Platelets: Small in Size But Essential in the Regulation of Vascular Homeostasis

– Translation From Basic Science to Clinical Medicine –

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Platelets are small blood cells that adhere to the site of vessel injury where von Willebrand factor (VWF) is expressed. Platelets bind to VWF through interaction with a membrane protein, glycoprotein (GP) Ibα. Next, the accumulated platelets are activated to change their morphological and biochemical characteristics. Various vasoactive substances, such as immune-regulatory CD40 ligand, are released locally from activated platelet cells to maintain homeostasis of the vascular system. Major roles played by platelets in the regulation of hemostasis and thrombus formation include local activation of the coagulation cascade. Translocation of negatively charged phospholipids to the surface of activated platelets helps in the formation of prothrombinase complex, which efficiently produces thrombin. Thrombin produces fibrin around the activated platelets and further activates the platelets through thrombin receptor stimulation. Of the various platelet-stimulating receptors and activation signals, cyclooxygenase-1, P2Y12 adenosine 5′-diphosphate receptor, and thrombin receptor (protease activated receptor)-1 blockers are used clinically as antiplatelet agents. In the future, precise understanding of the quantitative contribution of platelet function in hemostasis and pathological thrombus formation should lead to the development of effective antithrombotic agents without increasing the risk of serious bleeding complications. (Circ J 2015; 79: 1871–1881)

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Regulation of Vascular Homeostasis by Platelets

Initial Adhesion on Functionally or Physically Damaged Endothelium

In the microcirculation, large and heavy cells such as erythrocytes circulate in the center of the blood vessel, whereas platelets, with their relatively small diameter (2–5 μm) circulate near the vascular wall (Figure 1).1,2 Platelets do not have nuclei but contain various organelles, including mitochondria, Golgi apparatus, and endoplasmic reticulum.3 Of note, an open canalicul system is a platelet-specific micro-organ, which plays an important role in activation-related release of vasoactive substances such as von Willebrand factor (VWF) and fibrinogen.

In the physiological setting, the vessel walls are covered by endothelial cells with antithrombotic characteristics. Upon stimulation of endothelial cells (eg, oxidative stress) or their physical disruption, the thrombogenic matrix, which includes VWF, is exposed directly to the blood circulation (our proposed model is shown in Figure 2). The platelets immediately adhere to the exposed VWF through interaction with a platelet membrane protein, glycoprotein (GP) Ibα (Figure 2B).4 As reported previously, and simply summarized in Figure 2C, the localization of GPIbα on platelets is heterogeneous.5 Platelets expressing GPIbα circulate, ready to bind to VWF.6,7 Initial adhesion of platelets is regulated by the function of the vessel wall. Various stimulatory factors, such as oxidative stress, induce translocation of VWF from inside the endothelial cells to the intravascular wall of the cells.4 Recent investigation revealed the important regulatory role of the autophagy system in the translocation of VWF to the outside of endothelial cells.8 It is of note that binding of VWF and GPIbα generates enough power for platelets to stick to the vascular wall, even against strong detaching forces, with only a small number of involved molecules.9–11

For stable adhesion of platelets, even in the initial phase, another membrane protein, GPIIb/IIIa, is involved, but the essential role of GPIbα cannot be replaced by GPIIb/IIIa even after full activation of platelets.12 Of note, the addition of anti-GPIIb/IIIa in flowing blood makes platelet thrombi unstable, suggesting complementary roles of GPIbα and activated GPIIb/IIIa in stable platelet adhesion under blood flow conditions (Figure 3).13 Various other stimulation receptors and signals might also play roles in initial platelet adhesion,7,14–20 but the details are still to be elucidated.
Local Release of Bioactive Substances From “Activated” Platelets

Platelets contain various vasoactive substances in storage granules such as α- and dense-granules. Upon activation, platelets change their shape to release their contents. In other words, platelets act as a local source of vascular regulatory substances, including CD40 ligand, serotonin, adenosine 5′-diphosphate (ADP), von Willebrand factor (VWF), fibrinogen and so on.

VWF plays an essential role in capturing platelets at the site of endothelial damage. Soluble VWF also contributes to the initial adhesion process. In the clinical setting, the plasma concentration of VWF in acute myocardial infarction (MI) is reported to be 2-fold higher than in healthy volunteers. High plasma concentrations of VWF are reported also in patients with heart failure. It is important to note that approximately 20% of plasma VWF is released from platelets/megakaryocytes, and the rest originates from vascular endothelial cells. VWF released from activated platelets may play a role in the “positive feedback” of platelet activation. The exact effect of locally released VWF on the onset of thrombotic events is still to be elucidated.

