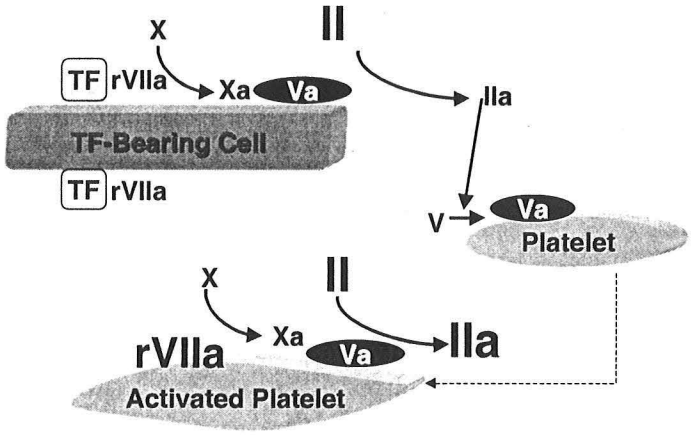
Recombinant factor VIIa: from concept to clinical practice



Cynthia J. Rutherford, M.B.,Ch.B.

Medical Grand Rounds
March 7 2002

University of Texas Southwestern Medical School

Dr. Cynthia Rutherford is Professor of Internal Medicine and a member of the Hematology-Oncology Division in the Simmons Comprehensive Cancer Center. She is Medical Director if the adult program of the North Texas Comprehensive Hemophilia Center.

Her main clinical interests are in "benign" hematologic disorders, particularly hemostatic disorders. Her current research centers around new immunologic approaches to treating immune thrombocytopenic purpura.

The synthetic clotting factor, recombinant Factor VIIa (rVIIa), was originally developed with a fairly narrow therapeutic focus, namely to treat patients with severe hemophilia who had antibodies to their deficient clotting factor. Once its mechanism of action was understood, it was recognized it could be helpful in a much wider range of hemostatic disorders, including treatment for patients with acquired inhibitors of clotting factors, congenital factor VII deficiency, platelet dysfunction, thrombocytopenia, the coagulopathy of advanced liver disease, warfarin overdose and even trauma. The medical scientist involved in rVIIa development recently christened recombinant factor VIIa, the "universal hemostatic agent. "[1]

Factor VII [2]

Naturally formed Factor VII, like most coagulation factors, is secreted by the liver. It is one of six hemostatic proteins whose synthesis is vitamin K-dependent: these include the coagulation factors, Prothrombin (Factor II), Factors VII, IX and X, and two anti-coagulant proteins, Protein C and Protein S. These share many similarities, both in gene and protein structure. All vitamin K-dependent factors circulate as inactive zymogens. Each needs to be activated by a specific factor to express its enzymatic activity. Factor VII circulates as a single chain zymogen of about 50,000 daltons; it has the shortest half-life of all the coagulation factors, about 2½ - 3 hours.

The different regions of factor VII confer different functions. When first synthesized by the liver, it has an amino-terminal leader sequence, which subsequently directs it to the endoplasmic reticulum. Here the enzyme, γ-glutamylcarboxlyase catalyses γ-carboxylation of 10 different glutamic acid (Gla) residues. This step requires the reduced form of vitamin K, which is converted to vitamin K epoxide during the reaction. A specific enzyme, vitamin K epoxide reductase, is required to reconvert vitamin K back to its reduced form. This is the enzyme inhibited by Warfarin, which produces its anticoagulant effect

by reducing the number of γ-carboxy-glutamic acid residues on Factor VII and the other vitamin-K dependent factors. These special Gla residues mediate binding of factor VII to lipid membranes. [3] A hydrophobic region follows the Gla region, with two epidermal growth factor (EDGF) like domains; these are involved in binding factor VII to its main cofactor, Tissue Factor. [4-6] The serine protease domain is in the carboxy-terminal of the protein.

Factor VII binds very strongly to its co-factor, Tissue Factor, even at very dilute concentrations. Once bound, factor VII can be activated to VIIa by a number of different proteases, with the most effective activator believed to be Factor Xa. When the protein is activated it is cleaved into two separate chains, although no portion is actually removed, because of the linking disulfide bond. Binding to Tissue Factor dramatically amplifies the enzymatic activity of Factor VIIa. [7] The Factor VII-Tissue Factor complex enzymatically activates Factor IX and Factor X. It has a natural inhibitor known as Tissue Factor Pathway Inhibitor (TFPI), which is activated when it forms a complex with Factor Xa. [8] TFPI acts as one of several important brakes on the coagulation system, providing negative feedback of this key initial step.

