

Theme Issue Article

Mechanisms of platelet activation: Need for new strategies to protect against platelet-mediated atherothrombosis

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Summary

Platelets are central mediators of haemostasis at sites of vascular injury, but they also mediate pathologic thrombosis. Activated platelets stimulate thrombus formation in response to rupture of an atherosclerotic plaque or endothelial cell erosion, promoting atherothrombotic disease. They also interact with endothelial cells and leukocytes to promote inflammation, which contributes to atherosclerosis. Multiple pathways contribute to platelet activation, and current oral antiplatelet therapy with aspirin and a P2Y₁₂ adenosine diphosphate (ADP) receptor antagonist target the thromboxane A₂ and ADP pathways, respectively. Both can diminish activation by other factors, but the extent of their effects depends upon the agonist, agonist strength, and platelet reactivity status. Although these agents have demonstrated significant clinical benefit, residual morbidity and mortality remain high. Neither agent is effective in inhibiting thrombin, the most potent platelet activator. This lack of comprehensive

inhibition of platelet function allows continued thrombus formation and exposes patients to risk for recurrent thrombotic events. Moreover, bleeding risk is a substantial limitation of antiplatelet therapy, because these agents target platelet activation pathways critical for both protective haemostasis and pathologic thrombosis. Novel antiplatelet therapies that provide more complete inhibition of platelet activation without increasing bleeding risk could considerably decrease residual risk for ischemic events. Inhibition of the protease-activated receptor (PAR)-1 platelet activation pathway stimulated by thrombin is a novel, emerging approach to achieve more comprehensive inhibition of platelet activation when used in combination with current oral antiplatelet agents. PAR-1 inhibition is not expected to increase bleeding risk, as this pathway does not interfere with haemostasis.

Keywords

Antiplatelet therapy, haemostasis, inflammation, platelet activation, thrombosis

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Introduction

Platelets play a key role in preventing blood loss in response to injury, but they are also responsible for the formation of pathogenic thrombi that cause acute manifestations of vascular atherothrombotic disease, such as acute coronary syndromes (ACS), including unstable angina, both non-ST-elevation (NSTEMI) and ST-elevation (STEMI) myocardial infarction (MI), ischaemic stroke/transient ischaemic attack and symptomatic peripheral artery disease (PAD). Furthermore, platelets are mediators of inflammation, contribute to atherogenesis, and have immunomodulatory activity. This article reviews the role of platelets in protective haemostasis and pathogenic thrombosis, and the platelet activation pathways involved in these processes. Recent insights in post-contact signalling, crosstalk between platelets and

the coagulation cascade, and the role of platelets in inflammation and atherogenesis will also be reviewed. Finally, the therapeutic implications of targeting platelet activation pathways with current and emerging oral antiplatelet agents for the treatment of atherothrombotic disease will be discussed.

Platelets in haemostasis and thrombosis

Several excellent reviews discuss the role of platelets in haemostasis and thrombosis (1–4). Platelet adhesion to the extracellular matrix is the initial step in primary haemostasis. Platelets roll, adhere, and spread on collagen matrix to form an activated platelet monolayer (Fig. 1) (1). Adhesion is mediated by the interaction between the glycoprotein (GP) Ib/V/IX receptor complex on the platelet surface to von Willebrand factor (vWF) and GPIIb/IIIa and GPIa to col-

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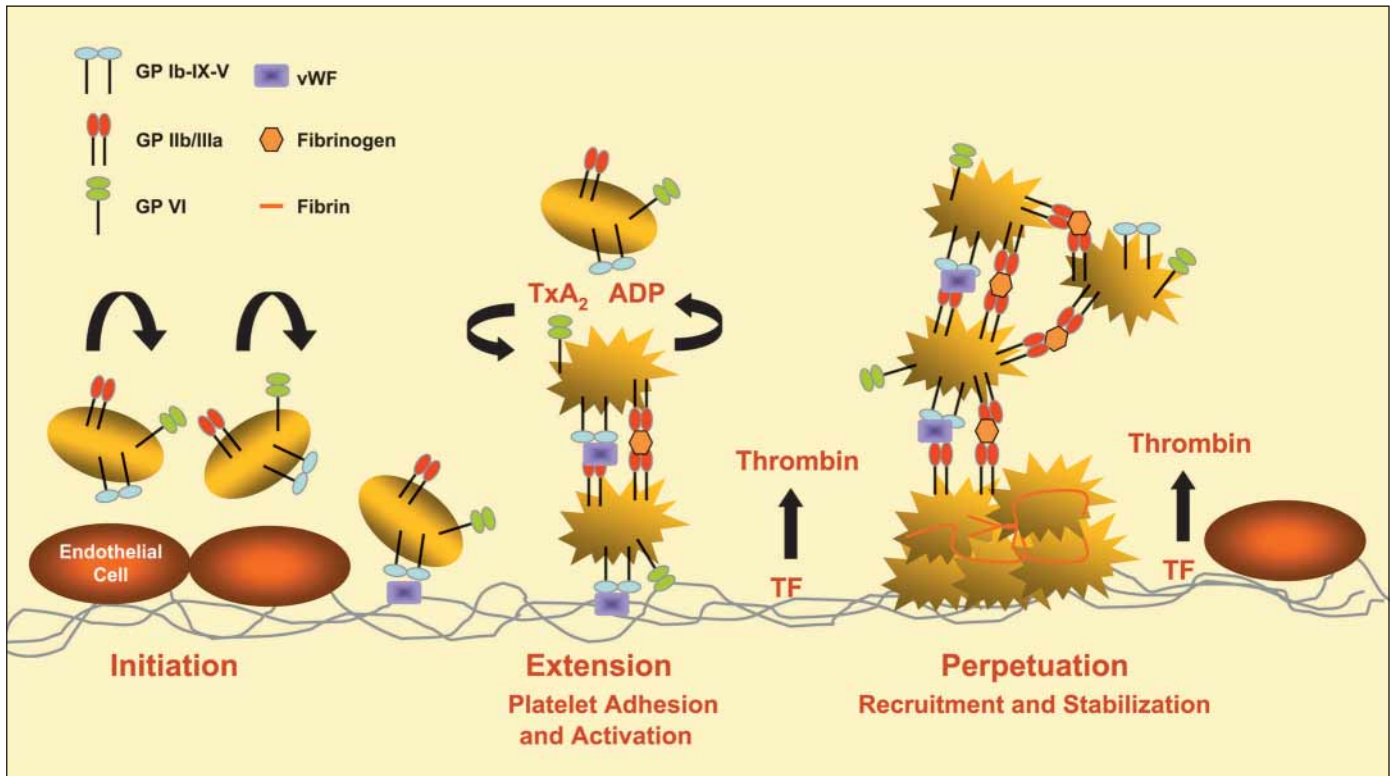


