

# Factor VIIa and its potential therapeutic use in bleeding-associated pathologies

Ulla Hedner<sup>1,2</sup>

<sup>1</sup>University of Lund, Lund, Sweden; <sup>2</sup>Research & Development, Novo Nordisk A/S, Bagsvaerd, Denmark

## Summary

Recombinant FVIIa (rFVIIa) was developed for treatment of haemophilia patients with inhibitors against FVIII/FIX. The haemostatic efficacy rate of 80–90% including major orthopaedic surgery (dosing of 90–120 µg/kg every other hour [h] for at least the first 24 h) was achieved in these patients. In a home-treatment setting the efficacy rate of haemostasis in mild-moderate bleedings was 92% (average number of 90 µg/kg doses was 2.2). A wide individual variation regarding recovery of rFVIIa (46 ± 12%; median 43%) as well as of clearance rate (36 ± 8 ml/kg/h; median 32 ml/kg/h in adults; children 2–3 times higher) has been observed. Thus children may require higher doses than adults. Accordingly the use of a dose of 270 µg/kg in one single injection was approved in the EU. Recent experience indicates that re-

peated doses of rFVIIa may decrease the number of bleeds in „target joints“, and thus may be useful as prophylaxis in severe hemophilia with inhibitors. Pharmacological concentrations of rFVIIa have been shown to enhance the thrombin generation on thrombin activated platelets in a cell-based model. By doing so a tight structured fibrin haemostatic plug resistant against premature lysis is formed. rFVIIa has been shown to induce haemostasis not only in haemophilia but also in other situations characterized by an impaired thrombin generation such as platelet defects, dilution coagulopathy developed as a result of trauma and extensive surgery. A special form of profuse bleeding, that may cause extensive problems is postpartum haemorrhage.

## Keywords

Factor VIIa, haemophilia therapy, haemostasis, haemophilia A / B, tissue factor / factor VII

**Thromb Haemost 2008; 100: 557–562**

## Introduction

Recombinant FVIIa (rFVIIa) was developed for treatment of haemophilia patients who have developed inhibitors against the protein they are missing, factor (F)VIII in haemophilia A and FIX in haemophilia B. These patients do not benefit from the administration of FVIII or FIX concentrates unless special measures to overcome or to remove the inhibitors are undertaken. The finding that pharmacological doses of rFVIIa provided haemostasis in patients with severe haemophilia in the absence of FVIII or FIX was a breakthrough in the understanding of the importance of FVII and tissue factor (TF) for haemostasis, and stimulated research into the TF- and FVII-dependent pathway of haemostasis. In the previous model, FVII and TF were recognized as the extrinsic pathway, and received little attention until demonstrated in the 1970s that the complex between FVII and TF activated not only FX but also FIX, which was part of the so-called intrinsic system (1). The previous cascade model did

not include cells and platelets and their role for the localization of the haemostatic process. Neither was it able to explain why patients with a FXII deficiency do not bleed excessively.

The role of platelets was identified already in the late 1800s. Exposure of phospholipids, especially phosphatidyleserine (PS) on the outer surface of thrombin-activated platelets was found to be important. PS is also required to maintain the anticoagulant activity of endothelium, as it enhances thrombomodulin activity. It was later recognized that platelets have complex coagulant activities that are not completely mimicked by phospholipids (2). The presence of potent inhibitors such as the tissue factor pathway inhibitor (TFPI) and antithrombin (AT) in the circulation also is important for regulation of the haemostatic process (2, 3).

Thus, the haemostatic process is primarily regulated by localization on cell surfaces and by plasma inhibitors. The so-called extrinsic and intrinsic pathways are much more complicated than realized previously, and the idea of two coagulation pathways – extrinsic and intrinsic – should be abandoned.

Correspondence to:  
Ulla Hedner, MD, PhD  
Novo Nordisk A/S  
Novo Allé  
Bagsvaerd 2880, Denmark  
Tel.: +45 4444 8888, Fax: +45 4449 0555  
E-mail: uhe@novonordisk.com

