Transient and persistent aspects of human platelet activation

Impact

Cardiovascular diseases still are a leading cause of death worldwide, and costed the European Union economy €282 billion in 2021 [1]. Platelets, the smallest blood cells, play a major role in arterial thrombosis as well as in physiological hemostasis. The use of antiplatelet therapy has become an essential component of cardiovascular disease treatment. While the inhibition of platelet responsiveness has decreased the occurrence of ischemic events, it also led to side effects of bleeding, thus perpetuating the therapeutic challenge of preventing thrombosis whilst preserving hemostasis. The future of antiplatelet therapy depends on finding new drugs that target specific platelet functions, based on better methods for assessing the risks of bleeding and thrombosis-induced ischemia [2]. The platelet integrin αIIbβ3, the collagen receptor GPVI, the thrombin receptors PAR1/4, and the ADP receptors $P2Y_{1/12}$ are all suitable targets. In this thesis, I focus on a better understanding of transient platelet activation pathways by investigating how platelets can respond to different agonists and inhibitors in a time-dependent way.

Integrin αIIbβ3, expressed at high level, stays in an inactive conformation on resting platelets. After platelet stimulation, it converts due to an inside-out signal from the low-affinity state into a high-affinity state, able to bind fibrinogen and other ligands. The ligand binding also promotes clustering of integrin molecules and, in turn, triggers the process of outside-in signaling [3]. This signaling cascade sets into motion a variety of cellular processes, crucial for platelet functions like spreading, aggregation, clot retraction, and the consolidation of thrombus formation. Thus, the integrin αIIbβ3-related signaling pathways are regarded as potential points for antithrombotic treatment. In Chapters 2-3, our objective was to enhance current understanding of the reversibility of integrin αIIbβ3 activation.

In Chapter 3, we showed that platelet stimulation via GPVI or PAR1 caused transient integrin αIIbβ3 activation, while stimulation via PAR4 led to permanent αIIbβ3 activation. We also confirmed that the protein kinase C pathway is crucial for integrin αIIbβ3 activation, and that autocrine responses via $P2Y_{12}$ are essential for a sustained integrin αIIbβ3 opening. Flow cytometry was employed in this chapter, which method can also be used for clinic testing, as it can rapidly analyze thousands of cells in complex mixtures per second. By detecting the degree of integrin activation, it assesses whether platelets are activated or not. Flow cytometry can also assist in determining the clinical dosage of drugs, and thereby improve the risk prediction of thrombosis and bleeding.

In various clinical situations, it can be needed to quickly assess the hemostasis process, for instance in patients with acute bleeding symptoms. In Chapter 4, we tested several agonists that rapidly trigger platelet aggregation. Our findings suggest that it is still effective to administer antiplatelet drugs, while aggregation is already occurring. However, when compared to PAR activation, GPVI activation shows a more restricted time frame for interference in the

aggregation process. This may be helpful in clinical treatment of thrombosis with suspected roles of different receptors.

In Chapters 5-6, we used a high throughput method to assess the GPVI and PAR-induced Ca^{2+} mobilization in platelets. In Chapter 5, we find that this response with weaker agonists relied more on secondary mediators, when compared to stronger agonists like collagen-related peptide and thrombin. Our findings also provide novel quantitative insight into the importance of Ca^{2+} entry for platelet activation. We confirmed that Na^{+}/Ca^{2+} exchangers and Orai1 channels are crucial in this process, thereby building upon earlier reports [4]. In the next Chapter 6, we investigated the roles of different protein kinase C (PKC) isoforms in the regulation of Ca^{2+} entry. We showed that overall PKC activation via GPVI led to a robust inhibitory effect on the entry through Orai1 channels. In addition, this inhibition also suppressed platelet procoagulant activity and the coagulation process. Notably, we discovered that PKC-dependent phosphorylation of the signaling proteins BIN2 and STIM1 associates with a decreased Orai1 Ca^{2+}-channel activity. Indeed, PKC isoforms served as potent negative regulators of the process of store-operated Ca^{2+} entry. These findings offer fresh perspectives on the exploration and creation of novel drugs aimed at modulating these molecular pathways.

In the last experimental Chapter 7, we describe a method for obtaining calibrated thrombin generation curves in 96-well plates, thereby providing the first comprehensive quantitative comparison of this coagulation process in whole blood and platelet-rich plasma from the same subject. Our results indicated that red blood cells play an initial role in the thrombin generation, whilst activated platelets later support this process. Thrombin generation accelerated as the hematocrit level increased, and delayed when the erythrocytes were treated with annexin A5, which blocks phosphatidylserine. These findings on red blood cell properties are valuable for assessing the risk of bleeding or thrombosis in a clinical setting.

Overall, this thesis elaborates how transient activation via GPVI and PARs is a major response function of platelets, and thereby likely is relevant for thrombosis and hemostasis. In addition, the thesis provides new insights into the regulation of platelet Ca^{2+} responses and thrombin generation. My expectation is that these discoveries will help to drive the development of antiplatelet medications and to enhance the clinical assessments of thrombosis and bleeding risks.

References

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