Figure 1: Platelet plug formation. Vascular injury results in the exposure of collagen and von Willebrand factor (vWF) in the vessel wall. Circulating platelets adhere and form a monolayer of activated platelets on the collagen matrix, which drives the release of adenosine diphosphate (ADP) and thromboxane (Tx) A₂ from the adherent platelets. Secretion of ADP and TxA₂ promotes changes in platelet shape and ampli-

fication of platelet activation. Thrombin, generated by locally produced tissue factor (TF), is the most potent platelet activator. In the perpetuation phase, platelet contacts promote growth and stabilisation of the platelet plug. Adapted with permission from Brass LF. *Chest*. 2003;124:18. © American College of Chest Physicians. All rights reserved.

lagen at sites of vascular injury. The interaction between vWF and GPIb/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). Under normal conditions, soluble vWF does not undergo significant interactions with GPIb/V/IX. However, when immobilised on exposed collagen at sites of injury, it becomes a strong adhesive substrate.

Platelet activation and recruitment is stimulated by bound platelet secretion products and local prothrombotic factors (tissue factor), which lead to generation of haemostatic plugs. Multiple pathways lead to platelet activation, including those stimulated by collagen, adenosine diphosphate (ADP), thromboxane A₂, epinephrine, serotonin and thrombin (1–4). The cumulative action of these activators results in recruitment of platelets from the circulation, which also leads to several distinct manifestations of platelet activation (Table 1). These include platelet shape change, expression of pro-inflammatory molecules such as P-selectin and soluble CD40 ligand (sCD40L), expression of platelet procoagulant activity, and conversion of GPIIb/IIIa (αIIbβ₃-integrin) into an active form, which allow platelet aggregation and the potential for pathologic thrombosis. Local accumulation of these agonists recruits circulating platelets into the growing, stable haemostatic plug. Thrombin-mediated generation of fibrin from fibrinogen also contributes to formation and consolidation of the haemostatic plug (1).

GPIIb/IIIa is the central platelet receptor mediating platelet aggregation. Upon activation of this receptor, it promotes platelet adhesion, aggregation and spreading on the exposed extracellular matrix of the injured vessel wall, as well as thrombus formation and stability. Bound fibrinogen to GPIIb/IIIa bridges activated platelets and contributes to thrombus stabilisation. Fibrin-rich clots are generated by thrombin, which is produced initially via tissue factor in ruptured or eroded atherosclerotic plaques. Through platelet activation by multiple pathways, a protective haemostatic plug may progress ultimately to an occlusive, platelet-rich thrombus.

Platelet activation pathways

Multiple pathways contribute to platelet activation (Table 1) (1–4). ADP is stored at high concentrations in dense granules and released from adherent platelets during platelet activation. ADP contributes to platelet activation occurring both during protective haemostasis (i.e. formation of the initial platelet monolayer) and during formation of occlusive platelet-rich thrombi. Release of thromboxane A₂ from adherent platelets enhances recruitment and aggregation to the primary plug and activates platelets during both protective haemostasis and pathologic thrombus formation. Collagen is a strong thrombogenic substrate. Under high-shear conditions, platelet adhesion is mediated by

binding of vWF immobilised on collagen or on the surface of activated platelets to GPIb. This interaction leads to activation of GPIIb/IIIa (which can not bind soluble ligands in its inactive state) and to stable vWF-mediated platelet aggregates (3). However, the binding between GPIb and vWF is insufficient for stable adhesion. GPVI is the major platelet-collagen receptor that mediates platelet activation, which is necessary for adhesion, aggregation, degranulation and coagulant activity on the matrix (3). GPIa acts cooperatively with GPVI and reinforces interactions between GPVI and collagen. Thrombin is the most potent platelet activator (5, 6). Thrombin activates platelets at extremely low concentrations (lower than those required for activation of the coagulation cascade) (5, 6). Thrombin binds the protease-activated receptor (PAR)-1 on the platelet surface, cleaving the receptor, and exposing a tethered ligand, which binds and activates the receptor (7–11). Platelets also express PAR-4, which requires higher concentrations of thrombin for activation (8). Signalling via PAR-4 is available for haemostasis when very high levels of thrombin are generated, thereby providing a protective mechanism in situations where this pathway may contribute to arrest bleeding, such as trauma. PAR-2 is not expressed on platelets and is not activated by thrombin. PAR-3 can bind thrombin on platelets, but the functional role of this receptor remains unclear. Thrombin also binds GPIb, which has been proposed to enhance the specificity of thrombin cleavage of PAR-1 (12). In addition to platelets, PARs are also expressed in other cells in the vasculature, including leukocytes, endothelial cells, and smooth muscle cells (10). In the vessel wall, PAR-1 and PAR-2 mediate responses involved in contractility, inflammation, proliferation, and repair. PAR-1 also has vasodilatory ef-

fects. Stimulation of PAR-1 on endothelium in normal arteries causes production of nitric oxide and smooth muscle cell relaxation (9). In arteries with severe atherosclerotic lesions, relaxation does not occur upon PAR-1 stimulation and contraction may be induced (9).