Received July 8, 2008  
Accepted after minor revision September 2, 2008

Prepublished online September 5, 2008  
doi:10.1160/TH08-07-0434

## Haemostasis

According to the current concept, haemostasis is localized to cell surfaces, mainly the TF-bearing cells and the thrombin-activated platelets. TF-expressing cells are present in the deeper layers of the vessel wall (4). As a result of injury to the vessel wall, TF is exposed to the circulating blood and forms complexes with FVII/FVIIa present in the circulation. Because TF is a receptor protein anchored to cells by a transmembrane domain the TF-FVII/FVIIa complex is localized by cells to the site of injury. Furthermore, it was suggested that a few functional FVIIa-TF-complexes are always present extravascularly and support a continuous TF-dependent extravascular generation of small amounts of FIXa and FXa (4). In fact, it was recently demonstrated that TF close to the vessels bound FVII or FVIIa in the absence of a clear injury (5). The coagulant activity seems to be restricted to a limited number of TF sites on cell surfaces. Thus, the vast majority of TF is non-functional (encrypted). There is still no consensus on how the encrypted TF is decrypted, although the exposure of anionic phospholipids especially phosphatidyleserine have been shown to be important in this process (4, 6). The importance of the membrane composition was also stressed by the finding that depletion of cholesterol from cell membranes impaired functional TF expression in fibroblasts (7). Recent observations suggest that protein disulfide isomerase (PDI) released from activated platelets, may ensure the formation of disulfide bonds claimed to be important for the coagulant-activity of TF (8). Although several publications have stressed the importance of PDI in regulation of the TF activity and thrombus formation (9–11), the importance of PDI in regulation of the TF activity is still being discussed (12). Encrypted TF may also be carried by cell elements such as white blood cells or microparticles. Furthermore, it has been reported that washed platelets incubated with TF were able to take up TF in a process involving traffic of vesicles through channels of the open canalicular system (OCS). TF was identified in the OCS and occasionally in the alpha-granulae of the platelets (13). Whether platelet-related TF is constitutively present in platelets (14) or transferred from other cells (14, 15) is the subject of debate. The FXa formed converts limited amounts of prothrombin into thrombin sufficient to activate FVIII and FV as well as FXI and platelets. The FXa activity is restricted to the TF-bearing cell surface. Any FXa that diffuses off the cell is immediately inhibited by AT. As soon as FXa is formed, a complex including TF-FVIIa and FXa is formed, inhibited by TFPI, and internalized. TFPI enhances the TF-specific internalization and degradation of FVIIa, which requires the C-terminal domain of TFPI and FXa. Most of the internalized FVIIa is degraded, but a small fraction recycles back to the cell surface as an intact protein. In the absence of TFPI, FVIIa bound to TF is internalized and degraded (16). The small amount of initial thrombin binds to platelets that have adhered to extravascular matrix components at the site of injury partially mediated by binding of von Willebrand factor to collagen (17). Thrombin enhances platelet activation leading to release of FV as well as activation of FV and FVIII splitting off von Willebrand factor (18). Thrombin also activates FXI. The platelets activated by the initially formed thrombin expose negatively charged phospholipids on their surface, which enhances the binding of the ac-

tivated coagulation proteins to the platelet surface. FIXa activated initially by the FVIIa-TF complex diffuses to the activated platelet and binds strongly to the negatively charged platelet surface where the most effective FX activation and thrombin generation take place (2). The binding of coagulation proteins on the platelet surface is facilitated by the combined stimulation of the platelet collagen receptor (GPVI) and thrombin receptor owing to the development of a subpopulation of platelets with an increased binding capacity (19, 20). Individual variations in such subpopulations may add to the variability of platelet procoagulant response. The content of phosphatidyleserine (PS) of the platelet membrane is important (2), although the binding of FXIa is also mediated by specific sites promoting the formation of active FIXa-FVIIIa (“tenase complex”) (21). The tenase-complex activates FX from the circulation into FXa on the platelet surface, associates with FVa and generates a burst of thrombin required to form a firm, well-structured fibrin haemostatic plug. The FX-activation occurs quickly and only small amounts of FX are necessary for a saturated formation of Xa according to results obtained in a cell-based in-vitro model (22). FXIa generated by the initial thrombin activates more FIX into FIXa on the platelet surface, thereby enhancing thrombin generation. All these reactions seem to be well regulated in terms of saturation of the activation processes. However, adding more prothrombin increases the thrombin generation without reaching any level of saturation (22). The gel network formed at the gelpoint (clotting time) has been found to be important for the scaffold into which the subsequently activated fibrinogen molecules are incorporated, the primary scaffold becoming more porous the lower the thrombin concentration. On the contrary, an increase in thrombin concentration and thereby a more rapid fibrinogen activation leads to formation of less porous fibrin gels with thinner fibers and small liquid spaces (23). A tight fibrin network makes the haemostatic plug more resistant against premature proteolysis thereby helping to maintain haemostasis (24). Also, a full thrombin burst is necessary for a full activation of the thrombin activatable fibrinolytic inhibitor (TAFI) further protecting the formed fibrin plug.

In situations with impaired thrombin generation, e.g. haemophilia, a loose fibrin plug is formed, which is less resistant towards proteolysis. As a result, these patients are characterized by a defective maintained haemostasis and repeated rebleedings. A full thrombin burst is also dependent on the number of platelets, resulting in the formation of loose fibrin plugs in thrombocytopenia. Patients having low platelet counts thus form fibrin plugs that are easily dissolved by fibrinolytic enzymes leading to the characteristic bleedings from tissues rich in fibrinolytic enzymes such as the gastric mucosa, the ear-nose-throat region and the urinary tract.

