1

General introduction
The present PhD thesis entitled “Coagulation factor VIIa: prohemostatic drug and biomarker for thrombosis” includes the topics hemostasis, thrombosis, and hemophilia with a central role for (recombinant) factor VIIa. These topics are addressed in this general introduction to understand the scope of this thesis.

**Hemostasis**

*Primary hemostasis*

Hemostasis is a physiological response in the body to minimize the loss of blood upon damage of the blood vessels. Blood clotting is accurately controlled in the body by an interplay between blood platelets, clotting factors, inhibitors of coagulation, and the fibrinolytic system. Upon damage of a blood vessel, subendothelial components, such as collagen or von Willebrand factor (VWF), are exposed to the blood. Platelets adhere to these subendothelial components and become activated. More platelets are attracted and aggregate to form a platelet plug sufficient to close an injured vessel. This initial hemostatic response is also known as primary hemostasis. The platelet plug needs to be reinforced by fibrin to be able to permanently close vessel wall damage. The platelets form a platform for secondary hemostasis, which comprises the cascade of enzymatic reactions of coagulation factors culminating in the formation of fibrin.

*Secondary hemostasis: classic cascade model of coagulation*

In the 1960s, two research groups independently proposed a theory about the mechanisms involved in secondary hemostasis in which they proposed that blood coagulation occurs via the cascade model of coagulation [1,2]. In this model, one coagulation factor will activate another coagulation factor and so on, in order to obtain thrombin which will subsequently cleave fibrinogen into fibrin. In this model, two separate enzyme pathways were proposed to form factor X(a), and subsequently a shared ‘common pathway’ will start to form fibrin as depicted in Figure 1. One pathway is the intrinsic pathway, also known as the contact activation pathway, and the other pathway is the extrinsic pathway, also known as the tissue factor (TF) pathway. This model with these pathways still forms the basis of coagulation screening: the activated partial thromboplastin time (APTT) which tests the functionality of the intrinsic and the common pathway, and the prothrombin time (PT) which tests the functionality of the extrinsic and the common pathway.
Figure 1 The classic cascade model of coagulation, consisting of the intrinsic pathway and the extrinsic pathway both resulting in the common pathway. The zymogen (not activated) coagulation factors are indicated with blue circles (black text labels), and become activated coagulation factors which are indicated with blue circles (white text labels). The cofactors FVIIIa, TF, FVa are indicated with the coloured circles. Abbreviations are explained in the abbreviation list. Original figure 14.1 [3], reprinted with permission of Oxford University Press.

Secondary hemostasis: cell-based theory

More recently, the cell-based theory has been proposed as a refinement of the classic cascade model of coagulation, which better reflects hemostasis in vivo [4,5]. The two most important proposed cell types involved in this model are TF-bearing cells and platelets. The model describes three distinct, but overlapping phases: an initiation, an amplification and a propagation phase.

The initiation phase comprises the start of coagulation by exposure of membrane bound TF, serving as receptor for FVII(a). This TF could be originated from vascular smooth muscle cells and fibroblasts located within a vessel wall and surrounding blood vessels which will be exposed upon vessel wall damage, and TF on the surface of macrophages,
monocytes, or microparticles present within the blood stream. Approximately 1% of the zymogen FVII circulates in its active form (FVIIa) and both forms can bind to TF. Factor VII becomes fully activated by TF-FVIIa upon binding TF as depicted in Figure 2, however the predominant physiological activator of FVII \textit{in vivo} is nowadays supposed to be FXa [6]. Activated FVII has little enzymatic activity to activate its substrates FIX and FX unless it is bound to TF. FVIIa thus obtains full proteolytic activity upon binding TF. This is illustrated in Figure 2. The FVIIa/TF complex activates small amounts of factor IX and factor X. The cofactor FVa of the activated FX is unavailable at this stage, so the reaction rate is relatively low. Nonetheless, FXa is able to convert prothrombin (FII) into trace amounts of thrombin (FIIa).