There are several other potential positive feedback mechanisms mediated by activation-dependent release of bioactive substances from platelets. Of them, an ADP-mediated mechanism is of particular importance because antiplatelet agents commonly used in clinical practice block ADP receptors, namely P2Y12. Initial platelet thrombi become unstable.

![Figure 1.](image1.png)

**Figure 1.** Platelets in the microcirculation. Relatively large and heavy cell such as erythrocytes circulate in the center of blood vessels, whereas smaller cells such as platelets flow in the periphery where the velocity is relatively slow compared with the center of blood flow.

![Figure 2.](image2.png)

**Figure 2.** Proposed model of platelet adhesion regulated by endothelial cells (EC) and blood flow. Thrombogenic subendothelial matrix containing von Willebrand factor (VWF) is not exposed in the healthy human (A). Injury to the ECs causes VWF to be exposed to the blood flow, and immediately circulating platelets are captured (B). Heterogeneous localization of GPIbα on the platelet cell membrane is thought to assist in the binding of GPIbα to VWF as the platelets roll across the ECs under blood flow conditions.
Role of Platelets

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**Figure 3.** Mechanism of platelet thrombus formation under arterial blood flow conditions. Once platelets have adhered to von Willebrand factor (VWF) expressed at site of vessel damage through binding with GPIbα, they become activated. Activated platelets change their shape, change the conformation of membrane protein GPIbβ/IIIa, translocate functional proteins such as P-selectin, and release various vasoactive substances and microparticles. VWF expressed or bound on the surface of activated platelets also captures circulating platelets, thereby growing in a three-dimensional manner. Various receptors on platelets play important roles in stabilizing platelet adhesion and thrombus growth.

when P2Y12 receptors are blocked by specific antagonists or in patients deficient in this specific receptor. We have proposed a model of ADP release from platelets upon activation (Figure 4) ([http://www.kurims.kyoto-u.ac.jp/~kyodo/kokyuuroku/contents/pdf/1752-08.pdf](http://www.kurims.kyoto-u.ac.jp/~kyodo/kokyuuroku/contents/pdf/1752-08.pdf)). In our model, we implemented the function of platelet activation induced by VWF. Once the activating signaling(s) reaches the dense-granules, degradation of ADP/ATP to the open canalicular system starts (Figure 4, Movie S1).

In addition to extracellular release by degranulation of α-and dense-granules, shedding of the platelet membrane upon activation also plays a role in local release of bioactive substances from platelets. Indeed, platelet membrane-derived microparticles may play essentially important roles in regulating vascular homeostasis. The bioactive substances released as microparticles are stable in plasma because they are surrounded by lipid bi-layers. These platelet-derived microparticles accelerate local “coagulation” by providing negatively charged phospholipid. Unlike substances released without lipid layers, platelet-derived microparticles may contain more than 1 molecule in each of them. If the microparticles contain both CD40 ligand and P-selectin, these particles efficiently regulate the functions of leukocytes because of the additive function of both proteins. Microparticles may also bring RNA from platelets to other cells. The vascular regulatory mechanism of platelets through release of microparticles is an important research topic for the future.

**Local Regulation of Inflammation and Immune System by Platelets**

Ross previously suggested the crucial role played by platelets in the progression of atherosclerosis. At that time, Ross provided us with the notion that platelet-derived growth factor (PDGF) should play an important role in atherosclerosis by inducing proliferation of intimal cells. Indeed, PDGF was shown to be present not only in platelets but also in various other cells. Later, the main focus shifted to the concept that “atherosclerosis is a kind of inflammation.”

Platelets have a regulatory role in controlling local inflammation and immunity. First, surface translocation of P-selectin occurs upon activation of platelets. P-selectin captures leukocytes from flowing blood to regulate local inflammation. Platelet-leukocytes aggregates often increase in thrombotic diseases, but the pathophysiological roles of these aggregates are not yet clarified.