## FVIIa as a haemostatic agent

Approximately 20% of haemophilia patients develop inhibitors against FVIII or FIX. These patients do not benefit from concentrates of FVIII or FIX unless special measures are undertaken to overcome or remove the inhibitors. In patients with inhibitors, agents which are haemostatically effective independent of FVIII/FIX are required. FVIIa occurs in the normal circulation in a

concentration corresponding to ~1% of the total FVII protein mass (25). Furthermore, purified FVIIa did not induce systemic activation of the coagulation system in a dog model used to identify potential thrombogenic factors in activated prothrombin complex concentrates (APCC) (26, 27). However, FVIIa as a haemostatic agent was a new concept in the 1970s. The vision at the time was to develop a therapy for haemophilia patients with inhibitors that would be as easily available and convenient as existing treatments for haemophilia patients without inhibitors. If the new agent were successful, there would be no further need for complicated, inconvenient, and expensive therapies such as induced immune-tolerance treatment.

In the past, elective surgery has been more or less contraindicated in haemophilia patients with inhibitors because of the risk of uncontrollable bleeding. However, rFVIIa was demonstrated to have an efficacy rate of 90–100% in major surgery, including major orthopedic surgery (28, 29). Also in patients with serious bleedings a similar efficacy rate was achieved using essentially the same dosing schedule as that recommended in surgery (30) (Table 1).

As part of the vision of providing a treatment for haemophilia patients with inhibitors that would make them similar to patients without inhibitors, the effect of rFVIIa in a home-treatment setting was explored. An efficacy rate of 92% was achieved. However, the number of doses to achieve haemostasis was 2.2, which indicates that the dose used might not be optimal (31, 32).

The mechanism of action of pharmacological doses of rFVIIa was studied in a cell-based model demonstrating that rFVIIa binds to thrombin-activated platelets through a low-affinity binding requiring a 10-fold higher concentration of rFVIIa in the absence of FVIII/FIX to generate a similar amount of thrombin as was formed in the presence of FVIII/FIX (33). This reaction was TF-independent confirming previous findings (34–36). The bound rFVIIa activates FX on the activated platelet surface independent of the presence of FVIII or FIX and a dose-dependent increase in the thrombin generation on pre-activated platelets was demonstrated (37, 38). Although the lag phase of the initiation of thrombin generation normalized as compared to the value obtained in the presence of physiological concentrations of clotting factors and platelets in the cell-based model, the height of the thrombin peak did not reach the same level as found in the physiological situation after the addition of rFVIIa in concentrations of up to 500 nM (25–30 nM of FVIIa is the estimated

plasma level following injection of the standard dose of 90 µg/kg, and 75–80 nM of FVIIa following the dose of 270 µg/kg) (37). However, doses of 90 µg/kg or 270 µg/kg induce clinical haemostasis in most patients, indicating that the peak of thrombin generated may not be the most important but rather the rate of thrombin generation. Furthermore, the clot lysis time *in vitro* in haemophilia plasma was found to be prolonged after addition of rFVIIa (37, 39). Increased TAFI activation as a result of enhanced thrombin generation was suggested to contribute to the increased resistance to lysis (39). However, also a normalization of the fibrin permeability as a result of the tighter fibrin network demonstrated after addition of rFVIIa to haemophilia A plasma in the presence of pre-activated platelets, is of major importance for the increased resistance to proteolysis (40).

The haemostatic effect of rFVIIa in pharmacological doses thus seems to be mediated by an enhanced rate of thrombin generation on thrombin-activated platelet surfaces. This will result in an increased further activation of platelets at the site of injury, and increased platelet adhesion that may involve an enhanced platelet-platelet interaction initiated by thrombin binding to platelet glycoprotein Ib (GPIb) as well as other mechanisms (41). The enhanced thrombin generation ensures the formation of a tight fibrin structure of the haemostatic plug, as well as full activation of TAFI and FXIII (both activated by thrombin) necessary for maintaining haemostasis (42).

### Dose adjustment

By increasing the physiological level of FVIIa, the non-specific binding of rFVIIa to activated platelets is exploited. However, the exact relationship between the plasma concentration of FVII:C and the thrombin generation at the site of injury is not known. A number of assays for the measurement of thrombin generation have been described, but most of them measure thrombin formation in circulating blood rather than the thrombin generated at the site of injury. The mean recovery of rFVIIa (FVII:C at 10 minutes after injection) was found to be 46% (median 43%) (43, 44). The recommended dose at 46% of recovery would then correspond approximately to 25 nM to 35 nM of FVIIa in plasma. However, clearance rate, recovery at 10 minutes after injection, and the capacity to generate thrombin on the platelet surface vary widely among individuals (32). Accordingly, the optimal dose might show a great individual variation. Furthermore, the clearance rate in children below 15 years of age