![Diagram](image)

**Figure 2** Tissue factor serves as receptor for FVII(a). Upon binding of FVII(a) to TF, FVII(a) becomes activated. See text for detailed explanation. Abbreviations are explained in the abbreviation list. Original figure 13.11 [3], reprinted with permission of Oxford University Press.

The amplification phase comprises the functions of the trace amounts of thrombin formed on TF-bearing cells in the initiation phase. First, the thrombin formed will activate platelets in addition to the already adhered and partially activated platelets as described above during primary hemostasis. Secondly, thrombin will activate FV and FVIII which serve as a cofactor for FX and FIX respectively. Finally, thrombin also activates FXI which in turn can also activate FIX.

The propagation phase is most effective on the surface of activated platelets and therefore shifting the hemostatic process from TF-bearing cells in the endothelial to activated platelets is essential for optimal hemostasis. In this phase, FIXa, activated during the
initiation phase, will bind to factor VIIIa on the surface of activated platelets. FIXa is not rapidly inhibited in solution, so it can freely diffuse from the TF-bearing cells to activated platelets. On the other hand, FXa is rapidly inhibited by antithrombin (AT) or tissue factor pathway inhibitor (TFPI) and cannot diffuse freely. Bound FIXa/FVIIIa subsequently activates FX at the surface of activated platelets, in which the FIXa/FVIIIa/FX complex is also known as the intrinsic tenase complex. Additional FIXa to activate the necessary FX is supplied by FXI(a) during the amplification phase. The FXa formed will bind to FVa on the surface of activated platelets and combines with prothrombin (FII), which is also referred to as prothrombinase complex, resulting in activation of prothrombin to thrombin (FIIa). The thrombin will convert fibrinogen into fibrin which eventually results in a fibrin clot. Furthermore, FXIIIa plays an important role in cross-linking fibrin and this improves the resistance of fibrin clots against fibrinolysis (explained in the paragraph ‘fibrinolytic system’ below).

**Inhibitors of coagulation**

There are several inhibitory mechanisms available to limit and control the clotting process, all acting on different steps in the coagulation cascade. Three inhibitory mechanisms will be described in this introduction. Tissue factor pathway inhibitor (TFPI) is an inhibitory protein active in the initiation phase. First, TFPI will bind to the active site of FXa, which results in inhibition of its proteolytic activity. Secondly, the TFPI-FXa complex will bind to FVIIa bound to TF. This results in the quaternary complex TF-FVIIa-TFPI-FXa, in which the enzymatic activities of both FXa and FVIIa are inhibited.

Antithrombin inhibits FIXa, FXa, FXIa, FIIa, as well as FVIIa as it is captured in the FVIIa-TF complex, by forming a stable 1:1 complex. The inhibitory effect of antithrombin is more efficient in the presence of heparin sulphates, which are present on the surface of most eukaryotic cells and in the extracellular matrix.

The protein C anticoagulant system inhibits FVa and FVIIIa. The system is activated when thrombin binds to the endothelial receptor thrombomodulin (TM), which inhibits the procoagulant activity of FIIa and allows activation of protein C (PC) and thrombin activatable fibrinolysis inhibitor (TAFI, explained in the paragraph ‘fibrinolytic system’ below). Protein C is localized on the endothelial surface by binding its endothelial protein C receptor (EPCR). Activated protein C (APC) inhibits coagulation factors FVa and FVIIIa, in the presence of two cofactors protein S (PS) and FV, by cleaving specific peptide bonds in these coagulation factors.
**Fibrinolytic system**

The fibrinolytic system serves to break down the clot once the endothelial layer has been restored. Fibrin is degraded by plasmin, which is formed by activation of plasminogen by tissue plasminogen activator (t-PA). The main mechanisms regulating fibrin degradation include the presence of plasminogen activator inhibitor-1 (PAI-1) and α2-antiplasmin, which are circulating inhibitors of t-PA and plasmin, respectively. In addition, removal of the lysine binding sites on fibrin, which facilitate plasminogen and t-PA binding, by thrombin activatable fibrinolysis inhibitor (TAFI) regulates fibrinolysis. This inhibitor is activated by thrombin, and this process is far more efficient when thrombin is in complex with thrombomodulin.