Platelet interactions in post-contact signalling

Resting platelets are not normally in stable contact with each other but develop contacts once activated. Platelet contacts provide an adhesive force and a secondary source of intracellular signalling. These events are referred to as “outside-in” signalling: events occurring downstream of integrin activation after ligand binding has occurred (13). Numerous signalling events mediate these interactions, including pathways modulated by integrins and other cell adhesion molecules, receptor/ligand interactions, and molecules secreted or shed from activated platelets (Table 2) (13). For example, GPIIb/IIIa accumulates at sites between activated platelets, and its high-affinity state interacts with the cytoplasmic cytoskeletal protein talin, which is a critical final step in integrin activation and stabilisation (14, 15). The adhesion protein kindlin-3 has also been shown to bind to the cytoplasmic tail of beta-integrin (at sites distinct from talin) and to cooperate with talin in integrin activation (16). Platelet endothelial cell adhesion molecule-1 (PECAM-1) binds the tyrosine phosphatase SHP-2 and bridges it to GPVI, providing an inhibitory effect on collagen signalling and preventing unwarranted platelet activation and thrombus growth (17). CD2 family members are expressed on both resting and activated platelets and are involved in maintaining platelet aggregate stability (18).

Platelet activator	Receptor(s)	Effect on platelets
ADP	P2Y₁ P2Y₁₂	Platelet shape change (P2Y ₁) Transient aggregation (P2Y ₁) Sustained irreversible aggregation (P2Y ₁₂) Expression of P-selectin (P2Y ₁₂) Release of thromboxane A ₂ (P2Y ₁ and P2Y ₁₂) Platelet recruitment to sites of injury (P2Y ₁₂) Induction of procoagulant activity and aggregation (P2Y ₁₂)
Thromboxane A ₂	TPα TP β	Platelet recruitment and aggregation to a primary platelet plug (TP α)
Serotonin	5HT-2A	Platelet recruitment to sites of injury Induction of procoagulant activity via retention of fibrinogen and thrombospondin on platelet surface
Epinephrine	α_{2a}	Supplementary role overlapping P2Y ₁₂ receptor signaling
Collagen	GPIb (high shear via vWF) GPIIb/IIIa (high shear via vWF) GPIa/IIa (low shear) GPVI (low shear)	Activation of GPIIb/IIIa Release of ADP and thromboxane A ₂ Platelet spreading Platelet aggregation Induction of procoagulant activity via release of Ca ²⁺
Thrombin	PAR-1 PAR-4	Platelet aggregation (PAR-1) Release of ADP, thromboxane A ₂ (PAR-4), serotonin (PAR-1) and epinephrine (PAR-1) Activation/mobilisation of P-selectin and CD40 ligand (PAR-1) Induction of platelet procoagulant activity (PAR-1)

Receptors primarily responsible for activation in platelets are indicated in **bold**. ADP, adenosine diphosphate; GP, glycoprotein; PAR, protease-activated receptor; vWF, von Willebrand factor.

Table 1: Agonists involved in platelet activation and their effects on platelets (1–4).

The tetraspanin CD63 and tumor-suppressing subchromosomal transferable fragment cDNA6 (TSSC6) interact with GPIIb/IIIa, contributing to platelet spreading on immobilised fibrinogen (19) and stabilisation of arterial thrombi (20), respectively. The receptor/ligand interactions between Eph receptor tyrosine kinases and ephrins are implicated in platelet aggregation, clot retraction and thrombus stability (21). Platelets also secrete or shed several molecules upon activation and aggregation. Shedding of GPIIb α , GPV, GPVI and P-selectin may serve to down-regulate responsiveness to collagen, whereas shedding of CD40L and sema4D stimulates platelets (13).

Crosstalk between platelets and the coagulation cascade

Platelet activation and activation of the coagulation cascade are complementary processes (22, 23). Coagulation factors bind platelets through either their glycoprotein receptors or through phospholipids that become exposed on the outer surface of the plasma membrane following platelet activation. For example, binding of collagen to GPVI activates platelets, exposes phosphatidylserine, and supports thrombin formation and stabilisation (24). The collagen-GPVI interaction also leads to shedding of membrane blebs into the circulation, which provides procoagulant microvesicles. Prolonged increases in intracellular calcium, a common final effect of platelet activation by ADP, thromboxane A₂, thrombin and collagen, is required for bleb formation and phosphatidylserine exposure (23). ADP also stimulates platelet procoagulant activity through interaction with P2Y₁ and P2Y₁₂ (25). Platelet secretion products contribute to the procoagulant activity of activated platelets by providing factor V, factor VIII and fibrinogen (4). Activated platelets support the initiation phase of coagulation by providing binding sites for factor XI and prothrombin. These functions reveal the dual role of platelets in activation and coagulation.

