Interplay of platelets, chemokines, and extracellular vesicles in the propagation of vascular inflammation

IMPACT

Platelets are vital blood components and despite their small size, control haemostasis and immune and inflammatory responses[1,2]. Platelets are able to perform immune actions by themselves or by the release of their bioactive modulators like chemokines or extracellular vesicles (EVs)[3,4]. These modulators are biologic messengers and transfer infor-mation from one cell to the other. In this way chemokines and EVs are able to control immune responses and support the clearance of injured tissue or unwanted intruders to initiate the healing process. This is a delicate balance and when disturbed, it can lead to excessive in-flammatory responses with devastating consequences such as transfusion related lung injury (TRALI) or cardiovascular diseases (CVDs) like a heart attack or stroke[5,6].

In this thesis, I focused on the communication between platelets and platelet derived modulators with the vasculature under inflammatory conditions. In **chapter 3**, the release of bioactive modulators through EVs after stimulation of platelets was investigated. We have found three main events in platelet activation that are responsible for EV release. These events are i) activation and subsequently deactivation of platelet integrin, ii) exposure of the pro-coagulant membrane phospholipid phosphatidyl serine, and iii) formation of filopodia's, which gives the platelet a spikey appearance. If one of these events is blocked, EV release is diminished. The consequences of circulating EVs were investigated in **chapter 4**. Here we found that interactions between EVs and endothelial cells stimulated intercellular calcium signalling, which is a central junction for the regulation of numerous functions of the endothelial cells. Under inflammatory conditions circulating EVs from platelets are able to upregulate the attraction of white blood cells and these cells are more prone to bind onto the endotheli-um thereby enhancing inflammatory responses and tissue damage leading to progression of atherosclerosis. Further analysis of the signalling pathways in platelets that regulate the three main events for EV release, identified in this thesis, could provide new insights in how to regulate this EV release. This could provide possibilities to therapeutically induce EV release to invoke the repair mechanisms after tissue injury. This, combined with the knowledge found in EV uptake by endothelial cells, could also prove useful in other scientific fields, for example the use of EVs for targeted drug delivery for example in the treatment of cancer.

Atherosclerosis, more commonly known as "hardening of the arteries", is an age related chronic inflammatory disease which is the underlying cause of many CVDs. According to the world health organisation about 18 million people die of CVDs each year[7]. CVDs is the leading cause of death and disability worldwide. Unfortunately, early detection

of fatty deposits (atherosclerotic plaques) in the vascular wall that could ultimately lead to cardiovascular event is not feasible at this time. Platelets are implicated to play a role in the process of atherosclerosis development[8]. Treatment of patients that suffered from a cardiovascular event depends on which type of event has occurred and if the patient has additional health problems like diabetes, obesity, or hypertension. Treatment ranges from lifestyle changes such as healthy diet, more exercise or to quit smoking, to different kinds of medications such as lowering of cholesterol, anti-coagulants, or anti-platelet drugs. In **chapter 5** and **6**, several anti-platelet medications are evaluated for their anti-inflammatory properties. One of the main findings was that phosphodiesterase -3 and -5 inhibitors (cilostazol and tadalafil, respectively), reduced the direct interaction of platelets with inflamed endothelial and that combination therapy with aspirin reduced the indirect platelet interactions by reducing the release of inflammatory modulators such as chemokines from platelets. Understanding the complexity of the different signalling pathways that regulate the responses of platelets may take years and research by different scientific groups is still ongoing. Presenting at conferences and publishing the results after being peer reviewed is important to share our knowledge with other groups, the clinic and pharmaceutical companies. The findings described in this thesis of anti-platelet drugs are important for clinicians who treat patients with the investigated medications, increasing the awareness that these medications do not only interfere with platelets blood clotting functions but also with the immune functions of platelets. Further investigation to tailor antiplatelet drugs to interfere only on one function is needed and collaborations between the pharmaceutical industry (for drug development and production), clinic (clinical trials), and scientists (fundamental research) is vital.

In nature, inflammatory modulators, *e.g.*, chemokines, are targeted by parasites to survive and remain undetected by the host immune system while feeding. Investigation of the saliva from the brown dog tick revealed proteins that could inhibit modulators that regulate the immune responses[9,10]. In **chapter 7**, the tick-derived peptides, Evasin-3 and -4, were investigated for their ability to inhibit white blood cell attraction by platelet-derived chemokines. Furthermore, these proteins can be made in the laboratory and can be modified by adding a fluorescent or radioactive probe without compromising their ability to bind to chemokines and interfere with the attraction of white blood cells. Since chemokines might be enriched at sites of developing plaques, this could be useful in the diagnosis of the early onset of atherosclerosis. There is evidence that nature can provide blueprints for the

development of new diagnostic tools for the identification and prevention of cardiovascular events. By locating vascular inflammation, the early onset of potentially dangerous plaques could be prevented and could thereby lower the risk on cardiovascular events.

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