**Table 1: Studies of rFVIIa in haemophilia.**

	Authors	No. of cases	No. of bleeds/ events	Dose (µg/kg)	Haemostasis (%)	Mean of inj. / total dose
Home treatment	Key et al. <sup>31</sup>	60	614	90	92	2.2
	Laurian et al. <sup>70</sup>	16	147	90–120	90	3.8
	Santagostino et al. <sup>71</sup>	21	53	90	79	2
	Kavakli et al. <sup>47</sup>	24	2/dose	270x1; 90x3	90 - 85	1; 3
	Young et al. <sup>48</sup>	21	3/dose	270x1; 90x3	91 - 90	1; 3
Surgery	Shapiro et al. <sup>29</sup>	29	29	35 q2; 90 q2	40 d3; 100 d3	135; 81
Serious bleeds	Lusher et al. <sup>30</sup>	158	713	90	76 - 90	

may be as much as three times the normal rate for adults (45, 46), which suggests that they may require higher doses of rFVIIa in order to ensure formation of the firm, tight initial haemostatic plug that is necessary for maintaining haemostasis. Recently, a dose of 270 µg/kg was approved in Europe on the basis of a study comparing 90 µg/kg three times per bleed with one single bolus of 270 µg/kg (47, 48).

Although treatment with rFVIIa is not a substitution therapy like FVIII/FIX therapy the feasibility of administration of rFVIIa in a continuous infusion (CI) was explored by Schulman and et al. (49) in two haemophilia patients with inhibitors. They initiated treatment with a bolus of 90 µg/kg and continued with a CI dosing adjusted by the pharmacokinetic of each patient. The plasma level of FVII:C used as the surrogate measurement for the extra rFVIIa necessary for generation of a tight, strong fibrin haemostatic plug at the site of injury in the absence of FVIII/FIX, is not well defined. More important than the actual FVII:C level in plasma may its ability to generate an effective thrombin generation on the activated platelets at the site of injury be. Unfortunately, no method for the evaluation of this localized thrombin generation is yet available, which may make an adjustment of an exact dose for a CI administration problematic. The initial experience of CI infusion of rFVIIa therapy pointed out the importance of the individual pharmacokinetics of each patient and recommended the dose schedule to be accordingly adjusted (49). During the last decade varying schedules for CI rFVIIa therapy have been reported and were recently reviewed (50, 51). The results include both successes and failures and may reflect the experience in haemophilia treatment at each center included, more than the dosing of rFVIIa. It is obvious that rFVIIa may be administered as a CI although the optimal dosing regimen is not known and may vary in different patients due to individual pharmacokinetics. Its success may also depend on the use of adjunct therapy like antifibrinolytics (50, 52). Extra bolus doses seem to be required in some cases to keep haemostasis which requires extra attention from the medical staff.

### Effect of rFVIIa as prophylaxis

In non-inhibitor patients with haemophilia regular prophylaxis with several doses of FVIII/FIX per week has been demonstrated to minimize the development of arthropathy, and therefore is the recommended treatment in severe haemophilia patients (53). Recently several haemophilia patients with inhibitors have been successfully treated with repeated doses of rFVIIa (54). The “target joints” in these patients characterized by an inflammatory synovitis require a stable haemostatic plug for full haemostasis. In a recently published randomized, prospective clinical trial, daily administration of rFVIIa in doses of 90 µg/kg or 270 µg/kg decreased the number of bleeds, not only during the three-month treatment period but also during the observation time that followed (three months of no regular treatment) (55). This outcome may mark another step toward the goal of making the treatment of haemophilia patients with inhibitors similar to that of non-inhibitor patients. The decrease in bleeding during the treatment period was probably due to amelioration of the inflammatory synovitis. However, it is not clear how this effect was achieved by once-daily administration of an agent with a plasma half-life of 2–3 hours. Neither is it clear why rFVIIa prophylaxis re-

duces the number of haemorrhagic events in the post-treatment period. Although this phenomenon may be due simply to a decrease in the inflammatory response, due to the decreased number of bleeding events, evidence related to the extravascular distribution of FVIIa may also play a role in the prolonged effect of rFVIIa. TF-FVII complexes may form continuously on extravascular TF-expressing cells surrounding blood vessel walls (4, 56). The bound FVIIa is internalized and partially degraded in the cell, while some of it will reappear on the cell surface and bind to TF. This process may occur continuously until all FVII/FVIIa is cleared and may continue for a long time if there is plenty of FVIIa in the extravascular compartment (57), which may be the case after administration of pharmacological doses of rFVIIa. Assuming that a similar process occurs *in vivo*, continuous formation of rFVIIa-TF complexes on cell surfaces extravascularly may facilitate thrombin generation on platelets that plug the leak in blood vessels in the joint tissues following the mechanical strain of movement. Another possibility would be that rFVIIa administered in pharmacological doses binds to some other protein or compound and serve as a reservoir for complex formation locally at any exposure of TF.