**Thrombosis**

Abnormalities in the balance of coagulation can result in excessive bleeding (hemorrhage) or in clotting of the blood (thrombosis). The formation of an occlusive thrombus can both occur within a vein or within an artery.

**Figure 3** A blood clot in an artery mainly consists of platelets (left), and a blood clot in a vein mainly consist of fibrin and red blood cells (right). Original figure 16.4 [3], reprinted with permission of Oxford University Press.
Arterial thrombosis

Blood clots in an artery mainly consist of platelets (Figure 3, left), and therefore treatment or prevention of arterial thrombosis often includes antiplatelet therapy. Examples of such antiplatelet drugs are aspirin and clopidogrel. Aspirin inhibits the enzyme cyclooxygenase (COX), and as a result there is no production of thromboxane A2 which is an important secondary activator of platelets. Clopidogrel irreversibly inhibits the receptor P2Y12, an adenosine diphosphate (ADP)-receptor present on the membrane of platelets, which is involved in secondary activation of platelets as well. Arterial thrombosis is often preceded by atherosclerosis, in which the artery wall thickens through deposits of atheroma (which includes lipids, macrophages, and connective tissue). Upon rupture of an atherosclerotic plaque there will be a rapid adherence of platelets resulting in a platelet-rich thrombus.

Venous thrombosis

Blood clots in a vein mainly consist of fibrin and red blood cells (Figure 3, right), and therefore treatment or prevention of venous thrombosis includes anticoagulation drugs such as vitamin K antagonists (VKA), low molecular weight heparin (LMWH), and direct inhibitors of FXa or thrombin. VKAs interfere with the action of vitamin K, which is essential for the production of functional vitamin K-dependent clotting factors (i.e., FII, FVII, FIX, FX, PC, PS, PZ). A drawback of VKA treatment is the requirement for monitoring of the extent of anticoagulation, and if necessary adapting the dosage to obtain the desired result. LMWHs do not require laboratory monitoring, but its subcutaneous route of administration limits its long-term use. The new generation oral anticoagulants including dabigatran, rivaroxaban, and apixaban do not require laboratory monitoring. LMWH enhances the inhibitory activity of antithrombin, and thus has multiple targets. In contrast, dabigatran inhibits FIIa, and both rivaroxaban and apixaban are inhibitors of FXa. The advantage of the latter three oral anticoagulants mentioned is the fact that they inhibit a single coagulation factor compared to the other drugs which inhibit multiple coagulation factors at once. There is ongoing research for novel anticoagulant strategies, as for example investigating glycoprotein (GP) Ib as an antithrombotic target. GPIb is the most important ligand binding protein of the GPIb-IX-V complex present on the membrane of platelets. GPIb is an interesting option for the development of novel antithrombotics, as it is involved in both the binding of platelets to the vessel wall and acts as a receptor for several coagulation factors. However, the physiological relevance of coagulation factor interaction with GPIb is yet uncertain.
Venous thrombosis occurs in 1-3 individuals per 1000 per year [7,8], and the most common forms are deep vein thrombosis (DVT) of the leg and pulmonary embolism. Venous thrombosis is a multicausal disease for which many risk factors have been firmly established [9]. These risk factors may be acquired (environmental) such as bed rest, surgery, oral contraceptive use, age or inherited such as a deficiency in protein C or S or a mutation in factor V (FV Leiden). A hypofibrinolytic status [10-12] and increased plasma levels of several clotting factors or low levels of inhibitors have also been indicated as risk factors for venous thrombosis, such as high levels of FVII and FXI, and low levels of TFPI [13-15]. Travelling is an established acquired risk factor for venous thrombosis as well, of which air travel is associated with a 2-4 fold increased risk of venous thrombosis [16,17]. This risk is even higher in individuals traveling by air and using oral contraceptives, having the factor V Leiden mutation, being short (<1.60 m) or being tall (>1.90 m), or having a high body mass index (>30 kg/m²) [18]. Individuals may have one risk factor or encounter the presence of multiple risk factors at once, leading to individualized variations resulting in some being at more risk than others.

