High throughput assessment of platelet signaling, function and inhibition

Impact

Platelet activation is considered a cornerstone in the pathogenesis of cardiovascular diseases [1]. Under physiological conditions, such as upon injury, excessive blood loss is prevented by the activation of hemostatic mechanisms, including the formation of a platelet aggregate (thrombus). The coagulation cascade is also triggered, which causes the formation of fibrin (blood clot) as a vital protective role. On the other hand, undesired platelet activation due to atherosclerotic plaque damage or blood stasis can lead to intravascular thrombosis and impacts major health issues such as heart attack, stroke, cancer, and infection. Cardiovascular diseases are still the major causes of death worldwide, estimated to take almost 18 million lives each year [2]. Antiplatelet drugs are essential in the treatment of cardiovascular diseases. One of the main questions in the treatment of thrombosis is how to balance the anti-thrombotic benefits of antiplatelet drugs against the negative side effects, in particular an increased bleeding risk [3]. In addition, malfunction of the hemostasis system can lead to a bleeding disorder, which is often difficult to diagnose due to the high heterogeneity among affected patients and the lack of molecular information on the exact cause of the disease [4]. Current diagnostic tests often lack sensitivity for an accurate bleeding or thrombotic risk prediction, because these do not integrate the various processes involved in thrombus formation, especially lacking blood flow. Therefore, additional research is needed to measure platelet functions in physiological and pathological conditions, as well as to develop advanced methods to find novel antiplatelet drugs. Such efforts can improve the current cardiovascular medicine and result in a more optimal diagnosis and treatment of patients.

The Horizon 2020 TAPAS consortium is a European Joint Doctorate program (No. 76118), which stands for Targeting Platelet Adhesion Receptors in Thrombosis. TAPAS aims to develop new expertise to identify, understand and test new molecular targets on blood platelets for the selective prevention and treatment of thrombotic diseases. TAPAS focuses on the research of two receptors on platelets, glycoprotein (GP)VI and CLEC-2, both of which play a major role in platelet-related thrombotic disorders and a lesser role in hemostasis. As an early-stage researcher of this consortium, my thesis focuses on the platelet collagen receptor GPVI, defining in particular how its activation mechanism differs from the stimulation of platelets by the coagulation protein thrombin, acting via protease-activated receptors (PARs) (**Chapter 2**). During this research, I focused on these two receptor signaling pathways to develop new high throughput methods that can be used for small molecule screening purposes, aiming to find novel inhibitors (**Chapters 3-5**).

Collagen as one of the major components of the extracellular matrix is a potent activator of platelets acting through interaction with the immunoglobulin-type receptor GPVI. GPVI is considered to be a promising antithrombotic drug target due to its restricted expression pattern in platelets and megakaryocytes and its relatively minor role in hemostasis. Yet, it is important to better understand the basic biology behind GPVI signaling and platelet activation in order to develop novel approaches to modulate GPVI receptor function. Another platelet receptor, GPIb, promotes platelet adhesion to collagen under shear conditions via collagen-bound von Willebrand factor (VWF). In addition, G-protein coupled receptors (GPCRs) for soluble platelet agonists are major contributors to platelet activation.

Currently, the majority of anti-platelet drugs target the latter receptors. This concerns the clinically used $P2Y_{12}$ receptor antagonists (clopidogrel, prasugrel, ticagrelor), the cyclooxygenase inhibitor aspirin preventing thromboxane receptor TP activation, and thrombin receptor PAR1 antagonists (vorapaxar). All these drugs have limitations such as an increased risk of bleeding, despite a clear reduction of secondary thrombotic events. In addition, there are partly genetically determined different interpatient responses linked to an incomplete mode of action. Here, I intended to find new small molecules (**Chapter 5**) or peptides (**Chapter 7**) that interfere with the platelet activation pathways and receptors mentioned above for the development of improved candidate drugs.

The diagnosis of patients with a (mild) bleeding phenotype remains a difficult issue. Bleeding symptoms can have various underlying causes, which in most cases remain unclear even when extensive genetic sequencing has been performed [5]. Current platelet function tests appear not to always pick up a likely platelet-related bleeding disorder. Therefore, in order to gain more insight into the phenotype of patients with a familial history of bleeding, we used a high-throughput whole blood microfluidic assay (**Chapter 8**). This microfluidic assay is performed under conditions of shear stress mimicking the physiology of arteries or veins and promises to be of added value to assess platelet responsiveness and functions in a clinical setting. Moreover, the use of multiple thrombogenic surfaces, mimicking the components present in the vessel wall, allows us to distinguish several processes of platelet activation and thrombus formation, to better characterize platelet defects in patients. Further research is still needed and modifications should be implemented. These include the use of physiological temperature, studying bigger cohorts of patients, establishing reference ranges of healthy controls, and the generation of prediction models to simplify the diagnostic approach. Nevertheless, the results of this thesis emphasize that whole blood flow measurements are valuable in clinical settings with patients with a familial history of bleeding and assumed abnormal platelet functions. The microfluidic assays are also relevant for basic research as a proxy measurement of thrombotic or hemostatic disturbances, when studying specific signaling proteins or molecular inhibitors to further understand their effects. I have studied all this in the context of thrombosis (**Chapters 3, 5**), when evaluating the blood from patients with mutations associated with a bleeding disorder (**Chapter 6**), or aiming to develop agents against thrombotic thrombocytopenia (**Chapter 7**). Taken together, the results obtained during this thesis have provided additional knowledge on method development in thrombosis and hemostasis, drug identification using platelet function testing, and the unraveling of defective platelet functions in patients with a bleeding disorder.

References

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