Platelets contain various immunoregulatory proteins. Of them, CD40 ligand is important for regulating production of antibodies from B cells. Platelets are one of the major sources of CD40 ligand. We have shown surface translocation of CD40 ligand on activated platelets, in addition to local release of its soluble form. We have also shown that activated platelets release undetermined proteins (not CD40 ligand), inducing maturation of dendritic cells with specific characteristics. The exact understanding of the relationship between platelet activation and local regulation of inflammation, immunity and progression of atherosclerosis is still a future target of research.

**Local Regulation of Coagulation by Platelet Cells**

The close link between platelet activation and the coagulation cascade was demonstrated by colocalization of platelet and fibrin in human coronary arterial thrombi causing acute MI.
Role of Platelets in the Onset of Various Thrombotic Diseases and Their Control by Antiplatelet Therapy

Role of Platelets in the Onset of Atherothrombosis

Arterial thrombosis disease, such as MI and ischemic stroke, occurs more in patients with atherosclerotic vascular disease than in those who only have risk factors. Though the symptoms differ, there is much homogeneity among the risk factors, accompanying diseases and pathophysiology of MI and ischemic stroke. These arterial thromboses occurring in patients with atherosclerotic vascular disease are often termed “atherothrombosis.”

It is important to separate “atherosclerosis” and “atherothrombosis” because in the majority of cases “atherosclerosis” is asymptomatic whereas ischemic organ symptoms occur in patients with “atherothrombosis.” Indeed, atherosclerotic endothelial injuries play an important role in the initiation of “atherothrombotic events”, just like in the animal models. Various factors then play additive roles in the process of propagation of thrombi.
Role of Platelets in the Onset of Venous Thrombosis

Similar to atherothrombosis, there is much clinical evidence demonstrating preventive effects of aspirin for the onset and recurrence of venous thrombosis. After clinical development of orally available antithrombin and anti-Xa, there was active promotion of the concept that “anticoagulant but not antiplatelet agents are effective for prevention of venous thrombotic events.” However, the previously shown results with aspirin in preventing so-called “venous thrombosis”, including stroke in patients with AF and recurrence of venous thromboembolism, should not be forgotten. Though warfarin prevents stroke more efficiently, the antiplatelet agent clopidogrel in addition to aspirin has been shown to reduce thrombotic cardiovascular events and stroke in AF patients. These clinical findings support the notion that platelets play an important role in venous thrombosis.

In a pathological study, both platelets and fibrin were found in thrombi causing deep venous thrombosis and in cardioembolic thrombi in patients with AF. It is still just speculation, but most likely the activated platelets are an important initiator for coagulation at the site of blood stasis. The clinical finding that the risk of venous thrombotic events increases in patients with functionally damaged endothelium, in older patients, and those with hypertension, heart failure and diabetes supported the idea. It is fair to state that the clinical evidence for antiplatelet agents preventing venous thrombosis is weaker than that for their role in arterial thrombosis.

Antiplatelet Therapy: Basic Science and Clinical Experience

Aspirin

There are many receptors and activation signaling in platelets. Some of them have been tested as antiplatelet drugs and currently, a few are clinically available as antiplatelet agents. Of these, aspirin is the most widely used especially in the secondary prevention of MI. Aspirin inhibits the function of cyclo-oxygenase (COX-1) by its acetylation. The process from COX-1 inhibition to prevention of vascular events has yet to be clarified.
Japan might be one reason for the even lower incidence of thrombotic CV events in this country.

Clopidogrel and Other P2Y12 ADP Receptor Inhibitors

There is now enough clinical evidence for the use of clopidogrel as the established standard of care in patients with acute coronary syndrome (ACS). It is histologically interesting to know that clopidogrel has been widely used in clinical practice for years based mostly on clinical evidence with- out understanding even its pharmacological target molecule(s). The target of active metabolite(s) of clopidogrel, namely the P2Y12 ADP receptor, was cloned lately. Even now, the exact role of P2Y12 receptor stimulation in the process of pathologi- cal thrombus formation is to be elucidated.

There still is ongoing discussion on how can we modify the P2Y12 receptor by drug intervention to achieve the best balance between antithrombotic effects and increased risk of serious bleeding.

New P2Y12 inhibitors (prasugrel and ticagrelor) were developed recently. Appropriate selection of the most suitable drug for a suitable patient population is an ongoing discus- sion.

