The extrinsic coagulation pathway in coronary artery disease and endotoxemia
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Chapter I

General introduction and outline of the thesis
Introduction
Coronary atherogenesis
Coronary atherosclerotic disease is a major cause of morbidity and mortality worldwide. The pathogenesis of atherosclerosis is complex and many different factors are involved. The initial event is characterized by the presence of monocytes/macrophages within the arterial intimal layer (type I lesion according to Stary et al., Figure 1). These lesions develop into raised fatty streaks that contain the typical foam cells, i.e. monocyte-derived macrophages enriched with lipids or free cholesterol.

Figure 1. Drawing of cross-sections showing sequence of atherosclerotic lesion types from Stary type I to Stary type VI (reproduced from Stary et al., with permission).
This is followed by the development of finely dispersed extracellular lipid (Stary type II and III lesions). In a more advanced stage, this extracellular lipid forms a central core with displacement of normal cells and extracellular matrix, the so-called atheroma (Stary type IV lesion) that is surrounded by a fibrous cap (Stary type V lesion). A progressively thinner cap with decreasing collagen content, inflammation within the cap, and disturbances and changes in flow and shear stress, make the fibrous cape more vulnerable to rupture, in particular at the shoulders of the lesion. This may lead to arterial thrombus formation (Stary type VI lesion). Plaque disruption with superimposed thrombosis is the main cause of acute coronary syndromes such as unstable angina, myocardial infarction and sudden cardiac death. The content of the plaque determines its thrombogenicity and tissue factor (TF), either synthesized by macrophages or from other sources, is thought to play a pivotal role.

The extrinsic coagulation pathway and ischemic coronary artery diseases

Tissue factor is a transmembrane glycoprotein that is normally not exposed to the circulation. In the vascular system, tissue factor is predominantly located in the adventitia. At sites of vascular injury, TF fulfils its hemostatic role by initiating the extrinsic blood coagulation after binding to circulating factor VII. The complex TF/factor VIIa subsequently activates factor X and, at a slower rate, factor IX. Activated factor IX, in the presence of its co-factor factor VIII, cleaves additional factor X to factor Xa. Factor Xa leads to the generation of thrombin, which catalyzes the formation of fibrin. Polymerization and cross-linking of fibrin contribute to the formation of a stable arterial clot. Tissue factor pathway inhibitor (TFPI) inhibits this process by inhibiting the complex TF/factor VIIa after binding to factor Xa (Figure 2).

Abundant TF protein and TFPI have been found in human atherosclerotic plaques. Different cell types within the plaque express TF including endothelial cells, vascular smooth muscle cells and particularly foam cells around the lipid core, which likely represents the most thrombogenic component of the plaque. The presence of TFPI is associated with reduced TF activity. In specific human coronary atherectomy specimens, concentrations of TF antigen and activity were found to be higher in plaques taken from patients with acute coronary syndromes compared to patients with stable angina, and thrombus was detected only in TF-positive plaques. Significantly increased TF plasma levels were demonstrated in patients with acute myocardial infarction and unstable angina compared to stable angina patients and healthy controls. A recent study showed that circulating TF contains procoagulant...
activity\textsuperscript{15}. The source of plasma TF is not known. It is suggested that it is partly derived from the vessel wall\textsuperscript{16,17}, where procoagulant TF containing microparticles are produced in high amounts in human atherosclerotic plaques due to apoptotic cell death of predominantly macrophages and lymphocytes\textsuperscript{18}. In addition, a substantial proportion of these particles are of endothelial origin which may reflect endothelial erosion at the site of plaque disruption\textsuperscript{17,19}. Plasma levels of total and free TFPI\textsuperscript{20-22} as well as of TFPI activity\textsuperscript{22,23} have also been demonstrated to be increased in patients with acute coronary syndromes compared to stable angina patients and healthy subjects. It is conceivable that these pre-treatment elevated levels of TFPI during the acute phase of an acute coronary syndrome, are important responses that inhibit an activated coagulation system.