Properties of factor VII

- **Synthesized by the liver**
- **Gene on chromosome 13, 13 kilobases**
- **Single chain zymogen, MW 50,000 daltons**
- **T½ = 2½- 3 hours**
- **Vitamin K-dependent synthesis**
- **Cofactor is Tissue Factor**
- **Substrates are Factor X and Factor IX**
- **Inactivated by Tissue Factor Pathway Inhibitor**

The Coagulation Pathway

To understand how factor VIIa works in hemophilia and other clinical situations, it is necessary to understand the current cell-based model of coagulation. Most of us learned a very different model, the coagulation cascade, to help understand the complexities of the coagulation mechanism. [9, 10] In the

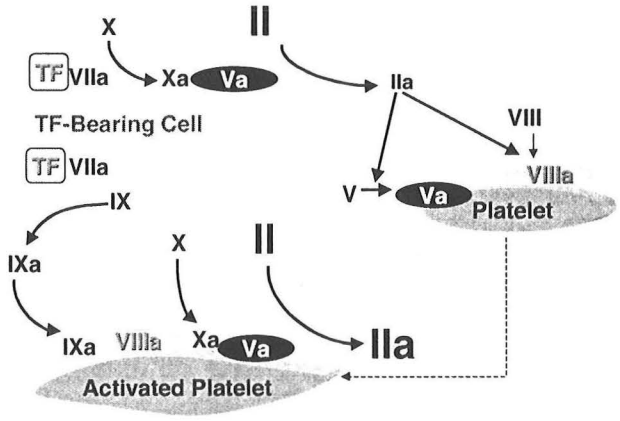
cascade model the circulating inert coagulation factors (zymogens) are successively activated in the plasma. Each newly activated form catalyzes, or acts as cofactor for, the activation of the next. This traditional cascade model is strictly divided into an Intrinsic pathway, in which the coagulation factors are activated within the vascular system, and an Extrinsic pathway, initiated by Tissue Factor outside the circulatory system. The Intrinsic pathway and the Extrinsic pathway do not interact until they finally merge to form the Common pathway, at the point of activation of Factor X to Xa. Following this Thrombin is generated from Prothrombin, and converts soluble fibrinogen to a fibrin clot.

This cascade concept remains useful, primarily in helping us remember the factors measured by the basic coagulation tests: the activated partial thromboplastin time (aPTT) screens the factors involved in the Intrinsic pathway and the Prothrombin time (PT) screens factors involved in the Extrinsic pathway. The concept of successive conversion of the inactive zymogens remains valid. However, this model does not stress that the central initiating event in hemostasis is the formation of the Factor VIIa-Tissue Factor complex at the site of injury. [11, 12] It also vastly under-represents the interactions between the various activated clotting factors: it is well recognized that the intrinsic and extrinsic pathways cannot operate without one another. It also omits the intricate control mechanisms that keep the brakes on coagulation, such as the Tissue Factor Pathway Inhibitor, which inactivates the Factor VIIa-Tissue Factor complex, [8] proteins C and S that inactivate Factors Va and VIIIa and antithrombin. Newer models of the coagulation system have corrected some of these shortcomings, but until recently general understanding has been that coagulation occurs in the plasma, albeit at the site of tissue injury, whereas we now believe that much of the system occurs on cell surfaces. [13, 14]

From their extensive studies of models of coagulation, Butenas and Mann describe three phases in the cell based coagulation including:

1) an initiation phase in which nanomolar amounts of Thrombin and femto- to pico-molar quantities of the other coagulation enzymes are generated, enough to begin platelet activation.
2) a propagation phase in which there is explosive prothrombin activation and generation of Thrombin – the Thrombin "burst."
3) a termination phase, where the procoagulants are rendered inactive by their various inhibitors.

Cell-based model of normal hemostasis
Initiation and propagation phases



Adapted from (14)

Two types of cells are integral to the current cell-based model, namely platelets and cells that express Tissue Factor on their surface. Epithelial cells, stromal cells and even astrocytes express Tissue Factor; moreover, inflammatory mediators can induce its expression in other cell types, including endothelial cells and monocytes.[15-18]

Once Factor VII binds to Tissue Factor, it is rapidly converted to VIIa. The resulting complex, localized to the site of injury, catalyzes two further reactions, namely Factor X to Xa on the cell surface, and Factor IX to IXa. Although Factor IXa is generated in the vicinity of the Tissue Factor-bearing cell it diffuses away from the cell, usually binding to activated platelets nearby.

Factor IXa has no natural inhibitors, unlike Factor Xa, which is inhibited by the Tissue Factor Pathway Inhibitor, and is rapidly inactivated immediately it leaves the cell surface.

Properties of Tissue Factor

- Unique structure in coagulation system
- Only transmembrane protein
- Expressed on many extravascular tissues:
  - adventitia of blood vessels, epidermal cells, stromal cells and astrocytes
- With inflammation may be expressed on:
  - vascular endothelium and monocytes
- Binds factor VII and VIIa
- Inactivated by Tissue Factor Pathway Inhibitor (TFPI)

On the cell surface Factor Xa interacts with its cofactor, Factor Va, to form a proteolytic complex ("Prothrombinase".) This generates a tiny amount of Thrombin from Prothrombin circulating in the vicinity of the cell. This tiny quantity of Thrombin immediately begins "priming" activities, including activating platelets, Factor V and Factor VIII. Thus events are set in motion to prepare the coagulation system to generate a subsequent powerful "burst" of Thrombin in the propagation phase.

