Basic Principles of Platelet Biology and Clinical Implications
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Platelet activation and subsequent accumulation at sites of vascular injury are the first steps in hemostasis. Excessive platelet activation after atherosclerotic plaque rupture or endothelial cell erosion may also lead to the formation of occlusive thrombi, which are responsible for acute ischemic events. Multiple pathways are involved in platelet activation, including those activated by adenosine diphosphate (ADP), thromboxane A2 (TXA2), serotonin, collagen, and thrombin. Antiplatelet agents used for prevention of atherothrombosis have focused on blocking the formation of TXA2 (eg, aspirin) and interfering with ADP stimulation mediated by the P2Y12 receptor (eg, clopidogrel). These agents, used alone or in combination, significantly decrease the risk for atherothrombotic events, but a significant residual risk for recurrent ischemic events remains. This has been, in part, attributed to persistence of elevated platelet reactivity despite the use of these agents. Several novel antiplatelet agents are currently under clinical development, with the goal of achieving more efficacious platelet inhibition. These include agents that more efficiently block TXA2-mediated effects, as well as more potent P2Y12 receptor antagonists. In addition, inhibition of the protease-activated receptor-1 platelet activation pathway stimulated by thrombin has emerged as a rational target for clinical development. An overview of the basic principles of platelet biology is given and currently available antiplatelet agents, as well as those under clinical development, are reviewed. (Circ J 2010; 74: 597–607)

Key Words: Hemostasis; Platelets; Thrombosis

Accumulation of platelets at the site of vascular injury is the first step in the formation of hemostatic plugs, and these elements play a key role in preventing blood loss after injury.1 However, platelets are also responsible for the formation of pathogenic thrombi in patients with atherothrombotic disease, such as acute coronary syndromes (ACS), ischemic stroke/transient ischemic attack (TIA), and peripheral artery disease (PAD). ACS includes a spectrum of clinical presentations, ranging from unstable angina to non-ST elevation myocardial infarction (NSTEMI) and ST-elevation MI (STEMI). Atherogenesis and the formation of thrombi mark the pathogenesis of ACS, which begins with the rupture of an atherosclerotic plaque, micro-lesions of the vascular lining, and contact between the thrombogenic matrix and platelets; this, in turn, leads to platelet adhesion, activation, and aggregation and the subsequent formation of a thrombus and microembolization.2,3 The key role of platelet-mediated thrombosis in the pathogenesis of atherothrombotic disease is confirmed by the proven clinical benefits of acute and chronic antiplatelet therapy; however, despite the proven benefits of medical therapy and revascularization procedures, the residual acute and long-term morbidity and mortality remain significant.3,4

We review the pathophysiology of atherothrombosis, the role of platelets in protective hemostasis and pathogenic thrombosis, platelet activation pathways and their contributions to thrombosis and hemostasis, and offer a general overview of current antiplatelet agents and novel agents in clinical development.

Pathophysiology of Atherothrombosis
Multiple biochemical, environmental, and genetic factors influence the progression of plaques, through various phases of evolution, from stable, asymptomatic, grossly invisible lesions to unstable, high-risk atheromas.2 Platelets have been implicated in atherogenesis, the developmental process of atheromatous plaques (reviewed in Davi et al1). Raised plaques located beneath the endothelium represent the initial phase of atherosclerosis and may regress, remain dormant, or progress to a more complicated atherosclerotic lesion.2 The fibrous plaque represents the second phase, when smooth muscle cells migrate from the media to the subendothelial space where they proliferate and produce connective tissue to form a fibrous cap. Ultimately, complicated lesions can develop, which can manifest as calcification, hemorrhage, ulceration, and/or thrombosis.2 Rupture of atherosclerotic plaque within the intima of...
large- and medium-sized arteries triggers platelet activation, leading to thrombotic occlusion of the blood vessels.\(^1\) The thrombi block blood flow and oxygen supply (ischemia) in the affected arteries, resulting in the clinical manifestations of atherothrombotic diseases.\(^2\) Platelet-activated thrombus or hemostatic plug generation proceeds in 3 stages: (1) an initiation phase involving platelet adhesion, (2) an extension phase that includes activation, additional recruitment, and aggregation of platelets, and (3) a perpetuation phase characterized by platelet stimulation and stabilization of clots (Figure 1).\(^1,5\) Platelet activation in the extension phase is necessary for hemostasis and thrombosis and can be induced by multiple pathways.\(^6\) The perpetuation phase of thrombus formation is mediated by cell-to-cell, contact-dependent mechanisms, mostly intermediated by von Willebrand factor (vWF) under conditions of high shear stress,\(^7\) that lead to changes in platelet morphology, expression of procoagulant and proinflammatory activities, and platelet aggregation.\(^5\) Thrombus in acute atherothrombotic events can be either partially or completely occlusive. The former is composed primarily of platelet aggregates, and the latter of platelet aggregates and a fibrin-rich clot generated by the coagulation cascade.\(^2\)