### Clinical experience with rFVIIa in other than haemophilia patients

The ability of rFVIIa to enhance thrombin generation on the surface of activated platelets makes it a potential haemostatic agent in any situation that requires the formation of a tight haemostatic plug (33, 41, 58). In the *in-vitro* cell-based model the addition of rFVIIa caused a dose-dependent shortening of the lag phase of platelet activation and thrombin generation on the activated platelets in the presence of platelet counts down to at least 10,000 µl<sup>-1</sup>. Also a tighter fibrin structure was observed in the presence of rFVIIa and low platelet counts (59). In a flow-chamber model the addition of rFVIIa to whole blood made thrombocytopenic increased the fibrin deposition (60). If the events observed *in vitro* also occur *in vivo*, these may contribute to the haemostatic effect of rFVIIa in situations characterized by low platelet counts associated with uncontrolled haemorrhage (25). Anecdotal reports of a haemostatic effect in thrombocytopenic patients have been published (25). Furthermore, successful use of rFVIIa in patients with thrombasthenia Glanzmann are reported (61), and its use in such patients, who do not benefit from platelet transfusion, is approved by the European Medicines Agency (EMA). The dosage recommended is similar to the haemophilia dosage, 70–120 µg/kg every other hour in serious bleeding and surgery (42). Also, in patients with von Willebrand's disease, type III as well as in type II of the disease, a successful use of rFVIIa has been reported. In a total of 48 patients with congenital von Willebrand's disease a success rate of 96% was reported (62).

In patients with a normal basal haemostatic process, an impaired thrombin generation may occur when they are subjected to severe trauma or extended surgery. In these patients, a combination of dilution coagulopathy and local release of proteolytic enzymes caused by extensive tissue damage degrading coagulation proteins may develop. As a result of the dilution coagulo-

pathy, loose, porous fibrin deposits may form, which will be easy targets for premature dissolution by released enzymes leading to profuse, diffuse bleeding at sites of tissue damage. This process may be mainly localized without signs of generally increased fibrinolytic activity in the circulation. Successful use of rFVIIa in severely traumatized patients has been reported and rFVIIa has also been found effective in patients with uncontrollable haemorrhage unresponsive to conventional therapy (25). A special situation characterized by profuse, massive bleeding is the postpartum bleedings. successful use of rFVIIa, often administered as one single dose (90–100 µg/kg), has been reported in such patients (63). Anecdotal reports on the successful use of rFVIIa in cardiac and vascular surgery (64, 65) as well as in uncontrollable postoperative bleedings (32) were published. Furthermore, anecdotal reports of successful use of rFVIIa in patients with increased risk of bleeding due to treatment with anticoagulants are available (42).

Successful prophylactic use of rFVIIa in patients without any preformed coagulation disorder, but were subjected to surgery expected to release an abundance of fibrinolytic enzymes such as surgical prostatectomy was reported. A single dose of rFVIIa administered immediately before the expected release of fibrinolytic enzymes may have helped to generate extra thrombin, resulting in the formation of tight fibrin plugs resistant to the fulminant fibrinolysis occurring locally. A single dose of rFVIIa was also reported to limit the growth of an intracerebral hematoma in patients with intracerebral haemorrhage. In these patients, the formation of a stable fibrin plug resistant to the fibrinolytic activity surrounding the primary hematoma may have contributed to the effect (32, 42).

An extensive report on 22 placebo-controlled, randomized trials using rFVIIa in non-haemophilia patients was recently published (73). The conclusion from this review was that the use of rFVIIa reduced the need for blood transfusion and may reduce mortality. Furthermore, it did not increase the risk of venous thrombosis, but may increase the risk of arterial thrombosis. This latter statement originated from the studies in acute cerebral haemorrhage in which more arterial thrombotic events occurred in the group treated with 80 µg/kg. The review of Hsia et al. (73) included seven trials in patients with liver disease (3 liver transplantation, 2 variceal bleedings, 2 liver resection), three in cardiac surgery, four in acute cerebral haemorrhage, two in trauma, two in stem cell transplantation, one in Dengue haemorrhagic fever, one in spinal fusion surgery, one in prostatectomy, and one in pelvic/acetabular fractures.

## Safety

No side effects have been observed in healthy volunteers (66, 32). A thorough review of all adverse events observed among the more than 700,000 standard doses (90 µg/kg) of rFVIIa administered between 1996 and 2003 was reported in detail (67). They concluded that in no case it could be clearly determined that rFVIIa was definitely causally related to the thromboembolic event. Furthermore, they concluded that the incidence of thrombotic events with the use of rFVIIa is extremely low (67). The same conclusion was drawn by Roberts et al. (68) who found the rate of serious adverse events to be less than 1% in spite of administered extensively in many situations that predispose to thrombosis. A report including 11,000 patients who had received rFVIIa, found a rate of 1.5% of thrombotic events. Almost all of these occurred in non-haemophilia patients with an underlying condition predisposing to thrombosis. The authors pointed out that the spontaneous reporting system data presented does not allow the determination of the frequency of thromboembolic adverse events (69). The localized effect of rFVIIa through the binding to tissue factor-expressing cells and activated platelets most probably makes the drug safe (70).

