Unraveling the role of factor XI and plasma prekallikrein in coagulation

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Reflecting on the scientific work and establishing a connection between the results and the significance for the scientific and economic society have gained in importance. However, it is often challenging especially for a basic research scientist to clearly identify a direct link between the scientific outcomes and the social and economic impact. In contrast, translational research deals with the applicability of scientific findings to concrete, practice-relevant approaches that may have a more obvious impact on industrial and clinical applications.

This thesis combines work from basic research as well as from research with a more translational perspective to unravel the role of the contact activation pathway, particularly of FXI and PK, in coagulation and to investigate their role in thrombotic diseases.

Thrombosis as the underlying event of myocardial infarction, ischemic stroke and venous thromboembolism is still the leading cause of mortality worldwide.[1] Coagulation involves various cellular and pro- and antithrombotic factors, which, if unbalanced, may cause thrombotic complications or severe bleedings. Scientific research has made great efforts and progress in the treatment of thrombosis in recent years. The clinical application of anticoagulants was initially dominated by vitamin K antagonists, which were largely replaced by the introduction of direct oral anticoagulants (DOACs).[2] While this has improved the treatment of thrombotic disease in patients, the general risk of major bleeding of about 2%/year, remained. Particularly in an aging population both the risks of thrombosis and bleeding increase, which makes long term anticoagulation a major challenge. In recent years interest in the factors of the contact activation system of coagulation as possible targets for the treatment of thrombosis has increased, since they were shown to play a role in thrombosis while having only little or no impact on hemostasis.[3, 4]

Chapter 2 elaborates on the role of the contact activation system, in particular of FXI and PK, in arterial and venous thrombosis. The clinical applicability of FXI(a) inhibitors or FXI antisense oligonucleotides is promising, since inhibiting FXI reduced thrombus formation in animal studies without increasing the risk of bleeding.[5] The impact of PK on thrombosis is not quite as clear. While some data from preclinical studies suggest that PK(a) could be a valuable target for the treatment of thrombosis, there is a lack of clinical studies to support this. Given its role as initiator of the kallikrein-kinin pathway that regulates inflammatory processes, inhibition of PK(a) may, however, be a useful approach to treat thrombo-inflammation.[6]

The well-known role of PKa, besides triggering the kallikrein-kinin pathway, is the reciprocal activation of FXII, a component of the contact activation pathway, to

FXIIa which in turn activates FXI.[7] In **chapter 3** it was demonstrated that PKa contributes to intrinsic coagulation in an alternative pathway by directly activating FIX. This discovery could lead to a revised model of the coagulation cascade in which FIX could be considered as the key enzyme that merges the intrinsic and extrinsic in the common pathway. Furthermore, this could draw attention to PKa, which was previously viewed as an initiator of inflammatory processes rather than a component of the coagulation cascade, as a possible target in the treatment of thrombosis. In order to interpret the physiological role of this alternative pathway, further studies need to be conducted. However, the finding that FIX can be activated not only by FXI but also by PKa could help to reveal the reason for the variation in bleeding phenotypes of FXI-deficient individuals. Since the bleeding phenotype in FXI-deficient individuals does not necessarily correlate with the FXI level, it is difficult to predict future bleeding complications, which occur most likely after surgery or trauma.[8] In **Chapter 4** variations in PKa:C1inh complex formation and PKa activity were found among the analyzed plasma samples upon initiation of the contact activation pathway. Highest PKa:C1inh complexes and PKa activity were most likely observed in FXI-deficient individuals indicating a greater role for PKa in coagulation in these individuals. Due to the ability of PKa directly activating FIX and thus contributing to intrinsic coagulation, variations in PKa activity could explain the cause for the different bleeding phenotypes. Ideally, testing the PKa activity may help to predict potential trauma related bleeding complications. Whether fluctuations in PKa activity can indeed be associated with the bleeding phenotype in FXI-deficient individuals requires further investigation.

Biomarker research is valuable for clinical applications as the obtained parameters allow a direct interpretation of a patient's disease status and thus enable rapid treatment. To address this, the development of methods to determine suitable biomarkers for a particular disease is required. Throughout this thesis, enzyme:inhibitor complex ELISAs were used to gain insights in the coagulation status of plasma from healthy donors and different patient cohorts. In **Chapter 5**, the aim was to assess the extent of FXI and PK activation in patients with acute VTE. Elevated PKa:C1inh complex levels were determined in patients as compared with controls and increased FXIa:C1inh complex formation was associated with an increased risk of mortality. These results indicate a contributing role of FXI and PK to VTE and points towards novel treatment approaches for thrombotic diseases.

Chapter 6 is another example of how biomarker research can be used to gain further insights into a disease, in this case the globally challenging disease COVID-19, and to identify potential targets for improved therapy. Elevated levels of activated coagulation factors in complex with their natural inhibitors and increased complement

and neutrophil activation were determined in patients with COVID-19 infection indicating a close interplay between neutrophils, coagulation and complement. Inhibitors of components of the contact activation system or of C5a of the complement system could find clinical use to reduce or prevent severe complications caused by COVID-19 infection.

Taken together the finding that PKa directly activates FIX may prompt scientists to look at the coagulation cascade from a different angle and may provide novel treatment options in thrombosis. In addition, it may help to explain the cause of the variations in the bleeding manifestations in FXI-deficient individuals that have been shown to have variations in PKa activity upon contact activation. The finding that PKa:C1inh complexes are elevated in patients with acute VTE and that FXIa:C1inh complexes are associated with the clinical outcome of death, may lead to novel approaches for the treatment of acute VTE. Moreover, the cause of severe complications in COVID-19 infection suggests an interplay between neutrophils, coagulation and complement which may be valuable targets to improve the treatment of the disease.

From a treatment perspective, the availability of anticoagulants with preserved antithrombotic activity but reduced bleeding risk, such as might be the case with inhibitors of FXIa and/or PKa, would be another step forward for society. Currently several clinical trials with such inhibitors are ongoing.[4, 9] In translational terms, the identification of the involvement of specific procoagulant elements in human disease, like in Covid-19, VTE or other cardiovascular diseases, is a crucial process pointing to targets for intervention that were previously unknown. Hence, for long term treatment of aging patients with (recurrent) VTE, in addition to FXI(a) inhibitors the use of a PKa inhibitor may become an alternative for a DOAC (aimed at FXa or thrombin), to further reduce bleeding risk. Alternatively, in settings with a high atherothrombosis risk, but a similarly increased bleeding risk, a combination of PKa inhibitor and aspirin may provide a safer combination therapy as compared to currently registered combination of rivaroxaban and aspirin. Since safer platelet inhibitors are also being developed, the array of antithrombotic agents and possible combinations of agents further increases. As it becomes impossible to study all such combinations in dedicated clinical trials, new strategies will be required to use biomarkers for risk stratification and to address benefit/risk ratios in other trial designs than the thus far obligatory randomized trials requiring thousands of subjects.

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