**Role of Platelets in Hemostasis and Thrombosis**

Under physiological conditions, platelets circulate in blood in a quiescent state and their activation is inhibited by both nitric oxide (NO) and prostaglandin I\(_2\) released from endothelial cells.\(^1,5,7\) However, when activated, platelets provide rapid protection against bleeding and catalyze the slower formation of stable blood clots via the coagulation cascade (Figure 1).\(^7\) In the initiation phase of primary hemostasis, platelets roll, adhere, and spread on the collagen matrix to form an activated platelet monolayer.\(^7\) During the rolling phase, adhesion is mediated by interaction between the glycoprotein (GP) Ib/V/IX receptor complex on the platelet surface with vWF and between the GP VI and GP Ia proteins with collagen at sites of vascular injury.\(^1,5,7\) These interactions allow the arrest and activation of adherent platelets. Interaction between vWF and GP Ib/V/IX is required for the initial adhesion of platelets to the subendothelium under conditions of high shear (as found in small arteries, arterioles, and stenosed arteries).\(^7\) Under normal conditions, soluble vWF does not undergo significant interactions with GP Ib/V/IX, except under high shear conditions.\(^7\) However, when immobilized on exposed collagen at injury sites, it becomes a strong adhesive substrate.\(^7\) The role of vWF in platelet adhesion is pivotal as it binds to both collagen and 2 major platelet

**Figure 1.** Platelet adhesion and aggregation. The interaction between glycoprotein (GP) Ib and von Willebrand factor (vWF) mediates platelet tethering, enabling subsequent interaction between GP VI and collagen. This triggers the shift of integrins to a high-affinity state and the release of adenosine diphosphate (ADP) and thromboxane A\(_2\) (TXA\(_2\)), which bind to the P2Y\(_{12}\) and TP receptors, respectively. Tissue factor (TF) locally triggers thrombin formation, which contributes to platelet activation via binding to the platelet protease activated receptor (PAR)-1. (Adapted from Varga-Szabo et al. *Arterioscler Thromb Vasc Biol* 2008.)\(^7\)
Principles of Platelet Biology

When activated platelets adhere to sites of vascular injury, the local platelet activating factors help to recruit additional circulating platelets to extend and stabilize the hemostatic plug. These platelet-activating factors include adenosine diphosphate (ADP), thromboxane A$_2$ (TXA$_2$), serotonin, collagen, and thrombin. Among these, thrombin is the most potent. Thrombin is produced locally at the surface of activated platelets by tissue factor and mediates generation of fibrin from fibrinogen, which contributes to the formation of the hemostatic plug and platelet thrombus growth. Thrombin also directly activates platelets through stimulation of the protease activated receptor (PAR)-1. The release of ADP and TXA$_2$ from adherent platelets contributes to recruitment of circulating platelets, and also leads to several distinct manifestations of platelet activation, including change in platelet shape, increased expression of proinflammatory molecules (including P-selectin, soluble CD40 ligand and others), expression of platelet procoagulant activity, and conversion of the GP IIb/IIIa receptor into an active form, effectively allowing platelet aggregation that can lead to pathological thrombosis.

Upon activation, GP IIb/IIIa (αIIb/β3 integrin) mediates platelet aggregation and spreading on the exposed extracellular matrix of the injured vessel wall by means of fibrinogen bridges. Fibrinogen forms bridges between activated platelets and contributes to thrombus stabilization. These platelet-rich, white thrombi are typically not completely occlusive and are often associated with NSTE ACS. Progression to completely occlusive thrombus mediated by the coagulation cascade involves the formation of a fibrin-rich, red clot superimposed on the underlying platelet-rich, white thrombus, and is usually found in STEMI patients.

