td>
<td>Injection</td>
<td>Treatment of MI and ACS</td>
<td>115, 116</td>
</tr>
<tr>
<td>Tirofiban (Aggrastat)</td>
<td>Prescription</td>
<td>Injection</td>
<td>For reduction of thrombotic cardiovascular events in patients with NSTEMI ACS</td>
<td>42, 115–117</td>
</tr>
<tr>
<td>Prostacyclin and analogues</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGI2 (Epoprostenol)</td>
<td>Prescription</td>
<td>Injection</td>
<td>Long-term intravenous treatment of primary pulmonary hypertension</td>
<td>118, 119</td>
</tr>
<tr>
<td>Iloprost (Ventavis)</td>
<td>Prescription</td>
<td>Oral inhalation</td>
<td>Treatment of PAH</td>
<td>118, 119</td>
</tr>
<tr>
<td>Treprostinil (Remodulin)</td>
<td>Prescription</td>
<td>Oral</td>
<td>Treatment of PAH</td>
<td>119–121</td>
</tr>
<tr>
<td>Direct thrombin inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bivalirudin (Angiomax)</td>
<td>Prescription</td>
<td>IV</td>
<td>For use in prevention of thrombosis in patients with ACS undergoing PCI</td>
<td>122, 123</td>
</tr>
<tr>
<td>Dabigatran (Pradaxa)</td>
<td>Prescription</td>
<td>Oral</td>
<td>Prevention of thrombosis post joint replacement and atrial fibrillation</td>
<td>124, 125</td>
</tr>
<tr>
<td>Phosphodiesterase inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipyridamole (Persantine)</td>
<td>Prescription</td>
<td>Oral</td>
<td>Use in adjunct to coumarin anticoagulants in the prevention of thrombotic complications post cardiac valve replacement</td>
<td>126, 127</td>
</tr>
<tr>
<td>Cilostazol (Pletal)</td>
<td>Prescription</td>
<td>Oral</td>
<td>Reduction of symptoms of intermittent claudication</td>
<td>126, 128, 129</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; ASA, acetylsalicylic acid; MI, myocardial infarction; NSTEMI, non–ST-segment–elevation myocardial infarction; PAH, pulmonary arterial hypertension; PCI, percutaneous coronary intervention; PGI2, prostaglandin; and STEMI, ST-segment–elevation myocardial infarction.
synthesis pathway (aspirin), the G-protein–coupled ADP receptor P2Y12 (clopidogrel, prasugrel, or ticagrelor), and inhibitors of \( \alpha_{IIb}\beta_3 \) (abciximab, eptifibatide, and tirofiban). Other targets are the prostaglandin receptor that can be blocked by prostacyclin mimetics and phosphodiesterase inhibitors that elevate intracellular cAMP/cGMP (Figure 1). Anticoagulants that are direct thrombin inhibitors\(^3\) can also suppress platelet activation by inhibiting thrombin-dependent platelet activation via GPIb\(\alpha\) and protease-activated receptor-1/4. Notably, platelet adhesion and aggregation induced at high shear stress are dependent on ADP and \( \alpha_{IIb}\beta_3 \), and they are inhibited by elevated platelet cAMP/cGMP.\(^13,15\) In contrast, cyclooxygenase inhibition by aspirin has minimal effect.\(^15,38\) This may explain the relative lack of efficacy of aspirin as treatment for arterial thrombosis and the common use of aspirin in combination with other drugs.

Dual antiplatelet therapy typically refers to a combination of aspirin and a P2Y12 inhibitor, targeting secondary platelet activation via TxA2 and ADP, respectively. The \( \alpha_{IIb}\beta_3 \) inhibitors block thrombus formation to all agonists because \( \alpha_{IIb}\beta_3 \) is essential for platelet aggregation. For these most widely used drugs, it is interesting to consider the specific targets—cyclooxygenase, P2Y12, and \( \alpha_{IIb}\beta_3 \)—and the role of these targeted pathways in thrombus formation, in terms of antithrombotic protection versus bleeding risk, and resistance to dual antiplatelet therapy.

