Transfusion and Apheresis Science 44 (2011) 305–319

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

In-vitro assessment of platelet function

Susanne M. Picker *

Transfusion Medicine, University Hospital of Cologne, Germany

ARTICLE INFO

Keywords:
Platelet function
Bleeding time
PFA-100
Platelet aggregometry
Shear induced platelet adhesion
Thrombelastography
Platelet metabolism
Platelet mitochondrial function
Platelet apoptosis

ABSTRACT

Platelets (PLTs), play a key role in hemostasis, clot stability and retraction as well as in vascular repair and anti-microbial host defense. Upon vessel wall damage, PLTs undergo a highly regulated set including adhesion, spreading, aggregation, release reactions as well as exposure of procoagulant surfaces to rapidly form a hemostatic plug that occludes the site of damage. When PLT function is impaired, the bleeding risk increases, but (hyperreactive) PLTs are also involved in many pathophysiological events like thrombosis, vessel constriction, atherogenesis, tumor growth and metastasis, inflammation including atherosclerosis and the subsequent formation of arterial thrombi resulting in stroke and myocardial infarction.

While hereditary PLT function disorders are very rare, acquired PLT function abnormalities occur in the course of many diseases and can be associated with many drugs, i.e., nonsteroidal anti-inflammatorics, antibiotics or heparin. Therefore, apart from disease diagnosis, severity, and prognosis, assessment of PLT function also serves for identifying the efficacy of anti-PLT therapy and PLT hyperfunction as a possible predictor for thromboembolic events. Since PLTs undergo a lot of measurable changes during storage ex-vivo, one effort of transfusion medicine is the quality monitoring of PLT concentrates (PCs), but also the detection of donors with PLT dysfunction and the determination of patients in which PLT transfusions are effective.

The majority of PLT tests focus only on PLT functions involved directly in hemostasis including adhesion/aggregation, coagulation, and clot retraction. Traditional tests, almost complex, time-consuming, and poorly specified, are meanwhile enriched by more user friendly and easy-to-use point-of-care tests on fully automated instruments within whole blood without the requirement of sample processing. These tests help identifying surgical patients at increased risk of post-operative bleeding or with resistance to anti-PLT therapy, therefore at increased risk of thromboembolism. However, up to now, no study shows real outcome benefits by including these tests into the disease management. To date, no function test is suitable to address all distinct steps of PLT activation or reliably predict PLT behavior in vivo following transfusion.

1. Normal platelet function

Human PLTs are anucleate, discoid small cells (2–4 μm by 0.5 μm) that normally circulate at concentrations of 150–400 $\times$ 10^9/L (higher in women than in men despite lower thrombopoietin levels [1]) for a maximum of 10 days. They are primed to undergo explosive liberation/activation following damage from the vessel wall. This leads to the rapid formation of a vascular plug to occlude the site of damage. PLTs are therefore enriched in signaling proteins and surface glycoprotein receptors (GP) that enable them to sufficiently respond to vessel wall injury.

Besides from lysosomes and endoplasmatic reticulum, PLTs contain nucleic acid remnants from megacaryocytes, from which they derived, and are rich in mitochondria

* Address: Transfusion Medicine, University Hospital of Cologne, Kerpener Str. 62, 50937 Cologne, Germany. Tel.: +49 221 478 4869; fax: +49 221 478 6179.

E-mail address: susanne.picker@uk-koeln.de

1473-0502/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.transci.2011.03.006

[2]. There are two major metabolic pathways to produce cellular energy in form of adenine nucleotides (adenosine-triphosphate ATP, adenine diphosphate ADP): the anaerobic glycolysis of the cytosol and the mitochondria-based oxidative metabolism via the tricarboxylic acid pathway that prefers substrates like fatty acids and acetate, rather than glucose. App. 60% of adenine nucleotides are stored within PLT dense bodies (storage pool). The remaining adenine nucleotides (metabolic pool) are present either free in the cytosol or bound to actin in mitochondria [3]. Unlike erythrocytes performing anaerobic glycolysis for energy delivery, PLTs mainly use the mitochondria-based, aerobic oxidative phosphorylation pathway. The latter is reported to contribute to 80% of total ATP in a stored resting PLT [4]. Oxidative phosphorylation is able to produce 19-fold more ATP than anaerobic glycolysis, which delivers a total net of two ATP and two lactate molecules per metabolized glucose molecule. Lactate molecules are released into the extracellular medium, where they lower pH levels after conversion to lactic acid. This could impair PLT viability during storage, particularly at pH values below international acceptance criteria (pH $\geqslant$6.2–6.4) [5,6].

