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Platelet–vessel wall interactions and drug effects

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ABSTRACT

Platelet–vessel wall interaction is necessary for hemostasis and vascular repair, but also plays a fundamental role in the early and late development of atherosclerosis and atherothrombotic vascular events. A plethora of adhesion molecules, biological mediators and receptors are engaged in the regulation of platelet function in hemostasis and thrombosis. Currently available antiplatelet drugs act on targets that are critical for both, physiological hemostasis and pathological intravascular thrombosis. Consequently, their major disadvantage is bleeding complications, especially when different antiplatelet drugs are combined or applied together with anti-coagulants, such as in antithrombotic therapy of acute coronary syndromes. Aspirin, clopidogrel and GPIIb/IIIa antagonists are commonly used inhibitors of platelet aggregation or secretion. In addition, they modify platelet interactions with the vessel wall, which may contribute to or modulate their antithrombotic action. Some commonly used drugs without primary antiplatelet effects, such as heparins or statins, also appear to modify platelet interaction with the vessel wall. Present research on antithrombotic drug targets aims to identify new pharmacological concepts which more specifically address the pathophysiological mechanisms leading to intravascular thrombosis, thus intending to reduce interference with hemostasis.
This review article summarizes the biological and pathological mechanisms involved in thrombogenic platelet–vessel wall interaction, describes the current knowledge on the clinically available drugs in this field and gives an outlook on emerging concepts and innovative pharmacological compounds, which may improve efficacy and safety of antiplatelet therapy in the future.

1. Introduction

Platelets are about 1 μm large, disk-shaped particles in the blood stream which are crucial for hemostasis and thrombus formation. On average, a healthy adult human generates 10^{11} new blood platelets per day, which after leaving the bone marrow continue to circulate in the blood for about 10 days. Unlike erythrocytes or leucocytes, platelets are not intact cells but cell fragments, which possess cell membranes, mitochondria, a complex metabolism, numerous cell surface receptors and intracellular signaling pathways, allowing them to fulfill many biological functions in vascular biology. Platelets are anucleate but 'inherit' RNA from megakaryocytes, their precursor cells in bone marrow, enabling them to synthesize proteins. Whether platelets can be considered cells or not is therefore a matter of debate.

The Italian pathologist Giulio Bizzozero discovered platelets about 130 years ago and was the first to describe their adherence to the vessel wall and aggregation (Ribatti & Crivellato, 2007). Much later, in the 1970s, platelets were identified as major causative factors of acute coronary syndromes and myocardial infarction. This discovery led to dramatic improvements in the understanding of atherothrombotic disease and to fundamental advances in medical therapy. Above all, numerous drugs became available to prevent uncontrolled platelet activation, including aspirin, clopidogrel and many others. Though these act on distinct pharmacological targets, they are not specific with respect to their pharmacological response: all these compounds inhibit many platelet functions at the same time. Not surprisingly, a fundamental problem of the available antiplatelet therapy is the resulting increase of bleeding complications, often at a rate not far from the achieved reduction in cardiovascular events. This is the major limiting factor of all currently available antiplatelet therapies. Hence, the development of more potent antiplatelet drugs acting on conventional targets can be expected to achieve, at best, only small therapeutic improvements. The principal solution of the dilemma will likely be to identify more selective pharmacological targets within the

Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; COX, cyclooxygenase; GP, glycoprotein; NO, nitric oxide; NET, neutrophil extracellular traps; PAR, protease activated receptor; PSGL-1, P-selectin glycoprotein ligand-1; TTP, thrombotic thrombopenic purpurea; TX, thromboxane; vWF, von Willebrand Factor.

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complex interaction of platelets and the vessel wall, allowing prevention of pathological events driven by platelets while preserving their hemostatic action.