Platelets in inflammation and atherogenesis

Platelets interact with the vascular endothelium and link inflammation, thrombosis and atherogenesis, which is characterised by interactions between platelets, endothelial cells and leukocytes (26–28). Indeed, many of the surface receptors involved in repair of vascular injury and thrombosis also enable platelets to perform immunomodulatory functions (Fig. 2) (29, 30).

Platelet receptors in inflammation and immunity

Intact, inactivated endothelium does not usually interact with platelets. However, intact and inflamed endothelial cells are adhesive for platelets. Studies in mice have shown that platelet adhesion to inflamed endothelium is a multi-step process similar to platelet adhesion at sites of vascular lesions, involving initial platelet tethering, rolling and firm adhesion (26). P-selectin and E-selectin on endothelial cells mediate initial “loose” contact between platelets and endothelium and platelet rolling via binding to GPIb and P-selectin glycoprotein ligand-1 (PSGL-1) (26, 31). Platelet P-selectin is not required for the initial tethering and rolling of platelets, as demonstrated by the normal rolling of pla-

telets in mice lacking platelet expression of P-selectin (32). These initial interactions are highly reversible. Firm adhesion is mediated by GPIIb/IIIa and $\alpha_v\beta_3$ via fibrinogen (Fig. 2) (33, 34). The involvement of GPIIb/IIIa in inflammation is supported by studies showing that GPIIb/IIIa antagonism reduces the rise in inflammatory markers such as C-reactive protein and interleukin (IL)-6 after coronary interventions (35). Platelets also express the Toll-like receptors (TLRs) which are involved in initial pathogen clearance in the innate immune response. TLRs are expressed on both resting platelets and in human coronary thrombi, suggesting an association between infectious immunity and arterial thrombosis (36). *Trans* interaction of the platelet junctional adhesion molecule-A (JAM-A) leads to deposition of chemokines in the lumen by platelets and leukocyte recruitment (37).

Activated platelets also expose CD40L, promoting endothelial inflammation. CD40L induces endothelial cells to produce reactive oxygen species (ROS), adhesion molecules, chemokines and tissue factor. The interaction between CD40 on endothelial cells and CD40L on platelets leads to release of interleukin (IL)-8 and monocyte chemoattractant protein-1 (MCP-1), which recruit neutrophils and monocytes (38). This interaction also stimulates endothelial expression of adhesion molecules (E-selectin, VCAM-1 and ICAM-1), which mediate adhesion of monocytes, lymphocytes and neutrophils to the endothelium (38). In addition, activated platelets release tissue factor on endothelial cells in a CD40-dependent manner, promoting thrombosis (39). Ligation of CD40 also results in release of matrix metalloproteinase (MMP)-2 and -9, which promote degradation

Table 2: Platelet interactions in post-contact signalling (13).

Contact-dependent signalling interactions	Mediator(s)
Integrin and cell surface molecules	GPIIb/IIIa ($\alpha_{IIb}\beta_3$)
	GPIb GPVI PECAM-1
	CTX family members (JAM-A, JAM-C, ESAM, and CD226)
	CD2 family members (SLAM/CD150 and CD84) Tetraspanins (CD9, CD151, TSSC6*, and CD63*)
Receptor/ligand interaction	Eph receptor tyrosine kinases and ephrins
	CD72 and plexin-B1 binding to sema4D
Molecules secreted or shed from activated platelets	GPIIb α GPV GPVI P-selectin CD40L sema4D

*Tumor-suppressing subchromosomal transferable fragment cDNA 6 and CD63 are expressed in dense granules and migrate to the cell surface upon platelet activation. ESAM, endothelial cell-selective adhesion molecule; GP, glycoprotein; JAM, junctional adhesion molecule; PECAM-1, platelet endothelial cell adhesion molecule-1; SLAM, signaling lymphocytic activation molecule; TSSC6, tumor-suppressing subchromosomal transferable fragment cDNA6.

of the extracellular matrix and remodelling of inflamed tissue (26). In turn, MMP-2 has stimulatory effects on platelet activation, whereas MMP-9 is inhibitory (40). Beyond these roles, ligation of platelet CD40L also induces dendritic cell maturation, B cell isotype switching, and enhanced CD8⁺ T cell activity (29).

Soluble immune regulators released by platelets

Adherent platelets become activated and secrete or expose multiple inflammatory factors including growth factors, chemokines, cytokines and coagulation factors (Fig. 2). Platelet-derived chemokines can potentiate thrombosis and inflammation (41). Regulated on activation, normal T expressed and secreted (RANTES or CCL5) is secreted by activated platelets and deposited on inflamed or atherosclerotic endothelium, leading to monocyte arrest (42). RANTES and platelet factor 4 (PF4 or CXCL4) form heterodimers, leading to enhanced monocyte arrest by RANTES (29, 43). Injection of activated platelets into atherosclerosis-prone mice leads to P-selectin-dependent PF4 and RANTES deposition and promotes atherosclerosis, whereas inhibition of RANTES receptors decreases lesion size (29). Disruption of PF4 and RANTES heteroaggregates with peptide antagonists inhibits monocyte recruitment and atherosclerosis (44). RANTES can also induce expression of other chemokines and cytokines in target leukocytes, while PF4 has angiostatic activity and can inhibit the proliferation of endothelial cells (29, 30). Secretion of the cytokine IL-1 β by platelets causes endothelial cells to release the chemokine MCP-1 and induces endothelial expression of the adhesion molecules ICAM-1 and $\alpha_v\beta_3$ (45). These events promote adhesion of neutrophils to inflamed

endothelium. Finally, activated platelets secrete ROS. The function of platelet-derived ROS is unclear, but has been proposed to enhance platelet recruitment to a growing thrombus (46) and lipid peroxidation of cell-membrane phospholipids and circulating low-density lipoprotein (26).