**Bleeding Risk Associated With Current Antiplatelet Therapeutics**

Although antiplatelet drugs are effective in reducing vascular deaths and nonfatal events by \(-50\%\) in high-risk cardiovascular patients, the main safety concern is excess hemorrhage.\(^39\) Primary prevention of cardiovascular events with aspirin is associated with a significant increased risk of extracranial bleeding (relative risk, 1.55; 95% confidence interval, 1.3–1.8; \( P<0.001 \)), which often negates the uncertain potential benefit

### Table 2. Antiplatelet Therapy: Drugs in Clinical Trials and Clinical Development

<table>
<thead>
<tr>
<th>Type/Name</th>
<th>Developmental Status</th>
<th>Route of Administration</th>
<th>Indication</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostacyclin analogue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beraprost</td>
<td>Ongoing clinical trials</td>
<td>Oral</td>
<td>Treatment of PAH and diabetic nephropathy</td>
<td>130, 131</td>
</tr>
<tr>
<td>GPIb(\alpha) antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abciximab</td>
<td>Phase I completed; phase II listed but not yet active</td>
<td>IV</td>
<td>For treatment of thrombotic events MI</td>
<td>71–73</td>
</tr>
<tr>
<td>Anti-(\alpha)VWF aptamers (ARC1779 and ARC15105)</td>
<td>…</td>
<td>IV</td>
<td>For treatment of TTP, WWD 2b</td>
<td>132–134</td>
</tr>
<tr>
<td>Anti-(\alpha)VWF nanobody (ALX-0081, caplacizumab)</td>
<td>Phase I, phase II TITAN trial</td>
<td>IV</td>
<td>For treatment of TTP</td>
<td>132, 135, 136</td>
</tr>
<tr>
<td>vWF-targeting (inhibit GPIb(\alpha) binding)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPVI bivalent soluble form</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Revatex (PR-15), humanized Fc fusion of GPVI ectodomain</td>
<td>Phase I completed; phase II recruiting</td>
<td>IV</td>
<td>For treatment of carotid artery stenosis, TIA, and stroke</td>
<td>74, 75</td>
</tr>
<tr>
<td>Thromboxane receptor antagonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terutoban</td>
<td>Clinical trials indicate no better outcome than aspirin</td>
<td>Oral</td>
<td>For secondary prevention of thrombotic events in cardiovascular disease</td>
<td>137–139</td>
</tr>
<tr>
<td>Ifetroban</td>
<td>Phase II</td>
<td>Injection</td>
<td>Treatment of hepatorenal syndrome</td>
<td>140</td>
</tr>
<tr>
<td>PAR-1 inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vorapaxar (SCH530348)</td>
<td>Recommended for approval by FDA’s Cardiovascular and Renal Drugs Advisory Committee</td>
<td>Oral</td>
<td>Reduction of atherothrombotic events in patients with history of MI</td>
<td>141–143</td>
</tr>
<tr>
<td>Atopaxar (E5555)</td>
<td>Phase II</td>
<td>Oral</td>
<td>CAD</td>
<td>141, 144, 145</td>
</tr>
<tr>
<td>ADP P2Y12 inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cangrelor</td>
<td>FDA denied approval and requested further clinical data</td>
<td>IV</td>
<td>For patients with ACS undergoing PCI; reduces MI and stent thrombosis events compared with other P2Y12 inhibitor drugs; associates with bleeding risks and lacks mortality benefit</td>
<td>146–148</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; CAD, coronary artery disease; FDA, Food and Drug Administration; IV, intravenous; MI, myocardial infarction; PAH, pulmonary arterial hypertension; PAR-1, protease-activated receptor-1; PCI, percutaneous coronary intervention; TIA, transient ischemic attacks; TTP, thrombotic thrombocytopenic purpura; vWF, von Willebrand factor; and VWD 2b, type 2b von Willebrand’s disease.
in otherwise healthy people. In patients with acute coronary syndromes, stable coronary artery disease, ischemic stroke or peripheral vascular disease, single-agent aspirin, or clopidogrel therapy is associated with an annual incidence of 2% to 4% gastrointestinal bleeding and 0.5% rate of intracranial hemorrhage. A 2014 analysis of 8 trials involving 41,483 individuals
showed daily aspirin treatment (160 or 300 mg) within 48 hours post ischemic stroke reduced the risk of early recurrent stroke, but it was connected with a small excess of intracranial bleeding.41 With individuals undergoing percutaneous coronary revascularization, administration of cIIB(3) inhibitors reduced the risk of death or myocardial infarction, however, with an increased risk of severe bleeding. These meta-analyses suggested the potential benefits of antiplatelet agents far outweighed the risk of bleeding complications. On the other hand, a Cochrane review of cIIB(3) inhibitors administered early after an ischemic stroke showed a link with a significant risk of intracranial hemorrhage with no reduction in mortality.42