It has been suggested that the initiation of venous thrombosis is by components within the bloodstream, as the endothelial lining of a thrombosed vein appears to be intact [19,20]. In contrast to arterial thrombosis, in which exposure of thrombogenic material present in atherosclerotic plaques initiates thrombus formation, such lesions are absent in veins. The risk factors previously being mentioned generally increase hemostatic potential by either enhancing activation of coagulation or by decreasing inhibition of coagulation. Having a hemostatic balance is therefore important, as alterations may contribute to the development of DVT. The initiating trigger in the development of DVT is, however, at present still not known. The exact mechanisms underlying air travel-related thrombosis are also incompletely understood. It is proposed in general that venous thrombosis is initiated via the extrinsic pathway, in particular via tissue factor-bearing microparticles [21-25]. It has been suggested that activation of the venous endothelial lining, for example by venous stasis, leads to the recruitment of TF-bearing microparticles via P-selectin expressed on the activated endothelium and P-selectin glycoprotein ligand-1 (PSGL-1) on the TF-bearing microparticles. This process results in accumulation of TF, which, possibly after fusion with platelets or endothelial cells, initiates venous thrombus formation [13]. In the last part of this thesis, we measured levels of FVII and FVIIa to assess the role of the tissue factor pathway in the initiation of air travel-related thrombosis (chapter 5) and in the initiation of DVT (chapter 6).
**Hemophilia**

Hemophilia A and B are X-chromosome linked bleeding disorders which comprise recessive mutations in the genes encoding for factor VIII or factor IX respectively, and therefore hemophilia almost exclusively occur in males. Hemophilia A has a prevalence of 1 in 10,000 males, and hemophilia B occurs in about 1 in 25,000-30,000 males. Hemophilia A can be classified as mild (FVIII levels >5-40%), moderate (FVIII levels 1-5%) or severe (FVIII levels <1%), which is also coherent with clinical severity and bleeding frequency. A similar subclassification in FIX levels is applied for hemophilia B. [3]

**Factor replacement therapy**

Back in the 1950s and 1960s, whole blood and plasma transfusions were the best treatment options for hemophilia patients. However, large volumes needed to be administered to achieve appreciable increases in FVIII or FIX levels. In 1964, cryoprecipitation was introduced which made it possible to make freeze dried concentrates that could be administered in relatively small volumes. The freeze-dried concentrates became available on large scale and could be easily stored, leading for example to the possibility for treatment at home. In the late 1970s and early 1980s it became clear that all that time, plasma-derived products were used sometimes containing viral contamination with viruses that were yet to be discovered. This unfortunately resulted in many HIV and hepatitis C infected hemophilia patients who unknowingly administered these contaminated products. Due to the technical developments in the production of plasma-derived products it became possible to eliminate viruses and pathogens and test for the presence of these pathogenic microbes. The risk of blood-borne virus transmission was thereafter strongly reduced [26]. At the same time, DNA recombination technology was developed to such a level that recombinant proteins could be used for human clinical use. In 1984, the human FVIII gene was cloned [27] and recombinant FVIII concentrates became commercially available in 1992 [28]. The human FIX gene was cloned in 1982 [29], and recombinant FIX concentrates became commercially available in 1998 [30].

**Inhibitor-complicated hemophilia**

The main problem with factor replacement therapy, plasma derived or recombinant, is the development of inhibitory antibodies against the supplemented FVIII or FIX. About 30-50% of hemophilia A patients and 1.5-3% of the hemophilia B patients develop inhibitors [31], making continuation of replacement therapy in these patients ineffective. Once an inhibitor titer is above 5 Bethesda Units (BU)/ml, bypassing agents are used to
treat hemophilia patients with inhibitors. First prothrombin complex concentrates (PCCs) were used, followed by activated PCCs, or factor eight inhibitor bypassing agent (FEIBA) which contains partially activated coagulation proteins. A decade later, recombinant factor VII became clinically available. These bypassing agents has been proven safe and effective for the treatment of bleeding episodes in hemophilia patient with inhibitors. The overall efficacy and outcome of bleeding episodes, however, were higher for recombinant FVIIa compared to the plasma derived FEIBA [32]. The treatment of choice is often based on expert opinions and personal experience, with taken individual responsiveness of patients into account. The treatment of inhibitor-complicated hemophilia patients has significantly improved by the introduction of rFVIIa.