Platelet Activation Pathways and Their Contributions to Hemostasis and Thrombosis

**ADP**

Two ADP receptors present on platelets are P2Y$_1$ and P2Y$_12$. Platelet shape change and transient aggregation are mediated by P2Y$_1$. Binding of ADP to the P2Y$_12$ receptor results in signaling cascades that culminate in platelet aggregation and thrombus growth and stabilization (Figure 2). P2Y$_12$ plays a critical role in the amplification of platelet aggregation induced by other agents, including serotonin, TXA$_2$, and thrombin. ADP contributes to platelet activation both during protective hemostasis, at the initial platelet monolayer formation, and in pathological thrombosis, when the occlusive platelet-rich thrombus is formed.

**TXA$_2$**

TXA$_2$ is released by adherent platelets, and amplifies the platelet adhesion response by binding to $\alpha$- and $\beta$-receptors (effects in platelets are mediated primarily through the $\alpha$ form). TXA$_2$ is produced from arachidonic acid through enzymatic conversion by cyclooxygenase-1 (COX-1) and
Thromboxane synthase. TXA$_2$ binds to TP receptors, which results in changes in platelet shape and enhancement of recruitment and aggregation of platelets to the primary platelet plug. The activity of TXA$_2$ is inhibited by the vasodilator, prostacyclin, and the concentrations of both TXA$_2$ and prostacyclin are increased in patients with unstable angina or following acute MI. The most important role of TXA$_2$ released from activated platelets and accumulated at the site of atheroma rupture is the contraction of microvessels downstream to disturb blood flow. TXA$_2$ activates platelets both during protective hemostasis and pathological thrombus formation.

Serotonin
Serotonin is a vasoconstrictor that binds 5HT-2$_a$ receptors and amplifies the platelet response by stimulating shape change and aiding platelet recruitment to sites of injury. It may also play a procoagulant role by promoting retention of the pro-coagulant proteins, fibrinogen and thrombospondin, on the platelet surface. Serotonin is implicated in shear-induced aggregation and thrombus propagation by positive feedback from intraplatelet serotonin stores. Microvascular contraction downstream from activated platelets may also contribute to occlusive thrombus formation.

Collagen
Subendothelial fibrillar collagen is a strong thrombogenic substrate. GP Ib$_a$ and GP IIb/IIIa interact with collagen indirectly via vWF. Under high shear, platelet adhesion is mediated by vWF, which associates with collagen by interaction with GP Ib$_a$. This interaction leads to activation of GP IIb/IIIa, which cannot bind its ligands in its inactive state, and subsequently to stable vWF-mediated platelet aggregates. Under low shear conditions, collagen serves as an adhesive substrate for platelets via the GP Ia/IIa (a2$\beta_3$ integrin) and GP VI collagen receptors on the platelet surface. GP VI is the major collagen receptor mediating the platelet activation that is necessary for adhesion, aggregation, degranulation, and coagulant activity on the matrix protein. Binding of collagen to GP VI triggers intracellular signals that shift platelet integrins to a high-affinity state, and induces release of ADP and TXA$_2$.

GP Ia/IIa acts cooperatively with GP VI. Platelet stimulation as a result of adhesion leads to spreading, further activation of GP IIb/IIIa enabling binding of soluble fibrinogen, and granule secretion. Collagen provides a strong stimulus for calcium release, which induces platelet procoagulant activity.

Thrombin
Thrombin is a very potent platelet agonist that activates platelets at extremely low concentrations (lower than those required for activation of the coagulation cascade). Thrombin-mediated platelet activation contributes to pathological thrombosis through the formation of an occlusive platelet-rich thrombus, but preclinical studies suggest it may not be required for protective hemostasis. Thrombin-mediated cleavage of fibrinogen into fibrin is more important for hemostasis than thrombin-mediated platelet activation. Thrombin activates platelets by binding PAR-1 on the platelet surface, cleaving the receptor, and exposing the tethered ligand that binds and activates the receptor. PAR-1 is the main thrombin receptor on human platelets, whereas PAR-4 requires a higher concentration of thrombin for activation. Platelet activation and stimulation of aggregation by PAR-1 is characterized by several processes leading to enhanced thrombus formation (Figure 3).

Overview of Antiplatelet Agents for Atherothrombotic Diseases

Aspirin (ASA)
ASA is an irreversible COX-1 inhibitor that blocks TXA$_2$ production. By preventing the formation of TXA$_2$, ASA diminishes platelet activation and aggregation promoted by TXA$_2$ but not by other agonists. Numerous large-scale clinical trials and meta-analyses have consistently demonstrated the benefit of ASA as a secondary prevention measure for recurrent ischemic events in patients with various manifestations of atherothrombotic disease. Although ASA has been used widely for the prevention of thromboembolism, and is recommended for management of patients who have experienced either MI or ischemic...
cerebrovascular events, it is associated with limitations, including a dose-dependent risk for bleeding and residual morbidity and mortality higher than those for more recently developed antiplatelet agents when used as monotherapy in patients who have experienced ACS and/or undergone cardiac surgery. In addition, there is also significant interpatient variability in the platelet inhibition achieved with a given dose of ASA, and decreased responsiveness is associated with a higher risk for atherothrombotic events.