The risk of bleeding has been shown to be dose related for all antiplatelet drugs, including aspirin (100 versus >325 mg daily),40 clopidogrel (300- versus 600-mg loading),43 and prasugrel (5 versus 10 mg daily).44,45 Combined antiplatelet therapy, with dual targeted inhibition of the platelet cyclooxygenase-1 pathway (aspirin) and the P2Y12 unit of the platelet ADP receptor (clopidogrel, prasugrel, and ticagrelor), is an effective strategy for reducing cardiovascular events, but it is associated with an increased relative risk of major bleeding by ≤40%.46 Rates of bleeding increase with the prolonged duration of dual antiplatelet therapy, percutaneous coronary intervention (PCI), or urgent coronary artery bypass surgery.46

More rapid and consistent P2Y12 platelet ADP receptor blockade by prasugrel and ticagrelor when compared with clopidogrel generally produces better outcomes, particularly in patients with acute coronary syndromes treated with PCI.46 However, this benefit comes at the cost of an increase in major life-threatening and fatal bleeding with prasugrel when compared with clopidogrel (2.4% versus 1.8%; hazard ratio, 1.32; P=0.03) as shown in the TRITON-TIMI 38 trial (TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet InhibitioN with prasugrel Thrombolysis In Myocardial Infarction 38).45 Patients with risk factors for bleeding were elderly (>75 years) and with low body weight (<60 kg). Dose reduction of prasugrel to 5 mg may improve the benefit/risk ratio in this susceptible group as shown in a subsequent study (TRILOGY ACS) where there was no difference in hemorrhage in a prespecified analysis of elderly patients (>75 years).44

Overall bleeding rates were similar with ticagrelor and clopidogrel in the PLAtete inhibition and patient Outcomes (PLATO trial); however, when bleeding from coronary artery bypass surgery was removed from analysis, ticagrelor had a higher risk of major procedure-related bleeding (4.5% versus 3.8%; hazard ratio, 1.19; P=0.03).47 Comparison between up-front and delayed (after PCI) administration of either prasugrel or antiplatelet cIIB(3) receptor antagonists (tirofiban and eptifibatide) showed little difference in efficacy, but early administration significantly increased bleeding and red-cell transfusion rates.48

Definitions of bleeding are poorly standardized and vary between different studies making direct comparison between various combinations of antiplatelet drugs more difficult. Dual oral antiplatelet therapy is now standard practice for treatment of acute coronary syndromes with and without PCI; however, the individual assessment of bleeding risk over time and risk of later thrombotic complications often determines the duration of combined antiplatelet therapy.46,49

Aspirin and Clopidogrel Resistance
It is accepted that there is a wide interindividual variation in response to aspirin and clopidogrel, which can be defined either through the patient experiencing recurrent platelet-mediated clinical events (recurrent in stent thrombosis, acute myocardial infarction, stroke, and death) or pharmacodynamically with various ex vivo platelet inhibition measures (agonist-induced platelet aggregation, serum and urinary thromboxane measurement, electric impedance aggregometry, shear-induced platelet functional analysis [PFA100], flow cytometry, and point of care agonist assessment [VerifyNow and VASP-P assay]).50 Controversy still exists as to whether any ex vivo test can simulate in vivo platelet response and there is uncertainty as to which test to preferentially perform. Observational studies suggest that at standard doses of aspirin (100 mg) or clopidogrel (75 mg), over a third of patients have predefined ex vivo resistance.51 Reasons for antiplatelet resistance include poor compliance, insufficient dose, slow pharmacodynamic effect, related gene polymorphisms that affect antiplatelet drug metabolism (eg, cytochrome P450 2C19 loss of function allele for clopidogrel) and ABCB1 polymorphisms, particularly 3435C>T, affecting efflux of clopidogrel via P-glycoprotein,40 and PEAR1 variants in patients exposed to aspirin.52 increased baseline platelet reactivity such as that found in diabetes mellitus and smoking, and increased platelet turnover.40 Whatever the reason, predefined high on-treatment platelet reactivity is consistently associated with 2- to 4-fold increased risk of acute myocardial infarction, stent thrombosis, and stroke.50