PLTs contain also specific granules: apart from ADP storage dense bodies serve to store serotonin and calcium, while α-granules store coagulation factors like von-Willebrand factor vWF, multimerin, thrombospondin-1, fibrinogen, IgE, and PLT cytokines. The granular contents mediate host defense, recruitment and activation of adjacent PLTs and leukocytes as well as regulation of tissue repair [7]. Degranulation and secretion of granular contents in PCs may cause adverse events in the recipient [8–10].

The normal vascular endothelium produces potent PLT inhibitors such as nitric oxide (NO), prostacyclin and natural ADPase (CD39). However, once subendothelial components like collagen, fibronectin, laminin, or vWF become exposed upon vessel wall injury, PLTs undergo a highly regulated set of functional responses including adhesion, followed by spreading, release reactions (degranulation), aggregation, induction of procoagulant activity, microparticle formation, and clot retraction. Subsequently, PLTs are also involved in fibrinolysis and repair of the vessel wall.

Due to specific binding between subendothelial aggregating agents and specific PLT surface receptors (including GP Ia–IIa and GP VI for collagen binding, GP Ib for vWF binding, and GP IIb–IIIa for fibrinogen binding), PLTs begin to slow down and transiently adhere or roll along the damaged area of the vessel wall. Under conditions of high shear, as found in the arterial circulation, the initial PLT–subendothelium interactions are exclusively mediated by vWF present in bridges between collagen and GP Ib. The following steps of activation are then mediated by other receptor–ligand interactions such as direct binding of collagen to its specific receptors. At lower shear rates found in the venous circulation, initial adhesion can also occur directly to subendothelial matrix proteins such as collagen or fibrinogen. The collagen–GP VI interaction results in firm adhesion, formation of a PLT monolayer and also in PLT activation via the elevation of cytoplasmatic calcium levels leading to the induction of various signal transduction pathways (outside-in signaling). This causes cytoskeletal changes that mediate shape change from discoid to sphere, pseudopod formation, and conformational change of the fibrinogen receptor GP IIb–IIIa. Only the activated GP IIb–IIIa complex is able to bind soluble plasma fibrinogen (and under some conditions, vWF) leading to ultimate aggregation and further spreading of the stimulated PLTs along the site of injury (inside-out signaling). Aggregation is therefore critically dependent on G-protein coupled receptors and is mediated by bridges between fibrinogen/vWF (under high shear) and the activated GP IIb–IIIa complexes on adjacent, stimulated cells. Simultaneous release of granule components (e.g., ADP and serotonin from dense bodies, vWF and p-selectin from α-granules), which then become exposed on the PLT surface, and cyclooxygenase-related TXA_2 formation/expression result in further recruitment, activation and aggregation of other PLTs near to the growing hemostatic plug. finally, internal, anionic, negatively charged phospholipids are exposed by transbilayer flip flop of the inner membrane leaflet and procoagulant microvesicles are generated. The exposure of anionic phospholipids, mainly phosphatidylserine (PS), provides a surface upon which PLTs can support thrombin generation by accelerating the tenase and prothrombinase reactions of the plasmatic coagulation pathway. Thrombin, the key enzyme of the coagulation cascade and the most potent PLT agonist, acts by cleaving protease-activated surface receptors. The resulting thrombin burst leads to further activation of GP IIb–IIIa, formation of TXA_2, secretion of granule contents, therefore to further activation and local recruitment of PLTs into the vicinity and inclusion of leukocytes (via their receptors for p-selectin). Thrombin also converts soluble fibrinogen into insoluble fibrin, which is cross-linked by the thrombin-activated factor XIII to confer stability of the otherwise fragile hemostatic plug/thrombus. After the clot has been formed, the activated PLTs rearrange and contract their intracellular actin/myosin cytoskeleton, which leads to clot retraction.

2. Indications for testing platelet function

When there is a defect in any of the distinct activating functions including GP-ligand interaction, degranulation and secretion reactions, cyclooxygease dependent TXA_2 formation, procoagulative function and thrombin formation, adhesion and activation of PLTs at sides of vascular injury is compromized. As a result of the impaired hemostasis (inherited or acquired), the risk of bleeding increases, which can be diagnosed by prolonged skin bleeding, the presence of very large PLTs (macrothrombocytopenia), defective agonist-induced PLT aggregation, and reduced prothrombin consumption.

In contrast to acquired PLT function disorders that occur in the course of many diseases including uraemia, liver function and myeloproliferative disorders or autoimmune diseases with anti-PLT antibodies, and also in association with many drugs, hereditary PLT disorders are very rare. They can be classified in receptor abnormalities for (1) adhesive proteins (e.g., Bernard–Soulier syndrome), (2) for soluble agents (e.g., Glanzmann's thrombasthenia) or