**P2Y₁₂ ADP Receptor Antagonists**

These agents include currently available thienopyridines (ticlopidine, clopidogrel, and prasugrel), as well as several compounds in late development (eg, ticagrelor [AZD6140], cangrelor, and cangrelor; Table 1). They exert their clinical benefit by inhibiting activation of P2Y₁₂-mediated platelet activation.

Clopidogrel, a second-generation thienopyridine, has largely replaced ticlopidine, a first-generation thienopyridine, because of its more favorable safety profile. Reduction in the risk of ischemic events observed in trials of clopidogrel plus ASA in patients with NSTE ACS, STEMI, and in patients undergoing percutaneous coronary intervention (PCI) have led to the use of dual antiplatelet therapy as standard-of-care therapy in these patient populations (Table 2). However, dual antiplatelet therapy is also associated with increased bleeding risk. In the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial, more patients in the clopidogrel plus ASA group than in the ASA alone group showed major bleeding (3.7% vs 2.7%, respectively; P=0.001). Although clopidogrel as a single agent demonstrated clinical benefit vs ASA for secondary prevention in the Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial in patients with atherosclerotic vascular disease, the addition of clopidogrel to ASA was not better than ASA alone in the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial and led to increased bleeding risk (Table 2). A post-hoc CHARISMA analysis showed that in patients with prior MI, stroke, or symptomatic PAD, the primary outcome was reduced from 8.8% with ASA to 7.3% with ASA plus clopidogrel (P=0.01); severe bleeding was not different between treatment arms.

Most recently, the Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for Intervention (CURRENT-OASIS 7) trial demonstrated no significant difference in the primary endpoint (composite of cardiovascular death, MI, or stroke) at 30 days between patients with ACS receiving double-dose vs standard-dose clopidogrel (Table 2). In patients who underwent PCI (n=17,232), double-dose clopidogrel was associated with a 15% relative reduction in the primary endpoint and a 42% relative reduction in definite stent thrombosis vs the standard-dose. There was a modest excess in CURRENT-defined major bleeds, but no difference in Thrombolysis In Myocardial Infarction (TIMI) major bleeds, intracranial hemorrhage, fatal bleeds or bleeds related to coronary artery bypass grafting (CABG) between treatment arms.

Response variability is also considered a potentially important limitation of clopidogrel, because inadequate inhibition of the ADP platelet activation pathway may leave patients at risk for thrombotic events. A large number of factors that may influence the response to clopidogrel have been identified, including poor compliance with treatment, possible drug–drug interactions involving cytochrome P450 (CYP) 3A4, genetic polymorphisms of CYP2C19 or the P2Y₁₂ receptor, and/or upregulation of other platelet activation pathways.

Prasugrel is a third-generation thienopyridine approved in Europe and the US for the prevention of atherothrombotic events in patients with ACS undergoing PCI (Table 1). Pharmacodynamic studies have shown more potent antiplatelet effects, lower interindividual variability in platelet response, and a faster onset of activity with prasugrel vs clopidogrel even when used at high loading and maintenance doses (Table 2). The Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel (TRITON) showed that prasugrel plus ASA provided a significant 19% relative risk reduction for ischemic events vs clopidogrel plus ASA in high-risk ACS patients undergoing PCI (Table 2), but it was also associated with significantly higher risks for life-threatening, fatal, and nonfatal bleeding. The benefit was mainly driven by a reduction in MI. A prespecified analysis of net clinical benefit (death from any cause, nonfatal MI, nonfatal stroke, and TIMI) favored prasugrel over clopidogrel in the overall patient cohort (12.2% vs 13.9%, respectively; P=0.004). However, patients with stroke or TIA had net clinical harm from prasu-
grel and patients aged ≥75 or weighing <60 kg had no net benefit from prasugrel. In contrast, prasugrel demonstrated net clinical benefit in patients with diabetes,36 in patients undergoing PCI for STEMI,37 and in patients receiving ≥1 stent.38