In the following, we will give a brief overview of biologically important mechanisms by which platelets interact with the vessel wall, how this contributes to the development of atherosclerosis, and how this may eventually precipitate acute atherothrombotic events such as myocardial infarction and stroke. Next, we summarize some of the knowledge on the ability of the presently available, approved drugs to alter platelet-vessel wall interaction. Finally, this review provides insight on new therapeutic concepts that are on the horizon and may, hopefully, extend and improve the pharmacological arsenal for prevention and treatment of atherothrombosis in the future.

2. Platelets — the small multi-talents: adhesion, aggregation and secretion

Vascular lesions are accompanied by injured endothelial cells and the exposure of extravascular tissue to platelets, which adhere to the vessel wall and ultimately form a hemostatic thrombus. The complex extracellular mediators, surface receptors, adhesion molecules and ligands converge in intracellular signaling that, in turn, triggers profound changes to the platelet surface. Further adhesion, aggregation, and release of storage granules lead to recruitment and activation of more platelets and finally culminate in the formation of a thrombus that also consists of immune cells and erythrocytes. Moreover, the accumulation of activated platelets next to a vascular lesion generates a matrix for the activation of the coagulation cascade (Fig. 1). Platelet activation and coagulation are counteracted by physiological inhibitors of platelet activation, such as endothelial cell-derived mediators (e.g., prostacyclin, nitric oxide). These prevent thrombus formation in the healthy vasculature, but are insufficient under pathological conditions (Smith et al., 2015; Neergaard-Petersen et al., 2016).

The contribution of platelets to the hemostatic response involves many interlaced processes (Fig. 1). Platelets initially adhere to the exposed adhesion proteins of the vessel wall. Among the most important are collagen and von Willebrand factor (vWF). Collagen, especially types I and III, is a major component of the subendothelial matrix. vWF is a large adhesive glycoprotein that forms multimers between 500–20,000 kDa and is present in blood, endothelial cells and platelets. Additional adhesion proteins are laminin, fibronectin, thrombospondin, and vitronectin (Broos et al., 2011). At high shear rate (arterial stenosis), vWF localizes towards sites of injury or is locally released by endothelial cells. It binds to subendothelial collagen via its A3 domain and exposes another specific binding site in its A1 domain, which allows binding to platelet glycoprotein GPIbα, a subunit of the GPIb/V/IX complex. This tethers platelets to the vascular wall. Due to the loose binding forces, platelets first keep 'rolling' on vWF in the direction of flowing blood. Thereafter, platelets attach to the vessel wall in a stable manner via binding of GPIa/IIa (integrin $\alpha_2\beta_1$) to collagen. P-selectin (see below) may also be involved in early platelet adhesion.

In the next step, adhesive proteins interact with platelet glycoprotein GPIIb/IIIa (integrin $\alpha_{IIb}\beta_3$), a highly abundant platelet protein located at the platelet surface and in platelet alpha granules. In an active (open) conformation, GPIIb/IIIa binds to fibrinogen, vWF, fibronectin, vitronectin and other ligands. This further enhances adhesion, allows for platelet 'spreading' and links platelets together (aggregation). These platelet integrins do not only attach platelets to the vessel wall but also serve as stimulatory receptors coupled to intracellular signaling cascades ('outside-in signaling'). Activation results in the release of platelet products and reorganization of the cytoskeleton. In addition, intracellular signal transduction also regulates integrin affinity to their binding counterparts ('inside-out signaling') (Lefkovits et al., 1995; Broos et al., 2011).

An important receptor involved in platelet–vessel wall interaction is GPVI, a member of the immunoglobulin family consisting of an Fc receptor gamma chain and an immunoreceptor tyrosine-based activation motif (ITAM) required for GPVI signal transduction (Broos et al., 2011). Upon stimulation by collagen, it mediates collagen-induced platelet activation via intracellular kinase cascades, resulting in the transactivation of platelet GPIa/IIa ($\alpha_2\beta_1$ integrin, VLA-2) and other integrins. GPVI is regulated secondary to integrin activation (GPIb/IX/V and GPIa/IIa). It is a crucial receptor in collagen-induced thrombus formation and is considered the most important platelet collagen receptor (Kuijpers et al., 2003; Versteeg et al., 2013).