Clopidogrel leads to a variable platelet inhibition and has a delayed onset of action, mainly because of the need for biotransformation in the liver via CYP450 isoenzymes (particularly the CYP2C19 isoenzyme) from the inactive parent drug to the active metabolite. Prasugrel is also a prodrug requiring CYP450-mediated metabolic activation, but it is less affected by the CYP2C19 polymorphism and has a faster onset of action. Ticagrelor is an orally active drug not requiring biotransformation and binds rapidly and reversibly to the P2Y12 receptor, making it an attractive direct P2Y12 inhibitor.46

Strategies for overcoming antiplatelet drug resistance include increasing initial loading doses (eg, aspirin: 100–300 mg; clopidogrel: 75–600 mg; prasugrel: 10–60 mg; ticagrelor: 90–180 mg)43,45,47 and personalized dose adjustment (eg, increase the maintenance dose of clopidogrel to 150 mg daily50,54 or drug switching from clopidogrel to prasugrel45,55,56 or ticagrelor) that may include adjustment based on regular testing platelet function monitoring.50

The lack of effect of individualized antiplatelet therapy by platelet function testing in recent large randomized studies in PCI and medically managed patients with coronary artery disease is disappointing.54–57 Studies that have not shown a change in outcomes include the TRILOGY-ACS (TaRgeted platelet Inhibition to cLarify the Optimal strateGy to mediCAlly manage Acute Coronary Syndromes) trial (n=2564; prasugrel versus clopidogrel),58 GRAVITAS (Gauging Responsiveness With a VerifyNow P2Y12 Assay: Impact on Thrombosis and Safety) increased dose (n=2214; clopidogrel 150 mg daily),54 and ARCTIC (Double Randomization of a
Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation, and Interruption Versus Continuation of Double Antiplatelet Therapy (standard versus monitoring antiplatelet drug/dose adjustment (n=2440 patients)). Although ≈20% of patients did not achieve optimal reduction in platelet reactivity, the event rates were low and the studies were underpowered.50 However, a recent meta-analysis of 9 pooled studies consisting of 12048 subjects showed significant benefit for tailored versus standard antiplatelet therapy with a risk reduction of ≈60% without an increase in bleeding.51 Smaller studies that demonstrated a consistent reduction in platelet reactivity in all patients below the threshold of high on-treatment platelet reactivity also showed significant clinical benefit of reduction in cardiovascular events.50

Observational studies have suggested that CYP2C19 polymorphisms associated with reduced activation of clopidogrel increase the risk of thrombotic events by 2-fold in patients treated with clopidogrel.58 However, analysis of 3 large randomized control studies showed no effect of the CYP2C19 or ABCB1 genotype on clinical outcomes in patients on clopidogrel.79,80 In an ongoing prospective post-PCI study assessing CYP2C19 genotype, antiplatelet therapy was changed from clopidogrel to ticagrelor or prasugrel in those with a loss of function polymorphism.83 These data will clarify the role of CYP2C19 genotype in selecting the type of antiplatelet therapy and will either confirm or refute the smaller studies suggesting an improvement in cardiovascular outcome with this strategy.

Recent clinical guidelines suggest that platelet function testing may have a limited role in selecting the choice of P2Y12 inhibitors in high-risk patients treated with PCI (class IIb) although routine testing is not recommended (class III).50 By better personalizing the drug therapy, there is an opportunity to better define high and low platelet reactivity to address the balance for the individual between bleeding and thrombosis in the context of antiplatelet therapy.

More Recent Targets for Antiplatelet Therapy

Interestingly, there are currently no inhibitors in routine clinical use targeting the primary platelet receptors, GPIbβ (or GPIb-IX-V), GPVI, or their ligands (vWF/collagen), interactions that initiate platelet adhesion and activation which become increasingly important as the shear rate increases. The limited distribution of these receptors to megakaryocytes/platelets should also offer advantages compared with targets with expression profiles that include other cells. On one hand, the rare congenital or acquired defects or deficiency of GPIb-IX-V (Bernard-Soulier syndrome) or GPVI results in variable bleeding risk, from relatively mild to more severe, which could argue against directly targeting these receptors.64–67 On the other hand, these cases often involve thrombocytopения or concurrent hemostatic disorders that complicate attributing bleeding to receptor dysfunction alone. Experimental studies suggest that GPIbβ has a far greater contribution to stable arterial thrombosis than vWF,67 whereas the GPIV-binding sites of collagen are not normally exposed to circulating platelets. GPVI also regulates thrombus formation in experimental models of arterial thrombosis or stroke,68,69 whereas the hemostatic consequences of GPVI deficiency on bleeding times are generally minimal. Overall, these findings support further development of antiplatelet therapies targeting either GPIbβ–vWF, GPVI–collagen, or both.