Ticagrelor is a nonthienopyridine forming part of a new class of P2Y12 inhibitors (Table 1).39 Compared with clopidogrel, ticagrelor exhibits a higher degree of platelet inhibition, a more rapid time to maximal inhibition of platelet aggregation, and more consistent inhibition of platelet aggregation.39–41 The efficacy and safety of 1-year treatment with ticagrelor plus ASA was reported in patients with ACS in the Study of Platelet Inhibition and Outcomes (PLATO) trial.42 At 12 months, cardiovascular death, nonfatal MI, or nonfatal stroke occurred in 9.8% of patients receiving ticagrelor as compared with 11.7% of those receiving clopidogrel plus ASA (relative risk reduction, 16%; P=0.001) (Table 2).42 Of note, the rate of all-cause death was 22% lower with ticagrelor (P=0.001). No significant difference in the rates of major bleeding using study definition criteria was found between ticagrelor and clopidogrel, but ticagrelor was associated with a higher rate of major non-CABG-related bleeding, including more instances of fatal intracranial bleeding. Rates of other adverse events were higher with ticagrelor vs clopidogrel: dyspnea (13.8% vs 7.8%; P<0.001), syncope (1.1% vs 0.8%; P=0.08), ventricular pauses ≥3 s during the first week of treatment (5.8% vs 3.6%; P=0.01) and increases in serum uric acid and serum creatinine levels at 1 month and 1 year, respectively (P<0.001 for each comparison).42

Cangrelor is a stable, intravenous adenosine triphosphate (ATP) analog and a highly selective P2Y1 receptor antagonist with a short half-life (~2.6 min) (Table 1).43 In patients with ACS or undergoing elective PCI, cangrelor nearly completely inhibited ADP-induced platelet aggregation while clopidogrel resulted in approximately 60% inhibition after 4–7 days of treatment.44 The phase 3 Cangrelor Versus Standard Therapy to Achieve Optimal Management of Platelet Inhibition (CHAMPION) PCI trial reported that in patients with ACS undergoing PCI pretreatment with intravenous cangrelor plus ASA was comparable to pretreatment with ACS, acute coronary syndromes; PCI, percutaneous coronary intervention; MI, myocardial infarction; PAD, peripheral artery disease; ASA, aspirin; NSTE, non-ST-segment elevation MI; CV, cardiovascular; FA, fibrinolytic agent; STEMI, ST elevation MI; CVD, CV disease. CAPRIE: Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events trial. CURRENT-OASIS 7: Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for Intervention trial. CURE: Clopidogrel for the Reduction of Events During Observation trial. PR日常: Cangrelor as Adjunctive Reperfusion Therapy trial. COMMIT: Clopidogrel and Metoprolol in Myocardial Infarction trial. CHARISMA: Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance trial. TRITON: Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel trial. PLATO: Study of Platelet Inhibition and Outcomes trial. CURRENT-OASIS 7: Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for Intervention trial. Clopidogrel was given as a loading dose of 300mg then 75mg daily in CURE, CREDO, PCI-CURE, CLARITY, CHARISMA, and TRITON. Clopidogrel 75mg daily was administered in CAPRIE and COMMIT. Selected patients also received heparin in CLARITY. In CURRENT-OASIS 7, double-dose clopidogrel was defined as a 600-mg loading dose and 150 mg once daily for 7 days, followed by 75 mg once daily. Standard-dose clopidogrel was defined as a 300-mg loading dose, followed by 75mg once daily. Prasugrel given as a loading dose of 60mg, then 10mg daily in TRITON. Ticagrelor was given as a 180mg loading dose, then 90mg twice daily in PLATO; clopidogrel was given as a 300–600mg loading dose, then 75mg daily.

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Table 2. Phase 3 Trials of Oral Antiplatelet Therapy for ACS, PCI or Secondary Prevention of Atherothrombotic Disease

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The rate of major bleeding (Acute Catheterization and Urgent Intervention Triage Strategy [ACUITY] definition) was higher with cangrelor (3.6% vs 2.9%; P=0.06). Rates of TIMI major bleeding were 0.4% with cangrelor and 0.3% with clopidogrel (P=0.39). The CHAMPION PLATFORM evaluated cangrelor vs placebo administered at the time of PCI followed by clopidogrel 600 mg. The trial was terminated prematurely after an interim analysis showed a lack of significant difference for the primary endpoint. The rate of death, MI or revascularization was 7.0% with cangrelor and 8.0% with placebo (P=0.17). Rates of in-stent thrombosis and all-cause mortality were lower with cangrelor, although the rate of ACUITY major bleeding was significantly higher with cangrelor (5.5% vs 3.5%; P<0.001). Rates of TIMI major bleeding were similar. The ongoing phase 2 trial Maintenance of Platelet Inhibition with Cangrelor After Discontinuation of Thienopyridines in Patients Undergoing Surgery (BRIDGE; NCT00767507) is evaluating the safety and effectiveness of cangrelor in patients who require short-term antiplatelet therapy prior to undergoing CABG.