Platelet-leukocyte interactions

The induction of inflammatory mediators by activated platelets culminates in the recruitment of leukocytes. Platelet P-selectin is crucial for the recruitment of immune cells through its adhesive activity and signalling properties (29). Leukocytes, including both monocytes and neutrophils, tether to adherent platelets via interaction between P-selectin and PSGL-1 on leukocytes, inducing upregulation and activation of β_1 and β_2 integrins and enhanced monocyte recruitment to activated endothelium (47). Platelet P-selectin is also required for RANTES deposition on inflamed endothelium (48). Firm adhesion is mediated by binding of Mac-1 (CD11b/CD18) on leukocytes to GPIb α and ICAM-2 (49). Platelet presentation of chemokines also leads to monocyte activation. Conversely, recruited leukocytes can contribute to enhanced platelet activation via recruitment of circulating activated platelets through binding of PSGL-1 to P-selectin (49). The interaction between platelets, leukocytes, and the endothelium can occur in variable ways: platelets can first form conjugates with leukocytes and support leukocyte recruitment to the endothelium via activation of leukocyte adhesion receptors. Alternatively, platelets adherent on the endothelium can chemoattract leukocytes and provide a sticky surface for neutrophil-endothelium interaction. The net result of these events is the infiltration of inflammatory cells into the vessel wall, which is im-

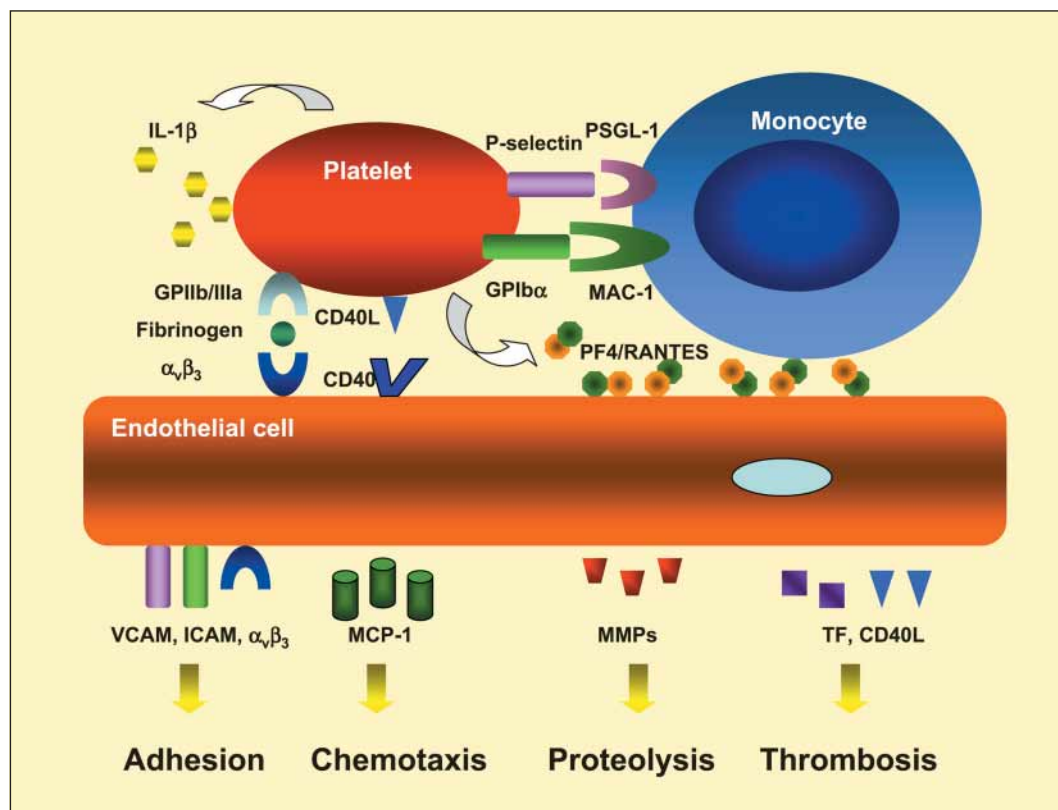


Figure 2: Platelet-stimulated inflammation of endothelial cells and monocyte recruitment. Firm adhesion between glycoprotein (GP) IIb/IIIa and $\alpha_v\beta_3$ induces release of interleukin (IL)-1 β and CD40L from platelets, leading to stimulation of inflammatory pathways in endothelial cells. Activated platelets deposit the chemokines PF4 and RANTES onto inflamed endothelium. PF4/RANTES heterodimers promote monocyte recruitment. MCP, monocyte chemoattractant protein; MMPs, matrix metalloproteinases; TF, tissue factor. Adapted from Gawaz et al. (J Clin Invest 2005; 115: 3378.) Copyright © The American Society for Clinical Investigation. 2005. All rights reserved.

portant in atherosclerosis (27, 49). Platelets therefore play additional roles beyond haemostasis and thrombosis. Platelet-mediated inflammation provides the basis for plaque formation before actual vessel occlusion. Platelets thus link diverse processes culminating in atherogenesis.