Thrombin Receptor Antagonists and Anticoagulants

As shown in Figure 7, platelets interact very closely with the coagulation cascade. Thrombin generated on the surface of activated platelets converts water-soluble fibrinogen to insoluble fibrin. Thrombin also activates platelets through thrombin...
role of platelets

par-1 inhibitors; that is, patients with prior mi.

even in prior mi patients, those with diabetes were shown to be the most appropriate patient population for vorapaxar.

the clinical development of vorapaxar and the assessment of the data by the us regulatory authority give us a clue to the selection of suitable small populations of patients for new drug interventions within the database of large-scale global trials in the future.

because thrombin generated on the surface of activated platelets plays a role in the activation of platelets through thrombin receptor stimulation, all agents inhibiting the coagulation cascade have the ability to inhibit platelet activation. indeed, the old oral anticoagulant, warfarin, demonstrated better efficacy in preventing arterial thrombotic disease after mi.

of the thrombotic events, thrombotic occlusion of coronary arteries after implantation of coronary stents was previously believed to be prevented only by antiplatelet therapy including p2y12 antagonists.

of the clinical trials applying newly developed direct oral anticoagulants (antithrombin and anti-xa) for patients with acs, the anti-xa therapy to lower cardiovascular events in addition to standard therapy in subjects with acute coronary syndrome-thrombolysis in myocardial infarction 46 (atlas-timi 46) is important because a reduction of “stent thrombosis” with the addition of anticoagulant (anti-xa) was demonstrated in this trial.

scientifically, this finding is important because it is the first demonstration that oral anticoagulants have the potential to reduce “stent thrombosis.” however, it is also important to realize that this study, along with other similar studies, taught us that the risk of serious bleeding complication increases with the addition of anticoagulant to standard antiplatelet therapy.
Anti-GPIIb/IIIa Agents
Experience with the clinical development of GPIIb/IIIa antagonists brought a lot of important insights into platelet and thrombotic events. GPIIb/IIIa is known to be an exclusively platelet membrane protein that mediates platelet aggregation. With the use of anti-GPIIb/IIIa agents, platelet aggregation can be blocked. Many platelet specialists supposed a relationship between "platelet aggregation" and "thrombotic events." It is natural that many relevant people (not only scientists and physicians, but also people working for pharmaceutical industries) had a strong expectation for anti-GPIIb/IIIa agents to block platelet aggregation and prevent thrombotic events.

Initial experience with the intravenous GPIIb/IIIa antagonist, abciximab (Fab fragment of humanized anti-GPIIb/IIIa antibody), revealed that thrombocytic complications after coronary intervention could be prevented by this agent. Other intravenous GPIIb/IIIa inhibitors demonstrated similar efficacy. However, serious and unpredictable bleeding complications, such as alveolar hemorrhage, were also noted later. In Japan, anti-GPIIb/IIIa is not registered because a mid-sized Japan stand-alone trial did not show efficacy, but suggested harm in the abciximab arm. Widespread use of loading-dose clopidogrel therapy reduces the leeway for intravenous anti-GPIIb/IIIa agents, even in patients with ACS. A recent publication demonstrated that the use of anti-GPIIb/IIIa in patients with ACS was less than 50% and its use was an independent predictor for serious bleeding complications.

Future Research Opportunities in Platelet and Antiplatelet Therapy
Our knowledge of the role of platelets in the regulation of homeostasis and thrombus formation is still small. All of the currently available "antiplatelet" agents (even low-dose aspirin) increase the risk of serious bleeding events with the reduction of thrombotic events. The main reason for this limitation to the development of antiplatelet agents is our poor understanding of platelets in "hemostatic" and "pathological" thrombus formation. There are huge numbers of essentially important publications regarding the biological functions of platelets. However, quantitative understanding of the relationship of the molecules comprising platelets to their function is still to be elucidated. Platelets play their biological roles under complicated blood flow conditions, which makes predicting their intra-vital behavior difficult. Extensive accumulation of quantitative biological research data, together with information technology (eg, computer simulation) may be helpful for precise constructive understanding of the biological function of platelets. Multiscale simulations of the human body with super-computers may be helpful technology. This bottom-up strategy may also have ripple effects on fundamental developments of indwelling medical devices such as "stents" and "vascular grafts" based on physiological phenomena and computational prediction.

Conclusions
In conclusion, extensive research opportunities still exist for understanding the precise role of platelet cells in the regulation of vascular homeostasis.

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