Recombinant activated factor VII

On 24-04-1981 the first hemophilia patient was successfully treated with purified human plasma-derived FVIIa [33]. Another few patients subsequently received this plasma-derived treatment. To proceed with this treatment for further clinical use of FVIIa, however, recombinant technology needed to be used for large scale production due to the low plasma concentration of FVIIa. This lead to the cloning of the human FVII gene in 1986 [34], and its transfection in baby hamster kidney cells [35]. It was shown that the FVIIa produced by transfected baby hamster kidney cells is very similar to human plasma FVIIa [35]. The first hemophilia patient was successfully treated with recombinant FVIIa on 09-03-1988 [36]. Clinical studies all showed that rFVIIa is safe and effective for the treatment of bleeding episodes as well as for the prevention of surgical bleeding in inhibitor-complicated hemophilia A and B [37,38]. rFVIIa was approved in Europe in 1996, in the United States in 1999, and in Japan in 2000.

Working mechanism of rFVIIa

During the development of rFVIIa the exact working mechanism was not yet completely understood. For example it was not clear why relative high plasma concentrations of rFVIIa are required for effective hemostasis in hemophilia patients. Research provided more insight in molecular mechanisms, but up till today there still is an ongoing debate about which working mechanism, the TF-dependent or independent, acts to enhance thrombin generation.

TF-dependent mechanism

Initially, it was thought that the mechanism of action of rFVIIa in hemophilia was based on tissue factor-dependent enhancement of thrombin generation. In 1993, results from an in vivo study in which non-bleeding chimpanzees received a bolus injection of rFVIIa
confirmed this hypothesis [39]. After rFVIIa infusion, plasma levels of the activation peptides of FIX and FX, and prothrombin fragment 1+2 increased. The elevations of these plasma markers could be abolished by infusing an inhibitory antibody against tissue factor just before the rFVIIa infusion. The results of these experiments provided evidence for the TF-dependent mechanism. However, they did not explain why relative high plasma concentrations of rFVIIa are required for effective hemostasis. The dissociation constant (Kd) of FVIIa for tissue factor is 0.5 nM, but relatively high concentrations of 10-20 nM of rFVIIa are needed for effective hemostasis in hemophilia patients. In vitro experiments with purified proteins revealed that, in the absence of FVIII, zymogen FVII significantly inhibits TF-initiated thrombin generation [40]. The inhibitory effect of FVII could be abolished by adding high concentrations (~10nM) of rFVIIa, resulting in thrombin generation as observed in normal plasma containing FVIII. The TF-dependent mechanism of thrombin generation by rFVIIa was confirmed in whole blood models [41], and in a FVII-deficient plasma model together with a mathematical model simulating reactions important for FXa-generated [42]. This latter study, however, also found evidence for the involvement of a TF-independent mechanism of action.

TF-independent mechanism

In the early phase of development of rFVIIa it was shown that rFVIIa has an effect on the activated partial thromboplastin time (APTT), as it shortened the APTT after addition of rFVIIa. This result indicated a TF-independent mechanism to activate factor X [43]. Experiments using purified coagulation factors showed indeed that rFVIIa is able to activate FX in the presence of phospholipid vesicles and Ca²⁺, however at a much lower catalytic efficiency compared to the same reaction in the presence of TF [44].

Research done over the years confirmed a TF-independent mechanism of thrombin generation by rFVIIa, as FIX and FX could be activated by rFVIIa on phospholipid vesicles, monocytes and activated platelets independently of TF [43,45-47]. Platelets expose anionic phospholipids on their surface upon activation. rFVIIa is able to bind to these negatively charged phospholipids and subsequently generates thrombin independently of TF [46].