Elnogrel (PRT060128) is a direct-acting, P2Y12 receptor antagonist (Table 1). In a phase 1 study, an oral formulation demonstrated dose-dependent and complete inhibition of ADP-induced platelet aggregation, which was fully reversible 24 h post-dose. A subsequent study conducted in patients on ASA and clopidogrel maintenance therapy with high platelet reactivity found that a single oral dose of elnogrel 60 mg could overcome high platelet reactivity. The Safety and Efficacy Study of PRT060128, a Novel Intravenous and Oral P2Y12 Inhibitor, in Non-Urgent PCI (INNOVATE-PCI; NCT00751231) is a phase 2 trial evaluating the safety and tolerability of elnogrel in patients undergoing elective PCI. The Safety and Efficacy Study of Adjunctive Antiplatelet Therapy Prior to Primary PCI in Patients With STEMI (ERASE-MI; NCT00546260) trial is a recently completed phase 2 placebo-controlled trial evaluating increasing intravenous doses of elnogrel administered prior to angiography in STEMI patients scheduled for PCI.

**Glycoprotein IIb/IIIa Inhibitors**

By competing with fibrinogen and vWF for GP IIb/IIIa binding, GP IIb/IIIa antagonists interfere with platelet cross-linking and clot formation. Investigation of oral GP IIb/IIIa inhibitors has been halted because of negative results from several large trials in patients with ACS or undergoing PCI. Parenteral GP IIb/IIIa inhibitors are associated with an increased risk of bleeding, are only administered within the hospital setting and are not used in the long-term care of patients with atherothrombotic disease. There are currently 3 parenteral GP IIb/IIIa antagonists in clinical use indicated only in patients with ACS undergoing PCI: eptifibatide, tirofiban, and abciximab; none of these agents is approved in Japan based on negative trial results in Japanese patients.

Eptifibatide is a small, reversible, and highly selective heptapeptide with a rapid onset and a short plasma half-life (2–2.5 h), which has demonstrated efficacy and safety in patients with NSTE ACS or undergoing PCI in a number of randomized clinical trials. Most recently, the Early Glycoprotein IIb/IIIa Inhibition in Non-ST-Stage Elevation Acute Coronary Syndrome (EARLY ACS) trial demonstrated that early administration of eptifibatide vs provisional eptifibatide after angiography (delayed eptifibatide) resulted in similar 30-day rates of death, MI, urgent revascularization or thrombotic complications during PCI in patients with NSTE ACS undergoing invasive management. TIMI major and minor bleeding rates were significantly higher with early eptifibatide vs delayed eptifibatide. Overall, those findings do not support the use of upstream compared with ad hoc GP IIb/IIIa inhibition in ACS patients undergoing PCI.

Abciximab is a large, chimeric monoclonal antibody with a high binding affinity and a biphasic plasma half-life (initial half-life <10 min, second-phase half-life =30 min). However, because of its high affinity for the receptor, it has a biological half-life of 12–24 min. The efficacy and safety of abciximab in patients undergoing PCI has been evaluated in several trials, including EPIC, EPILOG, and EPISTENT. The In Intracoronary Stenting and Antithrombotic Regimen—Rapid Early Action for Coronary Treatment (ISAR-REACT) trial showed that the rate of death, MI, and urgent revascularization at 30 days was low and comparable between patients undergoing elective PCI after pretreatment with clopidogrel 600 mg and allocated abciximab vs placebo. Rates of major bleeding were similar between groups, although abciximab was associated with a significantly higher rate of thromboembolism. ISAR-REACT-2 evaluated the same abciximab and clopidogrel treatment regimens in high-risk patients with NSTE ACS undergoing PCI. Death, MI, and urgent revascularization at 30 days occurred significantly less frequently with abciximab vs placebo (8.9% vs 11.9%; P=0.03). However, the treatment benefit of abciximab was confined to patients with elevated troponin levels. Rates of major bleeding were similar between groups. Overall, the findings suggest that in the modern era of interventional cardiology using high clopidogrel dosing regimens, GP IIb/IIIa inhibition should be reserved only for high-risk ACS patients with positive cardiac markers.