No indication of the formation of inhibitory antibodies against rFVIIa was seen in patients with haemophilia or in non-haemophilia patients treated with rFVIIa. However, FVII-deficient patients are at risk for development of antibodies against FVII (42).

Summarizing the experience of rFVIIa in bleeding-associated pathologies pharmacological doses have been found to be effective to an extent of allowing major orthopedic surgery in severe haemophilia patients with inhibitors, which is the most challenging potential bleeding situation known. As a result of its capacity to generate thrombin on the surface of activated platelets at the site of injury and thereby ensure an increased platelet activation and adhesion as well as the formation of a tight, well-structured fibrin haemostatic plug at the site of injury, it has also been shown to be an active haemostatic agent in other bleeding situations than those occurring in haemophilia patients. Thus, quite a number of placebo-controlled and randomized studies have demonstrated haemostatic effect in bleedings induced by trauma or extensive surgery without increasing the number of thrombo-embolic events. Of special interest is the efficacy in stopping postpartum haemorrhages, the most common complication of delivery with a high mortality among young women.

## References

1. Østerud B, Rapaport SI. Activation of factor IX by the reaction product of tissue factor and factor VII: Additional pathway for initiating blood coagulation. *Proc Natl Acad Sci USA* 1977; 74: 5260–5264.
2. Monroe DM, et al. Platelets and Thrombin Generation. *Arterioscler Thromb Vasc Biol* 2002; 22: 1381–1389.
3. Roberts HR, et al. Molecular biology and biochemistry of the coagulation factors and pathways of haemostasis. In: *Williams Hematology*, 6<sup>th</sup> Ed. McGraw-Hill Companies Inc. 2001; Chapter 112: p. 1409–1434.
4. Rapaport SI, Rao LVM. The tissue factor pathway: How it has become a "Prima Ballerina". *Thromb Haemost* 1995; 74: 7–17.
5. Hoffman M, et al. Tissue factor around dermal vessels has bound factor VII in the absence of injury. *J Thromb Haemost* 2007; 5: 1403–1408.
6. Shaw AW, et al. The local phospholipid environment modulates the activation of blood clotting. *J Biol Chem* 2007; 282: 6556–6563.
7. Mandal S, et al. Acute cholesterol depletion impairs functional expression of tissue factor in fibroblasts: modulation of tissue factor activity by membrane cholesterol. *Blood* 2005; 105: 153–160.
8. Chen VM, et al. Evidence for activation of tissue factor by an allosteric disulfide bond. *Biochemistry* 2006; 45: 1202–12028.
9. Manukyan D, et al. Protein disulfide isomerase as a trigger for tissue factor-dependent fibrin generation. *Thromb Res* 2008; 122 (Suppl 1): S19–S22.
10. Reinhardt C, et al. Protein disulfide isomerase acts as an injury response signal that enhances fibrin generation via activation of tissue factor. *J Clin Invest* 2008; 118: 1110–1122.