The effects of vascular intervention on TF expression in the vessel wall have been investigated in animal studies. Different models demonstrated increased TF expression by vascular smooth muscle cells after arterial balloon injury, which was associated by an enhanced TF activity that subsequently resulted in a procoagulant response on the luminal surface\textsuperscript{24,25}. The administration of specific inhibitors of TF or the TF/ factor VIIa complex, such as monoclonal antibodies to TF or recombinant TFPI, did not only reduce thrombus formation, but also decreased neointima development at the site of arterial injury in these models\textsuperscript{26-28}. Intimal smooth muscle cell expression of TF played an important role during both processes.
Chapter 1

Taken together, these findings suggest that the TF mediated pathway of coagulation is not only involved during the acute phase of unstable coronary syndromes, but may also contribute to prolonged thrombin generation and cellular processes, such as coronary restenosis after percutaneous coronary intervention.

The extrinsic coagulation pathway and disseminated intravascular coagulation

Besides the initiation of local thrombus formation in ACS, the extrinsic coagulation pathway is also involved in another disorder of widespread arterial coagulation activation, termed disseminated intravascular coagulation (DIC). Disseminated intravascular coagulation is a common disorder in critically ill septic patients. It is characterized by a widespread activation of coagulation that ultimately results in thrombotic occlusions of small and midsize vessels. As a consequence, organ perfusion can be compromised and may contribute to the failure of multiple organs, which is associated with an increased risk of mortality in patients with the septic syndrome. Besides impairment of physiological anticoagulant and fibrinolytic pathways, the TF-mediated coagulation appears to play a key role in patients with DIC. A significantly increased TF activity by monocytes has been demonstrated in endotoxemic patients, and the administration of inhibitors of the extrinsic coagulation pathway were shown to inhibit coagulation activation during experimental bacteremia and endotoxemia in primates and healthy humans. Recombinant TFPI also inhibited the interleukin-6 response and even prevented mortality in a lethal septic shock model in baboons. However, the clinical role of these inhibitors in patients with severe septicemia remains to be established.

Recombinant nematode anticoagulant protein c2

A novel inhibitor of the complex TF/factor VIIa, termed recombinant Nematode Anticoagulant Protein c2 (rNAPc2), is an 85 amino-acids containing peptide that was originally isolated from the saliva of the hookworm Ancylostoma caninum, a hematophageous nematode (Figure 3). There are 15000 known species of nematodes. They appear in large numbers. From species that live on the ground, 4,420,000 nematodes have been counted in 1 m² of a muddy beach at the Dutch coast, and a total of 90,000 have been found in a rotting apple. Hookworms, however, are nematodes that infect a wide range of mammalian hosts, including humans. They can cause anemia in their host by extracting their blood meal from lacerated capillaries in the mucosa of the small intestine over an extended period of time.
evolved highly effective anticoagulant strategies to facilitate the acquisition of their required blood meal and NAPc2 represents a major anticoagulant activity of the adult Ancylostoma caninum. It is made as a recombinant, secreted protein in yeast. Its mode of action is comparable to that of TFPI, however in contrast to TFPI, rNAPc2 binds to factor X at a site distinct from its catalytic center, which obviates the need for factor Xa formation. This implies that rNAPc2 can form a complex with circulating factor X and rapidly inhibit the complex TF/factor VIIa following a thrombogenic challenge (Figure 4)\textsuperscript{38}. The formation of a complex between rNAPc2 and factor X
results in a biologic half-life of more than 50 hours. In a recent clinical trial, rNAPc2 has been shown to be very effective in reducing the incidence of acute deep venous thrombosis, without hemostatic compromise, when administered prophylactically in patients undergoing knee arthroplasty\textsuperscript{19}. As TF/factor VIIa is thought to play an important role both in coronary thrombosis and disseminated intravascular coagulation, rNAPc2 may be effective to inhibit thrombin generation in patients suffering from acute coronary syndromes and/or undergoing percutaneous coronary interventions, as well as in patients suffering from septicemia.