Tirofiban is a tyrosine-derived, nonpeptide inhibitor associated with a rapid onset and short duration of action, with a plasma half-life of approximately 2 h. The efficacy and safety of tirofiban in PCI patients has been investigated in several trials.

**PAR-1 Inhibitors**

PAR-1 inhibitors block the binding of thrombin to PAR-1, thus inhibiting thrombin-induced activation and aggregation of platelets. Preclinical observations indicate that inhibition of the platelet PAR-1 receptor selectively interferes with thrombin-induced platelet activation, but not with the thrombin-mediated fibrin generation and coagulation that is essential for hemostasis. PAR-1 inhibitors may therefore represent a safe and effective mode of antithrombotic therapy.

Two PAR-1 inhibitors currently in clinical trials for the prevention of arterial thrombosis are E-5555 and SCH 530348. E-5555 is an orally active, potent PAR-1 antagonist that has demonstrated antithrombotic effects without increasing bleeding times in preclinical studies, and dose-dependent inhibition of thrombin receptor agonist peptide (TRAP)-mediated platelet aggregation in vitro studies. The safety and tolerability of E-5555 are under evaluation in patients with coronary artery disease and with NSTE ACS in 4 phase-2 clinical trials (NCT00619164, NCT00540670, NCT00548587, and NCT00312052).

SCH 530348 is a high-affinity, orally active, low-molecular-weight, nonpeptide, competitive PAR-1 inhibitor. SCH 530348 inhibits thrombin-induced platelet activation and aggregation without affecting the coagulation cascade or bleeding time. It does not inhibit platelet aggregation induced by ADP, collagen, a TXA2 mimetic or a PAR-4 inhibitor.
agonist peptide, indicating specificity for PAR-1 inhibition. The phase 2 TRA-PCI trial evaluated SCH 530348 used in combination with standard antiplatelet therapy (ASA+ clopidogrel) and an antithrombin agent (heparin or bivalirudin) over a 60-day treatment period in a total of 1,031 enrolled patients undergoing elective PCI. No significant increase was observed in TIMI major and minor bleeding (the primary endpoint) with SCH 530348 vs standard-of-care alone (2.8% vs 3.3%; P=0.58). Although not powered to demonstrate changes in efficacy, a nonsignificant trend for reduction in death/major adverse cardiovascular events was observed with SCH 530348 vs placebo (5.9% [all SCH 530348 groups] vs 8.6%). Specifically, a lower rate of non-fatal MI was observed compared with placebo (4.3% [all SCH 530348 groups] vs 7.3%). Of note, SCH 530348 did not block platelet activation mediated by ADP, arachidonic acid, or collagen, which stimulate pathways required for protective hemostasis. In a phase 2 clinical trial in Japanese patients with NSTE ACS (n=117), the addition of SCH 530348 to standard therapy with ASA and ticlopidine (and heparin) was not associated with an increase in the occurrence of TIMI major and minor bleeding or non-TIMI bleeding. Patients undergoing PCI (primary cohort) treated with SCH 530348 (n=71) experienced a significant reduction in periprocedural MI compared with patients receiving standard-of-care alone (16.9% vs 42.9%, respectively; 61% relative reduction; P=0.013). Furthermore, in a phase 2 trial in Japanese subjects with prior ischemic stroke, the addition of SCH 530348 to ASA was not associated with any episodes of TIMI major, TIMI minor, or non-TIMI bleeding.

Two phase-three trials for SCH 530348 are currently evaluating the drug’s safety and efficacy in addition to standard-of-care therapies vs standard-of-care alone in the prevention of ischemic events. TRA-2P-TIMI 50 will recruit approximately 25,000 patients with prior MI, stroke or PAD, and the TRA-CER trial will evaluate SCH 530348 in approximately 10,000 patients with NSTE ACS.

Phosphodiesterase Inhibitors
Cilostazol, an inhibitor of phosphodiesterase type III indicated for symptomatic relief of intermittent claudication in patients with PAD, possesses both antiplatelet and vaso-dilatory effects. Clinical efficacy of cilostazol as single antiplatelet therapy was shown in the Cilostazol Stroke Prevention Study. During PCI, cilostazol added to ASA and clopidogrel (“triple therapy”) was associated with a significantly reduced risk of stent thrombosis, angiographic restenosis, and in-stent late loss vs ASA plus clopidogrel without increased bleeding risk. The incidence of death, MI, and target lesion revascularization was also lower with triple therapy. The clinical benefits of cilostazol appear superior in diabetic patients with coronary artery disease. The addition of cilostazol to ASA and clopidogrel provided significantly greater inhibition of ADP-induced platelet aggregation in patients undergoing PCI. A US FDA warning indicates that cilostazol should be avoided in patients with congestive heart failure of any severity because of an increased mortality risk. Cilostazol is also associated with headache, palpitations, and diarrhea.