11. Cho J, et al. A critical role for extracellular protein disulfide isomerase during thrombus formation in mice. *J Clin Invest* 2008; 118: 1123–1131.
12. Pendurthi UR, et al. Tissue factor activation: is disulfide bond switching a regulatory mechanism? *Blood* 2007; 110: 3900–3908.
13. Lopez-Vilchez I, et al. Tissue factor-enriched vesicles are taken up by platelets and induce platelet aggregation in the presence of factor VIIa. *Thromb Haemost* 2007; 97: 202–211.
14. Panes O, et al. Human platelets synthesize and express functional tissue factor. *Blood* 2007; 109: 5242–5250.
15. Rauch U, et al. Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. *Blood* 2000; 96: 170–175.
16. Mandal SK, et al. Cellular localization and trafficking of tissue factor. *Blood* 2006; 107: 4746–4753.
17. Andrews RK, et al. Platelet adhesion receptors and (patho)physiological thrombus formation. *Histol Histopathol* 2001; 16: 969–980.
18. Pieters J, et al. In situ-generated thrombin is the only enzyme that effectively activates factor VIII and factor V in thromboplastin-activated plasma. *Blood* 1989; 74: 1021–1024.
19. Walsh PN. Platelet coagulation-protein interactions. *Sem Thromb Haemostasis* 2004; 30: 461–471.
20. Kjalke M, et al. Preferential localization of recombinant factor VIIa to platelets activated with a combination of thrombin and a glycoprotein VI receptor agonist. *J Thromb Haemost* 2007; 5: 774–780.
21. Melton LG, et al. Location of the platelet binding site in zymogen coagulation factor IX. *Blood Coag Fibrinol* 2001; 12: 237–243.
22. Allen GA, et al. Impact of procoagulant concentration on rate, peak and total thrombin generation in a cell-based model system. *J Thromb Haemost* 2004; 2: 402–413.
23. Blombäck B. Fibrinogen and fibrin – proteins with complex roles in haemostasis and thrombosis. *Thromb Res* 1996; 83: 1–75.
24. Collet JP, et al. Dynamic changes of fibrin architecture during fibrin formation and intrinsic fibrinolysis of fibrin-rich clots. *J Biol Chem* 2003; 278: 21331–21335.
25. Hedner U. Mechanism of action, development and clinical experience of recombinant FVIIa. *J Biotechnol* 2006; 124: 747–757.
26. Hedner U, et al. Studies on the thrombogenic activities in two prothrombin complex concentrates. *Thromb Haemost* 1979; 42: 1022–1032.
27. Hedner U, Kisiel W. Use of human factor VIIa in the treatment of two haemophilia A patients with high-titer inhibitors. *J Clin Invest* 1983; 71: 1836–1841.
28. Hedner U, et al. Successful use of recombinant factor VIIa in patient with severe haemophilia A during synovectomy. *Lancet* 1988; 2: 1193.
29. Shapiro AD, et al. Prospective, randomised trial of two doses of rFVIIa (NovoSeven) in haemophilia patients with inhibitors undergoing surgery. *Thromb Haemost* 1998; 80: 773–778.
30. Lusher J, et al. Clinical experience with recombinant factor VIIa. *Blood Coagul Fibrinolysis* 1998; 9: 119–128.
31. Key NS, et al. Home treatment of mild to moderate bleeding episodes using recombinant factor VIIa (NovoSeven) in haemophiliacs with inhibitors. *Thromb Haemost* 1998; 80: 912–918.
32. Hedner U, Erhardtsen E. Potential role of recombinant factor VIIa as a haemostatic agent. *Clin Adv Hematol Onc* 2003; 1: 112–119.
33. Monroe DM, et al. Platelet activity of high-dose factor VIIa is independent of tissue factor. *Br J Haematol* 1997; 99: 542–547.
34. Rao LVM, Rapaport SI. Factor VIIa-catalyzed activation of factor X independent of tissue factor: its possible significance for control of haemophilic bleeding by infused factor VIIa. *Blood* 1990; 75: 1069–1073.
35. Hedner U. Factor VIIa in the treatment of haemophilia. *Blood Coagul Fibrinolysis* 1990; 1: 307–317.
36. Telgt DSC, et al. Mechanism by which recombinant factor VIIa shortens the APTT: activation of factor X in the absence of tissue factor. *Thromb Res* 1989; 56: 603–609.
37. Allen GA, et al. A variant of recombinant factor VIIa with enhanced procoagulant and antifibrinolytic activities in an in vitro model of haemophilia. *Arterioscler Thromb Vasc Biol* 2007; 27: 683–689.
38. Allen GA, et al. Manipulation of prothrombin concentration improves response to high-dose factor VIIa in a cell-based model of haemophilia. *Br J Haematol* 2006; 134: 314–319.
39. Lisman T, et al. Inhibition of fibrinolysis by recombinant factor VIIa in plasma from patients with severe haemophilia A. *Blood* 2002; 99: 175–179.
40. He S, et al. The role of recombinant factor VIIa (FVIIa) in fibrin structure in the absence of FVIII/FIX. *J Thromb Haemost* 2003; 1: 1215–1219.
41. Lisman T, et al. Recombinant factor VIIa enhances platelet adhesion and activation under flow conditions at normal and reduced platelet count. *J Thromb Haemost* 2005; 3: 242–751.
42. Hedner U, Ezban M. Tissue factor and factor VIIa as therapeutic targets in disorders of haemostasis. *Ann Rev Med* 2007; 59: 29–41.
43. Lindley CM, et al. Pharmacokinetics and pharmacodynamics of recombinant factor VIIa. *Clin Pharmacol Ther* 1994; 55: 638–648.
44. Fridberg MJ, et al. A study of the pharmacokinetics and safety of recombinant activated factor VII in healthy Caucasian and Japanese subjects. *Blood Coagulation Fibrinolysis* 2005; 16: 259–266.