The need of high plasma concentrations of rFVIIa can be explained by the weak affinity of rFVIIa for phospholipids (Kd ~ 90nM). The explanation given by the TF-dependent thrombin generation theory elucidating that high doses of rFVIIa are required to overcome the endogenous FVII zymogen competition for TF, was rejected by results showing (auto) activation of zymogen FVII in the propagation phase of coagulation at high doses of rFVIIa [48].
Besides the inefficient TF-independent activation of FX by rFVIIa bound to the surface of platelets, there are differences in binding of wild-type rFVIIa and a TF-independent enhanced rFVIIa variant to platelets compared to synthetic phospholipid vesicles. The binding to synthetic phospholipid vesicles was similar for both rFVIIa and the rFVIIa variant, however the binding of the rFVIIa variant was higher compared to wild-type to the surface of activated platelets [49]. In addition, the rFVIIa variant also bound to not activated platelets. Therefore it is suggested that besides negatively charged phospholipids other components on the platelet surface might be involved in rFVIIa-mediated thrombin generation. Further research from our laboratory confirmed the involvement of a binding protein, as we identified glycoprotein Ibα as binding protein for rFVIIa present on the surface of activated platelets [50]. This interaction resulted in enhanced TF-independent thrombin generation on the activated surface of platelets. Recently, it was also demonstrated that the endothelial protein C receptor (EPCR) is expressed on the surface of activated platelets and contributes to the localization of rFVIIa to the surface of activates platelets [51].

In the development to improve treatment outcomes of hemophilia patients with inhibitors there is accumulating evidence in favor of rFVIIa variants with increasing TF-independent activity. In 2001, several FVIIa variants with increased intrinsic activity were made [52]. Two of those variants were tested in an in vitro plasma model and showed increased procoagulant and antifibrinolytic potential compared to wild-type rFVIIa [53]. These two variants, as well as an additional FVIIa variant, were also tested in vivo in a hemophilia A mouse model and showed increased hemostatic potential compared to wild-type rFVIIa [54]. Eventually, one of the tested rFVIIa variants, containing three amino acid substitutions (vatreptacog alpha), was further developed and tested in randomized clinical trials in hemophilia patients with inhibitors. The results looked very promising as the rFVIIa variant was superior over wild-type rFVIIa in secondary outcome measures, including the number of doses needed to treat a bleed and sustained bleeding control 1-2 days after the first dose [55-58]. Unfortunately, the clinical development of this rFVIIa variant was terminated due to the development of anti-drug antibodies.

Another rFVIIa variant, BAY 86-6150, was also in clinical development [59]. This variant contains six amino acid changes which resulted in increased binding to activated platelets as well as a prolonged half-life compared to wild-type rFVIIa. Unfortunately, also the clinical development of this rFVIIa variant was terminated due to development of antibodies to the drug.
Examples of other approaches to improve treatment outcomes of hemophilia patients are a glycoPEGylated rFVIIa variant or an albumin-fused rFVIIa variant. Both techniques lead to the prolongation of the plasma half-life of rFVIIa. Initial clinical results from the glycoPEGylated rFVIIa variant do suggest that this variant is well tolerated and safe, however no dose-response in inhibitor-complicated hemophilia patients was established and the clinical development of this product has been terminated [60,61]. The albumin-fused rVIIa variant is in clinical development [62,63].