**Outline of this thesis**

The aims of this thesis were to examine the responses of the extrinsic coagulation pathway during ischemic coronary diseases and to evaluate the effects of inhibition of this pathway by rNAPc2 on safety and on coagulation and/or inflammation during elective percutaneous coronary intervention and experimental endotoxemia.

**Chapter 2** outlines the current knowledge on the role of TF in atherosclerosis, arterial intervention and potential pharmacological approaches, with a focus on ischemic coronary syndromes.

The early diagnosis of acute coronary syndromes is a major challenge for physicians at the emergency department, especially in patients presenting with a non-diagnostic electrocardiogram. The main cause of these syndromes is atherosclerotic plaque disruption with superimposed arterial thrombus formation and TF induced thrombin generation appears to play a pivotal role in this process. Therefore, systemic markers of the coagulation and fibrinolytic pathway, including soluble TF and TFPI activity, may be useful in the triage of patients with chest pain presenting with a normal or non-diagnostic electrocardiogram. This is evaluated in **chapter 3**.

The importance of TF/factor VIIa in coronary thrombosis suggests that rNAPc2 could be effective for the treatment of patients suffering from acute coronary syndromes. In current practice, an increasing number of these patients undergo percutaneous coronary interventions. As an important first step in the evaluation of TF/factor VIIa inhibition by rNAPc2, a randomized, placebo-controlled, double-blind, multicenter trial with escalating dosages of rNAPc2 in patients undergoing elective coronary angioplasty was performed to evaluate the safety of the drug and its ability to inhibit thrombin generation. The results of this trial are reported in **chapter 4**.

Thrombotic complications after intracoronary stenting are considered to be a primarily platelet driven process. However, exposure of TF to the coronary circulation during
the intervention initiates coagulation activation and may contribute to the risk of these complications. Chapter 5 describes in detail the effects of TF/factor VIIa inhibition by rNAPc2 on coagulation and inflammation in patients who received intracoronary stent implantation, which is a subpopulation of the study patients described in chapter 4.

A potential drawback for any antithrombotic agent is the risk of bleeding complications. Activation of the extrinsic coagulation pathway by recombinant factor VIIa has been shown to restore thrombin generation during inhibition of the TF/factor VIIa complex by rNAPc2\textsuperscript{40}. Therefore, recombinant factor VIIa may be a good candidate to overcome the effects of rNAPc2. Another agent that is undergoing clinical development is the synthetic pentasaccharide fondaparinux\textsuperscript{41-43}. In contrast to other antithrombin-dependent anticoagulants, i.e. unfractionated heparin and low-molecular-weight heparin, fondaparinux selectively inactivates factor Xa without thrombin inhibition. Like rNAPc2, fondaparinux has a long biologic half-life of approximately 17 hours and a strategy to reverse the anticoagulant state in case of life-threatening bleeding or for acute surgery appears to be desirable also for this pentasaccharide. In chapter 6, activation of the extrinsic coagulation pathway by recombinant factor VIIa has also been used to investigate in human male volunteers, whether this strategy is able to neutralize the anticoagulant effects induced through selective factor Xa inhibition by fondaparinux.

Several inhibitors of the extrinsic coagulation pathway have been tested in studies of experimental endotoxemia and bacteremia. The different mechanism of action of rNAPc2 may result in an in vivo anticoagulant profile that is distinguishable from TFPI and other inhibitors of TF/factor VIIa. In a model of endotoxin-induced activation of coagulation and inflammation, chapter 7 and chapter 8 describe the ability of rNAPc2 to affect the enhanced response of these systems in chimpanzees and human volunteers, respectively. Instead of subcutaneous administration as performed in patients that underwent coronary angioplasty (see chapters 4 and 5), chapter 8 also describes the pharmacodynamics of rNAPc2 after intravenous administration in human volunteers.

Chapter 9 summarizes and briefly discusses the main findings derived from the studies described in the previous chapters.
References