45. Hedner U, et al. Pharmacokinetics of rFVIIa in children. 23<sup>rd</sup> Int Congress of the World Fed of Haemophilia. The Hague, The Netherlands, May 17–21, 1998.
46. Villar A, et al. Pharmacokinetics of activated recombinant coagulation factor VII (NovoSeven) in children vs. adults with haemophilia A. *Haemophilia* 2004; 10: 352–359.
47. Kavakli K, et al.; for the NovoSeven trial (F7HAEM-1510) investigators. Home treatment of haemarthroses using a single dose regimen of recombinant activated FVII in patients with haemophilia and inhibitors. *Thromb Haemost* 2006; 95: 600–605.
48. Young G, et al. Single 270 ug/kg-dose rFVIIa vs. Standard 90 ug/kg-dose rFVIIa and APCC for home treatment of joint bleeds in haemophilia patients with inhibitors: a randomized comparison. *Haemophilia* 2008; 14: 287–294.
49. Schulman S, et al. Feasibility of using recombinant factor VIIa in continuous infusion. *Thromb Haemost* 1996; 75: 432–436.
50. Schulman S. The onerous task of comparing treatments in inhibitor patients. *Thromb Haemost* 2007; 98: 710–712.
51. Pruthi RK, et al. Haemostatic efficacy and safety of bolus and continuous infusion of recombinant factor VIIa are comparable in haemophilia patients with inhibitors undergoing major surgery. *Thromb Haemost* 2007; 98: 726–732.
52. Hvas AM, et al. Tranexamic acid combined with recombinant factor VIII increases clot resistance to accelerated fibrinolysis in severe haemophilia A. *J Thromb Haemost* 2007; 5: 2408–2414.
53. Manco-Johnson MJ, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe haemophilia. *N Engl J Med* 2007; 357: 535–544.
54. Morfini M, et al. Prophylactic treatment of haemophilia patients with inhibitors: clinical experience with recombinant factor VIIa in European Haemophilia Centres. *Haemophilia* 2007; 13: 502–507.
55. Konkle BA, et al. Randomized, prospective clinical trial of recombinant factor VIIa for secondary prophylaxis in haemophilia patients with inhibitor. *J Thromb Haemost* 2007; 5: 1904–1913.
56. Rapaport SI, Rao LVM. Initiation and regulation of tissue factor-dependent blood coagulation. *Arterioscler Thromb* 1992; 12: 1111–1121.
57. Mandal S, et al. Cellular localization and trafficking of tissue factor in fibroblasts. *Blood* 2006; 107: 4746–4753.
58. Kjalke M, et al. High-dose factor VIIa increases initial thrombin generation and mediates faster platelet activation in thrombocytopenia-like conditions in a cell-based model system. *Br J Haematol* 2001; 114: 114–120.
59. He S, et al. The effect of platelets on fibrin gel structure formed in the presence of recombinant factor VIIa in hemophilia plasma and in plasma from a patient with Glanzmann thrombasthenia. *J Thromb Haemost* 2005; 3: 272–279.
60. Galan AM, et al. Increased local procoagulant action: a mechanism contributing to the favourable haemostatic effect of recombinant FVIIa in PLT disorders. *Transfusion* 2003; 43: 885–892.
61. Poon MC. The evidence for the use of recombinant human activated factor VII in the treatment of bleeding patients with quantitative and qualitative platelet disorders. *Transf Med Rev* 2007; 21: 223–236.
62. Franchini M, et al. The use of recombinant activated factor VII in congenital and acquired von Willebrand disease. *Blood Coag Fibrinol* 2006; 17: 615–619.
63. Karalappillai D, Popham P. Recombinant factor VIIa in massive postpartum haemorrhage. *Int J Obs Anesth* 2007; 16: 29–34.
64. Warren O, et al. Recombinant activated factor VII in cardiac surgery: a systematic review. *Ann Thorac Surg* 2007; 83: 707–714.
65. Warren OJ, et al. Recombinant activated factor VII: a solution to refractory haemorrhage in vascular surgery? *Eur J Vasc Endovasc Surg* 2008; 35: 145–152.
66. Friederich PW, et al. Ability of recombinant factor VIIa to generate thrombin during inhibition of tissue factor in human subjects. *Circulation* 2001; 103: 2555–2559.
67. Abshire T, Kenet G. Recombinant factor VIIa: review of efficacy, dosing regimens and safety in patients with congenital and acquired factor VIII or IX inhibitors. *J Thromb Haemost* 2004; 2: 899–909.
68. Roberts HR, et al. Safety profile of recombinant factor VIIa. *Sem Hematol* 2004; 41 (Suppl 1): 101–108.
69. O’Connell KA, et al. Thromboembolic adverse events after use of recombinant human coagulation factor VIIa. *J Am Med Assoc* 2006; 295: 293–298.
70. Hedner U. Recombinant factor VIIa: its background, development and clinical use. *Curr Opin Hematol* 2007; 14: 225–229.
71. Laurian Y, et al. Use of recombinant activated factor VII as first-line therapy for bleeding episodes in haemophiliacs with factor VIII or IX inhibitors (NOSE-PAC study). *Blood Coagul Fibrinolysis* 1998; 9 (Suppl 1): 155–156.
72. Santagostino E, et al. Home treatment with recombinant activated factor VII in patients with factor VIII inhibitors: The advantages of early intervention. *Br J Haematol* 1999; 104: 22–26.
73. Hsia CC, et al. Use of recombinant activated factor VII in patients without haemophilia. A meta-analysis of randomized control trials. *Ann Surg* 2008; 248: 61–68.