Dipyridamole selectively inhibits the cyclic guanosine monophosphate (cGMP) phosphodiesterase type V enzyme, thus augmenting the antiplatelet effects of the NO/cGMP signaling pathway. In patients with prior ischemic cerebrovascular disease, use of dipyridamole alone or in combination with ASA for secondary prevention of recurrent strokes has yielded conflicting results. However, in the largest trial, the European Stroke Prevention Study (ESPS II), dipyridamole with or without ASA effectively prevented stroke recurrence, and a meta-analysis of 7 randomized trials was consistent with the ESPS II results. The European/Australian Stroke Prevention in Reversible Ischemia Trial (ESPRIT) demonstrated that dipyridamole plus ASA may not only provide protection against stroke recurrence but also against other vascular events, such as MI or death from vascular causes. Results of the recent PROFESS trial showed that there was no significant difference in the risk of fatal or disabling stroke in patients receiving dipyridamole plus ASA vs clopidogrel.

Therapeutic Implications of Inhibition of Platelet Activation by Oral Antiplatelet Agents
Despite the extensive use of ASA and P2Y12 ADP receptor antagonists across a wide range of patients at risk for atherothrombotic events, the residual morbidity and mortality among those receiving these medications remain high. Strategies to further decrease risk for atherothrombotic events include combining a P2Y12 receptor antagonist with ASA or using more potent P2Y12 inhibitors. However, in TRITON and PLATO, residual risk with prasugrel and ticagrelor, respectively, in addition to ASA was almost 10%. The lack of comprehensive platelet activation inhibition in the presence of ASA and a P2Y12 receptor antagonist effectively allows accumulation of thrombotic events mediated by other platelet activators, such as thrombin.

Apart from high residual morbidity and mortality, ASA and P2Y12 receptor antagonists are associated with an increased bleeding risk that may be attributed to the inhibition of the TXA2 and ADP platelet activation pathways that are essential for normal hemostasis. These considerations underscore the need for agents that provide more comprehensive platelet inhibition without interfering with hemostasis, for greater protection against thrombotic events with no incremental bleeding risk. Inhibition of PAR-1 may provide more comprehensive inhibition of platelet-mediated thrombosis when used in combination with the current standard-of-care (ASA and a P2Y12 ADP receptor antagonist), and thus has the potential to incrementally reduce morbidity and mortality in atherothrombotic diseases. Importantly, this benefit of PAR-1 inhibition may not be accompanied by increased risk of bleeding, because this pathway may not be critical for normal hemostasis.

Summary
Platelet activation at sites of vascular injury leads to the formation of a hemostatic plug. Activation of platelets is therefore crucial for normal hemostasis. However, uncontrolled platelet activation may also lead to the formation of occlusive platelet-rich thrombi that can cause ischemic events such as MI, ischemic stroke/TIA, or symptomatic PAD. Platelets can be activated by multiple pathways, including those stimulated by thrombin, TXA2, ADP, and collagen. Each of these agonists stimulates a distinct platelet activation pathway leading to platelet-mediated thrombosis and associated clinical ischemic events. These considerations underscore the need for inhibition of multiple platelet activation pathways in patients at risk for atherothrombosis. ASA and P2Y12 ADP receptor antagonists each target a single platelet activation pathway and when used either alone or in combination,
significantly decrease the risk for atherothrombotic events. However, these agents, particularly when used in combination, may increase the risk of bleeding. The high residual risk for atherothrombotic events in patients treated with ASA and a P2Y12 ADP receptor antagonist may be related to the lack of comprehensive inhibition of platelet-mediated thrombosis, including the absence of an effect on PAR-1-mediated platelet activation induced by thrombin. Therefore, inhibition of the PAR-1 platelet activation pathway is a rational target for development of novel antiplatelet agents. Several other agents blocking other key platelet signaling pathways are also under clinical development. Ultimately, ongoing clinical trials will provide the safety and efficacy information to define the best combination of antiplatelet treatment strategies to treat patients with atherothrombotic disease.

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