Additional platelet activities

Despite their anucleate status, platelets are able to synthesise certain proteins *de novo* upon activation through a novel mechanism called signal-dependent pre-mRNA splicing. (For a review of the mechanisms involved, see Weyrich et al. [50]). Synthesised proteins include IL-1 β , tissue factor, plasminogen activator inhibitor-1 (PAI-1), cyclooxygenase (COX)-1, and B-cell lymphoma 3 (Bcl-3) (50). The physiologic significance of protein synthesis by platelets remained unclear until recently. Secretion of IL-1 β increases platelet adhesiveness towards leukocytes, although most of the newly synthesised IL-1 β remains in the platelet (51). Aspirin-treated platelets can synthesise COX-1 and recover the capacity to regenerate thromboxane A₂ (52). Bcl-3 promotes clot retraction in human platelets (53). Thus, protein synthesis by platelets can alter functional events relevant to thrombosis and inflammation. In addition to protein synthesis, platelets are also capable of programmed cell death (apoptosis) (54). Recent studies suggest that the physiological lifespan of platelets is regulated between the pro-survival and pro-apoptotic signals Bcl-x_L and Bak, respectively (54). Thrombin, at high concentrations, has also been implicated in platelet apoptosis through induction of Bak expression as well as other pro-apoptotic mechanisms (55, 56).

Therapeutic implications

Antiplatelet therapy

Inhibition of platelet activation pathways with oral antiplatelet therapy (aspirin and a P2Y₁₂ ADP receptor antagonist) is critical for the acute and chronic treatment of atherothrombotic diseases. Aspirin is an irreversible COX-1 inhibitor that blocks thromboxane A₂ production and thereby reduces platelet activation stimulated by thromboxane A₂ (57, 58). Clinical investigations with aspirin have consistently documented the benefit of aspirin in ACS (59, 60), percutaneous coronary intervention (PCI) (61, 62), and secondary (59) and primary prevention (59, 63) of acute ischaemic events. However, aspirin use is associated with bleeding risk, which may be attributed to inhibition of thromboxane A₂-mediated effects in haemostasis. Aspirin minimally inhibits other platelet activation pathways, allowing platelet activation by other agonists and exposing patients to risk of thrombotic events.

P2Y₁₂ ADP receptor antagonists inhibit the activation of the P2Y₁₂-mediated platelet activation pathway induced by ADP. These agents include ticlopidine and clopidogrel, as well as several compounds in late development (prasugrel, ticagrelor [AZD6140] and cangrelor) (64). By preventing ADP-induced activation of the P2Y₁₂ receptor, these agents reduce platelet activation mediated by ADP. The clinical efficacy of P2Y₁₂ ADP antagonists has been demonstrated in several clinical scenarios both as single antiplatelet therapy (CAPRIE) (65) and as an add-on to aspirin: CURE (66), CREDO (67), CLARITY (68), COM-

MIT (69), CHARISMA (70, 71) and TRITON (72). Despite this clinical benefit, residual morbidity and mortality remains high. In TRITON, the most recent large clinical trial of antiplatelet therapy in patients with ACS undergoing PCI, the incidence of death, nonfatal MI or nonfatal stroke at 15 months was 12.1% in patients treated with aspirin and the P2Y₁₂ receptor antagonist clopidogrel, and 9.9% in those receiving aspirin in combination with the more potent P2Y₁₂ receptor antagonist prasugrel ($p < 0.001$) (72). Thus, approximately 10% of patients experience recurrent thrombotic events or death with current antiplatelet treatment approaches. Furthermore, bleeding risk is an important limitation of P2Y₁₂ receptor antagonists because it contributes both to morbidity (i.e. need for transfusions) and is an independent predictor of short- and long-term mortality in patients with ACS and in those undergoing PCI (73–75). Results of TRITON suggest that bleeding risk increases proportionally with the degree of platelet inhibition (72) and can be attributed to the essential role played by the P2Y₁₂ pathway in haemostasis. Rates of TIMI major bleeding in TRITON were 2.4% with aspirin plus prasugrel versus 1.8% with aspirin plus clopidogrel ($p = 0.03$) (72). Rates of life-threatening bleeding, fatal bleeding, and bleeding requiring transfusion were also significantly higher with prasugrel. In addition, variability of response has been demonstrated with clopidogrel (76). Persistent platelet reactivity may increase the risk of thrombotic events due to inadequate inhibition of the ADP platelet activation pathway (77). Finally, minimal inhibitory effects on platelet activation pathways other than the one stimulated by ADP may allow platelet activation by other agonists (including thrombin) and thereby potentially lead to thrombotic events.

The aggregation of activated platelets, regardless of the agonist, is mediated by the cross-linking of the GP IIb/IIIa receptor on adjacent platelets by fibrinogen (4). GPIIb/IIIa inhibitors prevent platelet aggregation and subsequent thrombus formation by preventing the interaction of GPIIb/IIIa receptors with fibrinogen. Available GPIIb/IIIa inhibitors include eptifibatid, tirofiban, and abciximab. A meta-analysis of six trials evaluating GPIIb/IIIa inhibition for NSTEMI ACS in patients treated with aspirin and heparin demonstrated a significant reduction in the combined rate of death or MI at 30 days with GPIIb/IIIa inhibitors compared with control (10.8% vs. 11.8%, $p = 0.015$) (78). The rate of major bleeding was higher in patients treated with GPIIb/IIIa inhibitors (2.4% vs. 1.4%, $p < 0.0001$), but intracranial bleeding rates were similar (0.09% vs. 0.06%, $p = 0.40$). Because of the associated bleeding risk, these agents are only administered within the acute/hospital setting and are not used in the long-term care of patients with atherothrombotic disease.