Currently used antiplatelet drugs are based on natural products (aspirin), toxins (snake venom–based inhibitory peptides that blocked ligand binding to αIIbβ3), inhibitory antibodies (derived from anti–αIIbβ3 monoclonal antibodies), or small molecules (irreversible P2Y12 inhibitors developed as orally available prodrugs). Inhibitory toxins and antibodies, ligand mimetics, as well as nucleotide-based aptamers and soluble recombinant forms of receptor, have already been substantially developed as means of modulating GPIbβ–vWF, GPVI–collagen, or other platelet targets, including the prostaglandin receptor, TxA2 receptor, and protease-activated receptor-1 (Table 2; Figure 1). The GPIbβ–vWF axis has been targeted using aptamers or antibody-based agents selective for the GPIbβ-binding A1 domain of vWF that prevent vWF-binding platelet GPIbβ. Snake venom proteins have been long known to target human platelet GPIbα by metalloproteinase-mediated proteolysis of the vWF/ligand-binding domain (moc Hagin, natural killer protease) or by binding of C-type lectin family proteins to the leucine-rich-repeat domain of GPIbα (echicetin and alboaggregin-B).70 Antifibrin is a C-type lectin-like protein that binds GPIbα and inhibits vWF-binding GPIbα–dependent platelet adhesion and activation, important with potent activity at elevated shear rates.71–73

The GPVI–collagen axis has been targeted using a bivalent recombinant soluble form of the GPVI ectodomain expressed as a humanized Fc fusion protein.11,74,75 The ≈55-kDa soluble ectodomain of GPVI is shed from the human platelet surface by the membrane-expressed sheddase, ADAM-10.76,77 This process is tightly regulated, with essentially all the GPVI on healthy circulating platelets in an intact form, with shedding rapidly induced by ligand binding to GPVI, by platelet activation, by platelet exposure to high shear stress,78 by coagulation/factor Xa,79 or by other triggers, releasing plasma soluble ectodomain of GPVI, which is quantifiable by immunosassay as a platelet-specific marker.80 Native soluble ectodomain of GPVI shed from platelets is monovalent and binds collagen weakly compared with the bivalent form, which shows promise as a novel antithrombotic.11

Indeed, these new agents against GPIbβ–vWF, GPVI–collagen, and other platelet targets have all shown merit as potential antiplatelet drugs experimentally or in clinical settings associated with pathological thrombosis (Table 2). The type and properties of these agents, in terms of oral availability, reversibility, bleeding risk, and antithrombotic or off-target effects, will prove critical to their clinical application in different patient groups and will require longer term trials to establish viability.

Future Targets for Antiplatelet Therapy

On the basis of recent research discoveries, there is far wider scope for therapeutic regulation of platelet targets, including αIIbβ3,81 integrin α6β1,82 GPIb-IX-V, and GPVI adhesive function and signaling. In addition to vWF- and GPIbα-targeting inhibitors in development (Table 2), alternative strategies could have advantages, for example, small molecules with increased scope for bioavailability. In this regard, allosteric inhibitors can overcome the inherent difficulties of a small
molecule inhibiting a protein–protein interaction in which a large multisite interface occurs as for the interaction between the ligand-binding domains of GPIbα and vWF. As proof of this concept, a short cyclic peptide, CTERMALHNLC, which binds GPIbα, potently inhibits vWF binding not through competitive inhibition but by preventing formation of a ligand-receptive structural conformation.83,84

An alternative strategy targeting the ADP-P2Y1/P2Y12 activation pathway is the use of a soluble form of CD39 (solCD39), an ectonucleoside triphosphate diphosphohydrolase.85 This approach uses the ability of CD39 to hydrolyze ADP, dissimilar to the current ADP-P2Y12 drugs clopidogrel, prasugrel, and ticagrelor that block ADP-mediated platelet activation by binding to the receptor directly. An administration of a fusion protein consisting of solCD39 and a single-chain Fv fragment of an antibody specific for the cILbβ3 active form showed promising antithrombotic effects with no prolonged bleeding in an experimental mouse model. The drug is designed to allow a layer of platelets to firmly adhere to the wound and cILbβ3 to become activated before exerting its function, thus minimizing the amplification phase of platelets in developing thrombus.