P-selectin (CD62P) is an adhesive protein stored in α-granules of platelets and together with vWF in Weibel–Palade bodies in endothelial cells (Table 1). Its counter-receptor P-selectin glycoprotein ligand (PSGL-1) also occurs on platelets. P-selectin is released and integrated into the cell membranes upon platelet activation. P-selectin and PSGL-1 contribute to platelet rolling on the endothelium, where platelet GP Ib/IX/V appears to be a ligand interacting with endothelial P-selectin (Romo et al., 1999). Deficiency in P-selectin slightly inhibits hemostasis while increased P-selectin levels accelerate this process (Subramaniam et al., 1996; Andre et al., 2000). Importantly, platelet P-selectin mediates platelet binding to leukocytes during inflammatory responses, which in turn promotes coagulation by the induction of tissue factor expression on monocytes and macrophages (Siegel-Axel & Gawaz, 2007; Fuentes et al., 2013).

As mentioned, platelet activation involves several intracellular signaling pathways (Fig. 2). These include the activation of cytosolic phospholipase A_2 that releases arachidonic acid, which is converted to thromboxane (TX) A_2 by platelet cyclooxygenase (COX-1) and thromboxane synthase. TXA_2 is an important platelet feedback-activating autacoid, mediates vasoconstriction, migration and growth of smooth muscle cells, and activates platelet secretion of pro-thrombotic,

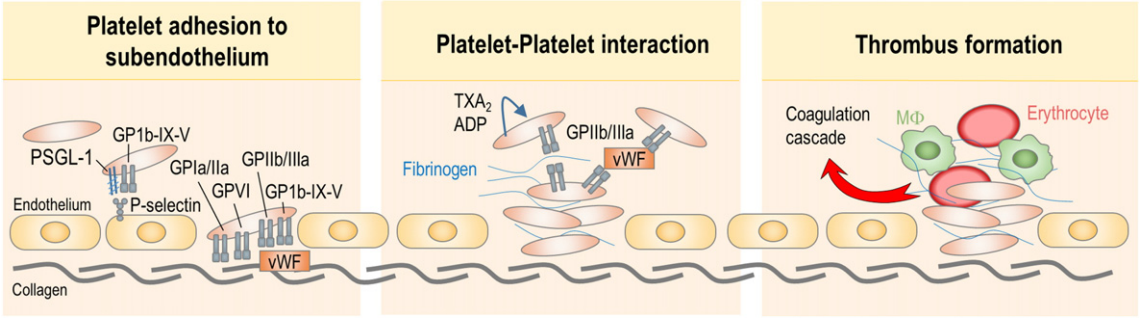

Fig. 1. Platelet–vessel wall adhesion and thrombus formation. Left: In a first step, platelets loosely adhere to the exposed subendothelium via interaction between the GP1b-IX-V and collagen-bound von-Willebrand Factor (vWF). Subsequently, adherence is enhanced by GPIa/IIa (integrin $\alpha_2\beta_1$) binding to subendothelial collagen and GPIIb/IIIa ($\alpha_{IIb}\beta_3$ integrin) binding to vWF. GPVI facilitates outside-in signaling. Platelet–endothelial cell interaction is mediated by platelet GPIb/IX/V and PSGL-1, which bind to P-selectin on endothelial cells. Middle: In a next step, more platelets are recruited and activated via e.g. GPIIb/IIIa interaction and release of autacoids, such as TXA_2 or ADP. Right: Finally, a compound thrombus is formed, which additionally consists of immune cells (e.g. macrophages, MΦ) and erythrocytes. Eventually, the coagulation cascade is activated. See Sections 2 and 3 for further description.