Platelet PAR-1 receptor for thrombin:

Novel therapeutic target for atherothrombotic disease

The risk for recurrent thrombotic events and bleeding with current therapies underscores the need for novel agents that provide more comprehensive platelet inhibition when used in combination with current oral antiplatelet agents without interfering with haemostasis. Inhibition of PAR-1 platelet activation by thrombin, the most potent platelet activator, could provide more comprehensive inhibition of platelet-mediated thrombosis when used in combination with current oral antiplatelet therapy.

PAR-1 inhibition is not expected to increase bleeding risk, because the PAR-1 platelet activation pathway may not be essential for normal haemostasis, as several preclinical studies have suggested (79–81). First, the non-peptide PAR-1 antagonist (FR171113) inhibits occlusive thrombus formation in a dose-dependent manner in a guinea pig model of arterial thrombosis without prolonging bleeding time (80). In addition, in this model PAR-1 antagonism did not prolong activated partial thromboplastin time, prothrombin time, or thrombin time, suggesting that PAR-1 inhibition does not affect the coagulation cascade. In contrast, treatment with the direct thrombin inhibitor argatroban inhibited thrombus formation but resulted in significantly increased bleeding time and clotting time (80). Furthermore, PAR-1 inhibition with FR171113 did not inhibit ADP-induced or collagen-induced platelet aggregation, suggesting that PAR-1 antagonism does not affect platelet activation pathways required for protective haemostasis. The P2Y₁₂ ADP receptor antagonist AR-C69931MX (cangrelor), and to a lesser extent the thromboxane A₂ antagonist indomethacin, have been shown to inhibit platelet adhesion to immobilised collagen, demonstrating that inhibition of these pathways disrupts normal haemostasis (82). Inhibition of PAR-1 activity in cynomolgus monkeys with the selective, small molecule antagonist RWJ-58259 results in significantly reduced platelet deposition at existing thrombi (79). PAR-1 inhibition did not affect haematologic parameters, including platelet counts, nor did it affect the coagulation cascade. In mice, PAR-4 is the receptor necessary for platelet activation by thrombin and is analogous to PAR-1 in humans (8). Mice lacking PAR-4 (*Par4*^{-/-}) exhibit markedly reduced platelet accumulation and thrombus growth after laser-induced arteriolar injury (81). However, these mice have no spontaneous bleeding and normal fibrin deposition, suggesting that this pathway is required for thrombus formation but not for haemostasis (81). Finally, thrombin-mediated cleavage of fibrinogen to fibrin is more important for haemostasis than thrombin-mediated platelet activation, as suggested by a substantially more dramatic bleeding phenotype in mice lacking fibrinogen (*Fib*^{-/-}) compared to *Par4*^{-/-} mice (8, 10, 83). Taken together, these findings suggest that PAR-1 inhibition should permit the formation of the initial monolayer of platelets which is necessary for arrest of bleeding, but block thrombus propagation.

Inhibition of PAR-1 with a thrombin receptor antagonist (TRA) is a novel approach in clinical development for the prevention of arterial thrombosis (9). SCH 530348 is an orally active, low-molecular-weight, non-peptide, competitive PAR-1 antagonist (84). Pre-clinical functional assays have shown potent inhibition of thrombin and thrombin receptor activating peptide (TRAP)-induced platelet aggregation by SCH 530348 (84). In addition, SCH 530348 is inactive in functional assays with PAR-4 and does not affect clotting parameters such as prothrombin time. Studies in cynomolgus monkeys revealed no bleeding risk with the administration of SCH 530348 (1 mg/kg or 10 mg/kg) alone or in combination with aspirin plus clopidogrel (85). These results suggested that SCH 530348 is a potent and selective PAR-1 antagonist which does not impact bleeding, and supported further clinical evaluation.

In the phase 2 Thrombin Receptor Antagonist – Percutaneous Coronary Intervention (TRA-PCI) trial, we evaluated the safety

and efficacy of SCH 530348 used in combination with standard oral antiplatelet therapy (aspirin and clopidogrel) and antithrombin agent (heparin or bivalirudin) over a 60-day treatment duration period in 1,031 patients undergoing non-urgent PCI or coronary angiography with planned PCI (86). Patients were randomised to receive one of three oral loading doses of SCH 530348 (10 mg, 20 mg or 40 mg) or placebo in addition to aspirin plus clopidogrel. Patients that underwent PCI (n = 573) were randomised to receive one of three oral daily maintenance doses of SCH 530348 (0.5 mg, 1 mg or 2.5 mg) or placebo. There was no significant difference in the primary end point of rate of the incidence of TIMI (Thrombolysis In Myocardial Infarction) major bleeding and minor bleeding between patients that underwent PCI in the two treatment arms at the end of the 60-day treatment period (2.8% in the collective SCH 530348 treatment arms vs. 3.3% with standard care therapy). The rate of death, major cardiovascular events (MACE) or stroke was assessed as a secondary endpoint and was not significantly different between SCH 530348 and placebo groups. However, there was a non-significant trend for dose-dependent reduction in MACE, specifically for non-fatal MI in the SCH 530348 groups versus the placebo group (4.3% vs. 7.3%) (86). We also assessed the effect of SCH 530348 on platelet aggregation induced by TRAP. SCH 530348 provided rapid, potent, dose-dependent, and durable inhibition of TRAP-induced platelet aggregation (86). SCH 530348 did not have any measurable effects on platelet aggregation induced by other agonists, including ADP, arachidonic acid or collagen (87). These results suggest that SCH 530348 has no activity on platelet activation pathways required for normal haemostasis.