**Mechanism of action of rFVIIa**

Recombinant FVIIa enhances thrombin formation via a TF-independent mechanism, in which thrombin has several downstream effects such as platelet activation, fibrin formation and activation of TAFI as described before. Therefore the working mechanism of rFVIIa can be explained following these downstream effects of thrombin in hemostasis. First, thrombin generation by rFVIIa results in enhanced platelet activation [64,65]. Furthermore, thrombin also has effect on fibrin formation as well as on fibrin structure. The quantity and rate of thrombin generation determines the structure of the fibrin clot. Fibrin clots composed of thin and highly branched fibrin fibers are less susceptible to fibrinolysis, as fibrin clots composed of thick fibrin fibers are more prone to be lysed by components of the fibrinolytic system [66]. Hemophilia patients have impaired thrombin generation and therefore their fibrin clots consist of thick fibrin fibers and have a high clot permeability [67]. Hemophilia patients also have a reduced TAFI activation, resulting in premature fibrinolysis [68]. Addition of rFVIIa increases thrombin generation, thereby normalizing fibrin structure by means of thinner fibrin fibers and increasing clot stability by preventing premature lysing of the fibrin clot by activation of TAFI [67,69-71].

**Treatment of rFVIIa**

Recombinant factor VIIa (rFVIIa) has been shown in several clinical trials to be safe and effective for treatment of bleeding episodes in inhibitor-complicated hemophilia A and B, and has been registered for this purpose [72-76]. It is recommended to administer either an intravenous bolus injection of 90 µg/kg body weight rFVIIa repeated every second hour or a single injection of 270 µg/kg body weight rFVIIa. Both dosing regimens have been proven to be equally effective and safe in several clinical trials [77,78]. However, the single-dose regimen may be more convenient and may improve patient compliance, in particular in the setting of home therapy [79,80].
Prophylactic treatment of rFVIIa

Besides the use of rFVIIa in the treatment of bleeding episodes in inhibitor-complicated hemophilia, recent clinical data demonstrated that rFVIIa is also useful to prevent spontaneous bleeding episodes in patients with inhibitors. In 2007, Konkle et al. published the first and until now only randomised controlled trial of the prophylactic use of 90 or 270 μg/kg body weight rFVIIa [81]. For both dose administrations a reduction in the number of bleeding events (27-59%) was observed during and three months after the prophylaxis period compared to the pre-prophylaxis period. In these studies, rFVIIa appeared to provide hemostatic efficacy for a time frame much longer than expected based on the plasma half-life. This prohemostatic effect of rFVIIa during prophylactic treatment is difficult to explain given its half-life of 2 hours. The mechanism of action of prophylactic administered rFVIIa is at present still not completely understood. In the first part of this thesis, mechanisms explaining the prophylactic efficacy of once-daily rFVIIa administration are investigated.
Aim of this thesis

In this thesis, coagulation factor VIIa plays a central role, in which the aim of the research was two-fold. First, potential working mechanisms of recombinant factor VIIa (rFVIIa, NovoSeven) were assessed when prophylactically administered to inhibitor-complicated hemophilia patients. Second, the use of FVIIa as biomarker for venous thrombosis was examined.

The first part of this thesis will focus on the prohemostatic effect of rFVIIa when given prophylactically. In chapter 2, potential mechanisms by which rFVIIa may exert a prolonged hemostatic effect have been investigated by administering a single bolus administration of rFVIIa to six non-bleeding pigs. During the time frame of prophylaxis, up to 48 h, plasma was collected and platelets were isolated and both were analysed for FVIIa levels and associated hemostatic activity. Chapter 3 describes the uptake of rFVIIa by megakaryocytes which subsequently produce platelets which contain hemostatically active rFVIIa.

In chapter 4, the interaction and functional consequence of the binding of FIX(a), a homologous protein to rFVIIa, to the GPIb-IX-V receptor is described as part of an ongoing study in our laboratory.

The second part of this thesis will focus on the use of coagulation factor VIIa as biomarker for venous thrombosis. In chapter 5, the initiating trigger of coagulation activation after air-travel has been investigated. Blood was drawn from individuals before, during, and after an 8 h flight, movie marathon or daily life routine and FVII(a) activity and antigen levels were measured and used as biomarker for extrinsic coagulation activation. In chapter 6, the initiating trigger of venous thrombosis has been investigated. Patients with acute deep venous thrombosis and controls were included, and antigen levels of FVII and FVIIa were measured to identify the role of the TF pathway.

In chapter 7, the results described in this thesis are discussed in a broader perspective, including the meaning for both patient and physician in clinical practice. Recommendations for future research are given.
References


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