Platelet activation and microparticles in the pericardial cavity during cardiopulmonary bypass
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Summary

As described in the Introduction of this thesis, cardiovascular disease remains the leading cause of death in The Netherlands. Cardiac surgery is frequently performed to improve the condition of the patient suffering from cardiac dysfunction due to coronary heart disease, heart valve malfunction, etc. One of the problems of the use of the cardiopulmonary bypass (CPB) during cardiac surgery is the activation of blood, leading to an inflammatory syndrome and blood loss. Originally, CPB circuit was presumed to be the main cause of the activation of blood cells and protein cascades, such as the coagulation and complement systems. Recently, interest has grown in the activation processes occurring in the pericardial cavity, i.e., the surgical wound field. This so-called pericardial blood can play a role in systemic blood, because it is returned into the patient during the operation procedure to limit blood loss.

The aim of this thesis is to investigate platelet activation with associated microparticle formation in pericardial blood, as well as the possible procoagulant and prothrombotic role of these microparticles.

Glycoprotein Ib (GPIb) is one of the main adhesion molecules on the platelet surface. Upon platelet activation this molecule partly disappears from the platelet surface by moving into the open canalicular system, which are channels of the cell membrane tunnelling deeply into the platelet. Several investigators have already reported the disappearance of GPIb from the platelet surface in systemic blood. They postulated this as a possible cause of platelet dysfunction with subsequently excessive intra-operative and postoperative blood loss. However, the situation in pericardial blood is unknown. In the study described in chapter 2, blood was collected at various time intervals during the operation, both from the systemic circulation as well as directly from the pericardial cavity. Evidence was gathered that 10 – 30% of GPIb disappears from the platelet surface in systemic blood, and up to 50% from platelets in pericardial blood. No other evidence was found for platelet activation in pericardial blood, such as a lack of surface appearance of GP53 (indicates lysosomal secretion) or P-selectin (marker for α-granule secretion), or increased binding of PAC-1 (marker for GPIIb-IIIa activation). This indicated the absence of a more general platelet activation in pericardial blood, although GPIb disappearance was pronounced.

In the study presented in chapter 2, flow cytometry was used to establish the
activation status and GPIb exposure of platelets. In this study it was noticed that material with a size smaller than platelets was present especially in pericardial blood. These were microparticles derived from platelets, as it stained with platelet antibodies. From pericardial blood microparticles were isolated and further characterised (chapter 3).

These microparticles were derived from platelets, erythrocytes, and to a lesser extent from monocytes. They generated thrombin in an in vitro thrombin generation assay via the TF and factor VII-dependent pathway and independent of factor XII pathway. Microparticles from systemic blood also generated thrombin, but to a lesser extent than pericardial microparticles.

Aprotinin is frequently used in cardiac surgery to reduce postoperative blood loss and thus the requirement for blood transfusions. Its working mechanism is still unclear, although a GPIb preserving effect has been described. Aprotinin is applied either solely in the priming fluid of the CPB circuit, or in the priming circuit combined with a continuous intravenous infusion. In the previous chapters, especially in pericardial blood, an extensive disappearance of GPIb from the platelet surface as well as increased production of microparticles was observed. In the double-blind randomised study described in chapter 4, the effect of per-operative local administration of aprotinin in the pericardial cavity on these processes was investigated. An effect of aprotinin, however, on these platelet responses was not observed in pericardial blood nor systemic blood. The topical use of aprotinin, however, may still be useful if one considers its effect on fibrinolysis.

Chapter 4a is a Letter to the Editor by Landis and Taylor in response to the study presented in chapter 4. These authors questioned whether an effect of topical aprotinin administration on platelet activation may be anticipated. They argued that aprotinin prevents platelet activation by thrombin via the PAR-1 and PAR-4 receptors, but not platelet activation by ADP, collagen or epinephrine. They stated that in pericardial blood the concentration of ADP is increased and subendothelial collagen is exposed. On the other hand, in systemic blood thrombin will mainly be formed by contact with the CPB circuit. They therefore proposed systemic administration of aprotinin. In chapter 4b we reply with three arguments that the topical administration of aprotinin should not be dismissed a priori. First, the contribution of blood activation by the CPB circuit to thrombin generation is questionable, considering the much higher thrombin concentration.
Summary

in pericardial blood than in systemic blood. Secondly, studies on the inhibition of thrombin-induced platelet activation by aprotinin, but not activation by ADP or collagen, were performed in vitro and with washed platelets, and are therefore not easily extended to the in vivo situation. Here, agonists will simultaneously stimulate the platelet and in a synergistic process activate the platelet to a larger extent than each individual agonist. Finally, plasmin, which activates platelets and is present at high concentrations in the pericardial blood, is also inhibited by aprotinin.

The presence of increased concentrations of soluble, non-cell bound, TF in pericardial blood was recently reported. However, it was unknown whether this TF is capable of thrombin generation. In combination with our previous findings we questioned whether this non-cell bound TF is associated with microparticles, because TF is only thrombogenic when it is membrane associated. In chapter 5 it is shown that non-cell bound TF in pericardial blood is 45 – 77% microparticle-associated. The microparticles initiated thrombin generation in vitro through the TF-factor VII pathway, whereas the non-microparticle associated, the so-called fluid-phase, form of non-cell bound TF did not generate thrombin. So we observed two forms of non-cell bound TF, one form which is microparticle-associated and supports thrombin generation via factor VII. The other form, which is fluid-phase, does not stimulate thrombin generation. The cellular source of the TF-bearing microparticles could not be established because the detergent used to visualise TF on microparticles prohibited the detection of other antigens such as those of platelets and erythrocytes. Microparticles from systemic blood were unable to generate thrombin through the TF-factor VII pathway, except at the end of the bypass procedure. This suggested that microparticles from pericardial blood upon their return into the patient remain active and thus pro-coagulant in the systemic circulation.

Chapter 6 shows that in the in vivo generated human cell-derived microparticles, isolated from pericardial blood, are highly thrombogenic in a venous thrombosis model in rats. This thrombus formation was initiated by TF present on the microparticles. In fact, evidence is provided that the extent of TF exposure determines their thrombogenicity in vivo.

In summary, the studies in this thesis demonstrate extensive coagulation activation and microparticle formation in blood collected in the pericardial cavity during
cardiac surgery with the use of extracorporeal circulation. The two processes even appear to be interlinked as thrombin may activate platelets and other cells, leading to formation of microparticles. The microparticles will in return stimulate thrombin formation via TF on their surface. The activated pericardial blood may therefore be responsible for some of the adverse effects associated with CPB, because it is reinjected into the patient during the operation to limit blood loss. It may especially contribute to thrombotic events after cardiac surgery, such as early graft occlusion, cerebrovascular accidents or (clinically silent) deep vein thrombosis. Further studies should address the question whether re-administration of activated pericardial blood to the patient leads to systemic hypercoagulation. Furthermore, the effectiveness of cell savers to remove procoagulant microparticles should be investigated.