The safety and tolerability of SCH 530348 was also confirmed in a recent phase 2 clinical trial in 117 Japanese patients with NSTEMI ACS (88). Addition of SCH 530348 (either 20 mg or 40 mg loading dose, followed by 1 mg or 2.5 mg maintenance dose) for 60 days to standard of care (aspirin, ticlopidine, and heparin) was not associated with an increase in the occurrence of the primary safety endpoint of TIMI major and minor bleeding or non-TIMI bleeding versus patients receiving standard-of-care therapies plus placebo, confirming previous findings in elective PCI. Patients undergoing PCI (primary cohort) treated with SCH 530348 (N = 71) experienced a significant reduction in periprocedural MI compared to the 21 patients receiving standard of care alone (16.9% vs. 42.9%; 61% relative reduction p=0.013). There were no deaths or any other MACE (88). An additional phase 2 trial in 90 Japanese patients with prior ischaemic stroke revealed no significant difference in the rate of either TIMI major or minor bleeding in patients allocated aspirin plus SCH 530348 (1.0 mg/d or 2.5 mg/d) versus aspirin plus placebo for 60 days (89). The data from these phase 2 trials demonstrate the potential clinical benefit of SCH 530348 when incorporated into the standard-of-care therapy for patients with vascular atherosclerotic disease.

Taken together, these results indicate that SCH 530348 is a novel antiplatelet agent with a unique mechanism of action that selectively targets the PAR-1 receptor for thrombin. Our findings and results from other studies suggest that inhibition of PAR-1 by SCH 530348 does not affect pathways required for haemostasis, as evidenced by pre-clinical functional data and platelet aggregation studies from treated patients showing no effect on aggre-

gation induced by ADP, arachidonic acid, or collagen. SCH 530348 is thus not expected to expose patients to increased bleeding risk. Results of three phase 2 trials demonstrated no increased risk of bleeding with SCH 530348 used together with current therapies, and suggest a potential benefit towards lower thrombotic events, thus improving upon the benefit/risk ratio of current antiplatelet therapy. These findings provide a rationale for evaluation of SCH 530348 used in combination with current standard-of-care dual antiplatelet therapy in phase 3 trials. Two phase 3 trials for SCH 530348 are currently ongoing: The Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischaemic Events (TRA-2P-TIMI 50; clinical trials.gov identifier NCT00526474) is a multinational, double-blind, randomised placebo-controlled trial that will evaluate the efficacy of SCH 530348 plus standard-of-care therapies, which includes aspirin and/or clopidogrel therapy in the secondary prevention of ischaemic events in patients with prior MI, stroke or PAD and will recruit approximately 20,000 patients (90). Patients will receive a 2.5 mg maintenance dose of SCH 530348 or placebo. The primary endpoint is the composite of cardiovascular death, MI, urgent coronary revascularization, or stroke. The key secondary end point is cardiovascular death, MI, or stroke. Patients will be followed for a minimum of one year. The phase 3 Thrombin Receptor Antagonist Clinical Event Reduction in acute coronary syndrome (TRA-CER; clinical trials.gov identifier NCT00527943) trial will be a multinational, randomised, double blind, placebo-controlled study and will evaluate the prevention of ischaemic events in patients with NSTEMI ACS in approximately 10,000 patients for ≥ 1 year of follow-up with a loading dose of 40 mg SCH 530348 and a maintenance dose of 2.5 mg SCH 530348 in addition to aspirin and clopidogrel. The primary endpoint is the composite of cardiovascular death, MI, stroke, rehospitalisation for ACS, and urgent revascularisation; the secondary end point is the composite of cardiovascular death, MI, and stroke (91).

Conclusion

Platelet activation is critical for normal haemostasis but may also lead to the formation of occlusive platelet-rich thrombi. Interac-

tions between activated platelets, endothelial cells and leukocytes promote vascular inflammation, which contribute to the development and progression of atherosclerosis. Platelet activation is a multifactorial process, with platelet-platelet contacts providing a secondary source of intracellular signalling downstream of integrin activation. Platelets thus contribute to important pathologic conditions leading to acute ischaemic events and chronic inflammatory processes, and represent an important therapeutic target.

Multiple pathways activate platelets, including those stimulated by thrombin, thromboxane A₂, ADP and collagen. Excessive platelet activation may lead to platelet-mediated thrombosis and associated clinical ischaemic events, such as death, MI, ischaemic stroke/transient ischaemic attack or symptomatic PAD. Aspirin and P2Y₁₂ receptor antagonists each target a single platelet activation pathway and minimally inhibit other platelet activation pathways. While the use of aspirin alone has demonstrated significant clinical benefit, and the addition of a P2Y₁₂ receptor antagonist provides incremental benefit, residual morbidity and mortality remain substantial. High residual risk may be due to the lack of comprehensive inhibition of platelet-mediated thrombosis, including the absence of inhibition of PAR-1-mediated platelet activation induced by thrombin, the most potent platelet activator. Additionally, the use of current therapies has been associated with bleeding risk, which may be due to the essential role of the thromboxane A₂ and ADP platelet activation pathways in normal haemostasis. Inhibition of the PAR-1 platelet activation pathway is a rational approach to development of novel antiplatelet agents with an improved therapeutic index, because this pathway is a key contributor to platelet-mediated thrombosis but does not appear to play a critical role in haemostasis. For this reason, PAR-1 inhibition may reduce clinical events driven by platelet-mediated thrombosis, without increasing bleeding risk.

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