Innovative new approaches for targeting cILbβ3 signaling include the recent report of short myristoylated peptides based on cytoplasmic sequences of β3 that selectively regulate the dynamic interaction between the receptor and intracellular binding partners, including the G protein Gz13. Notably, it seems feasible to dissect out precise signaling functions of cILbβ3 involving Gz13 or talin associations, which control platelet activation, adhesion, and aggregation/clot contraction, and to inhibit thrombus formation in mice comparable with current cILbβ3 inhibitors, yet without adverse bleeding.81

Platelet integrin α6β1 also represents a novel antiplatelet target. Recently, using platelet-specific knockout of integrin α6, the main vascular laminin receptor was indicated to play an important part in platelet adhesion, activation, and in arterial thrombosis without significant effect on bleeding risk.82 Future research will need to address possible detrimental effects of α6β1 blocking as the protein expression is not only limited to platelets but it is also found on various cell types including glial cells, neuronal stem cells, and endothelial cells.86-88

Signaling protein targets downstream of GPIb-IX-V/GPVI may also be worthwhile considering. First, Syk inhibitors have been tested clinically as selective, reversible anti-inflammatory/cancer therapeutics, without inhibitory activity toward other tyrosine kinases, such as Src, Lyn, or Btk.89 Syk inhibition also blocks GPVI-dependent activation of human platelets.14 Second, Btk mediates signaling downstream of GPIb-IX-V in platelets, and an irreversible Btk inhibitor (ibrutinib) is approved for treatment of the lymphoproliferative diseases, chronic lymphocytic leukemia, and mantle cell lymphoma. Therapeutic concentrations of ibrutinib inhibit collagen/GPVI-dependent platelet aggregation,91 and although it is associated with clinical bleeding in patients with chronic lymphocytic leukemia,92 this could be related to vulnerability resulting from decreased GPVI expression in lymphoproliferative disease.92

Third, recent evidence that platelet Nox-1/2-dependent ROS production is important for GPIb-IX-V/GPVI-dependent platelet function, including adhesion to collagen, and is inhibited by Nox-1/2 inhibitors93,94 raises the possibility that such inhibitors could be antithrombotic. An inhibitor targeting NOX1 and NOX4 (GKT137831) is in clinical trial as a treatment for diabetic nephropathy,93 unrelated to any possible antiplatelet effects. Evidence for the value of antioxidants in treating cardiovascular disease seems limited and requires further attention. Although direct therapeutic targeting of Nox-1/294 is viable as a general inhibitor of ROS and inflammation, it is not selective for platelet ROS pathways.95 However, an attractive possibility exists for these ROS inhibitors, as well as nonplatelet-specific targets mentioned above, in drug-eluting stents where their actions will likely be more localized. Other intracellular binding partners for GPIb-IX-V in addition to the p85 subunit of phosphatidylinositol 3-kinase21,22 include the regulatory protein, 14-3-3ζ, and the phosphorylation dependence, functional role, and potential for future targeting of this interaction have been previously reviewed in detail.15

Finally, antiplatelet strategies could focus on the expression and function of platelet-specific GPVI. In addition to blocking ligand binding or inhibiting signaling, either the requirement for GPVI to dimerize to elicit signaling (by cross-linking FcRy ITAM) or the controlled ectodomain shedding mediated by ADAM10 could also potentially be targeted.10-12,96 Further understanding of how shear stress and membrane properties control dimer formation and ADAM10 accessibility and cleavage could enable alternative therapeutic control of platelet reactivity toward collagen.

Conclusions

Current antiplatelet therapy has no doubt prevented incalculable death and disability in people at risk of athero/thrombotic/cardiovascular disease by preventing thrombotic events and quelling the proinflammatory role of activated platelets. Nevertheless, the lack of efficacy/resistance, reliable clinical guidelines, and increased bleeding risk in individuals remains an ongoing problem. New experimental evidence and increased understanding of platelet function and regulation are revealing new antiplatelet approaches to potentially improve clinical outcomes and monitoring.

The readers may also find additional information on antiplatelet and antithrombosis therapy from other reviews in the ATVB series New Targets of Antiplatelet Drug Development.14,28,36,97

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Disclosures

None.

References


sine kinase is essential for botrocetin/VWF-induced signaling and GPIb-α/VWF antagonism.


Arterial thrombosis precipitating heart attack and stroke is a major global cause of morbidity and mortality. Current antiplatelet therapies to detail in other reviews in this series.

is associated with an increased risk of a major bleeding event. An increasing body of evidence has identified several potential therapeutic


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