Catch FXIa

Impact paragraph

FXIa is a member of the intrinsic route of the coagulation cascade that has gained great interest over the past 10 years in the field of thrombosis and hemostasis. Inhibition of FXIa in patients that received total knee-replacement resulted in a decreased risk of thrombosis after surgery. In contrast, the admission of FXIa-contaminated intravenous gamma globulin (IVGG) packs to patients increased the risk on thrombotic events. These phenotypes originating from variations in FXIa levels suggest that FXIa is an important player in thrombus formation.

The main goal of this thesis was to develop a FXIa quantification assay that can be used in complex mixtures such as IVGG-packs and plasma. Such an assay would allow researchers to obtain more information about the effect of variation in FXIa-levels. Studies have shown that patients suffering from chronic artery disease or acute myocardial infarction have significantly increased FXIa-levels, thereby again suggesting that FXIa plays a role in thrombosis. Currently thrombosis is detected by a D-dimer test, which shows elevated concentrations when a thrombus has already been formed and subsequently been degraded, while elevated FXIa concentrations is suggested to cause thrombosis. By using elevated FXIa concentrations as a potential biomarker for thrombosis, medical doctors get a better view on patients' procoagulant status, which allows them to start antithrombotic medication at an earlier stage preventing thrombosis and reduce overall healthcare costs. Additionally, quantification of FXIa in circulation is important for the development of new anti-thrombotic medication. Nowadays, big pharma companies focus on the inhibition of FXIa as a new and safe anticoagulant therapy. Additionally, to gain more insight in the potency and safety of the drug, close monitoring of patients in clinical studies is critical. Currently, global coagulation assays are used to monitor the pro-coagulant state of these patients upon admission of the drug; however, direct quantification of FXIa activity could give more specific information on the inhibition of FXIa in circulation itself. Chapter 3 describes a proof-of-concept of an innovative catch-and-release assay to quantify FXIa. The assay is based on a two-step mechanism that first binds FXIa, and subsequently releases it in a buffered environment. In contrast to other FXIa assays, this set-up is independent of serpin inhibition, making it more reliable for clinical use, and will allow quantification of FXIa in a large group of patients. Apart from its clinical benefits, the catch-and-release mechanism is an innovative concept that could also be used to isolate other dimeric proteins from complex matrixes, ultimately allowing quantification. For example, the exchange of Fasxiator into a specific binder for a

different homodimeric protein such as 14-3-3, could result in the isolation of various homodimeric proteins from plasma. Furthermore, the development of a heterogeneous inhibitory construct could result in a quantification assay of a monomeric protein with multiple allosteric binding sites.

After a successful proof-of-concept, multiple studies were set up to optimize the catch-and-release concept. In chapter 5, peptide nucleic acids (PNA) were studied for their ability to form a dynamic multivalent construct. Unlike the construct from the proof-of-concept study, PNA's are not affected by plasma components. Therefore, development of a PNA based catch-and-release construct would allow addition of the construct directly to the blood-collection tube, where it could directly bind and protect FXIa from serpin inhibition. This concept would make the assay more straightforward to use in the clinic and less prone to external influences.

Finally, a novel reversal strategy was studied. Coupling of steric bulk to Fasxiator hindered the interaction with FXIa, and thereby resulted in an *off*-version of Fasxiator. The reversal strategy was studied to optimize the catch-and-release assay, but the development of an *off*-switch for inhibitors could be an interesting concept in multiple fields of research. Such a concept would allow the isolation of non-dimeric proteins from plasma by catching the protein *via* the *on*-state of the inhibitor, and an interesting strategy to study the effect of a specific inhibitors (e.g. novel anticoagulants) in biophysical and biological experiments.

Overall, the development of an FXIa-quantification assay will result in a better understanding, and treatment of thrombosis. Patients who are at risk of thrombosis could start anti-thrombotic medication at an earlier stage which will result in less hospital admissions reduce overall health costs, and an increase the quality of life for those at risk of thrombosis.