Once adjacent platelets become activated, membrane changes expose phosphatidylserine residues, and provide a surface on which further coagulation complexes can be assembled. Factor IXa combines with its cofactor, Factor VIIIa to form "tenase", which cleaves circulating Factor X. Factor Xa combines with its cofactor, Factor Va on the platelet surface to form "Prothrombinase." Now there is enough enzyme power to convert Prothrombin to Thrombin in the larger quantities needed to coagulate fibrinogen and begin the formation of the fibrin-platelet clot.

Congenital Hemophilia

In congenital hemophilia there is a defect in production of Factor VIII (Hemophilia A) or Factor IX (Hemophilia B.) Either of these defects leads to

problems generating sufficient factor Xa on the platelet surface and results in an inadequate Thrombin burst. Patients with either type of inherited hemophilia have an identical clinical phenotype, in which joint, muscle and to a less extent soft tissue bleeding occur, with clinical severity closely related to the level of the affected factor. Those patients defined as severe hemophiliacs have less than 1% of the normal factor level, and usually have frequent spontaneous joint and deep muscle bleeds. Modern management with recombinant or highly purified clotting factors has dramatically altered the course of the disease, and the majority of patients now treat their disease at home. Prophylactic treatment, and early treatment of bleeding episodes means the majority of hemophilia patients can live a nearly normal life, in both quality and life span.

The development of an antibody to a clotting factor in a patient with hemophilia is a very serious event, and dramatically changes the management and clinical course of the disease.[19-21] Such antibodies, usually known as inhibitors, are most likely to develop in patients who have severe hemophilia, with baseline factor levels less than 1%. The patient's immune system recognizes transfused clotting factor as "foreign" and generates antibodies. These inhibitors make subsequent treatment very difficult as transfused clotting factor is destroyed immediately. Many such patients end up having serious bleeding problems and progressive joint disease. Factor VIII inhibitors are proportionately more common than Factor IX inhibitors.

A number of strategies have been developed for both long and short and term management of these inhibitors. Long-term management involves the induction of immune tolerance, in which daily doses of factor are administered, usually for a period of weeks to months. [22, 23] In some patients, especially in children, and particularly if the inhibitor has not been present for long, this is successful; after an initial rise in inhibitor titer, it subsequently falls, hopefully to zero, despite ongoing administration of factor. There is no consensus about the best way to induce immune tolerance, either the dose of factor, or the use of

concurrent immune suppression. The only consensus is that it is very expensive and relatively unpredictable.

Management of acute bleeding depends on the titer of the inhibitor, and whether in the past the patient has been shown to have a strong anamnestic response to clotting factor. If the antibody titer is less than 5 Bethesda units, and the patient is a "low responder," it is sometimes possible to "flood" the inhibitor, using very high doses of factor replacement. If the inhibitor is to human Factor VIII alone, a porcine Factor VIII concentrate can be used. If the inhibitor titer is greater than 5 Bethesda units, or the patient has a history of very quickly boosting inhibitor titers, it is usually necessary to "bypass" the inhibited factor, by using pre-activated clotting factors.

Treatment of inhibitors in congenital hemophilia

- **Long term**
  - **Immune tolerance induction – daily doses of factor ± immunosuppressive drugs**
- **Short term**
  - **"Flood" the inhibitor, if titer low (<5 BU)**
  - **Porcine factor VIII in hemophilia A**
  - **Prothrombin complex concentrates, containing (activated) factors II, VII, IX and X**
  - **Recombinant factor VIIa**

Prothrombin complex concentrates were developed for this purpose in the 1970's. [24, 25] These concentrates consist of the Vitamin K-dependent proteins, factors II, VII, IX and X. The manufacturing process usually partially activates the factors, and in some of these concentrates they have been deliberately activated. These activated clotting factors, especially IIa, VIIa and Xa bypass the factor affected by the inhibitor. They produce variable effects clinically, and one cannot assess response quantitatively: for example the aPTT is rarely corrected. They carry a high risk of thrombotic complications. Moreover, they often contain low levels of other clotting factors, including Factor VIII, which can boost the titer of the existing inhibitors.

In the 1980's it was recognized that Factor VIIa alone would be a more suitable substitute to bypass the inhibited Factor VIII or IX. Unlike the other activated vitamin K-dependent factors, Factor VIIa cannot initiate coagulation at physiologic concentrations, unless it is bound to its cofactor, Tissue Factor. Theoretically it would be less likely to be thrombogenic. Hedner, a Swedish hematologist, then involved in the care of hemophilia patients, laboriously purified Factor VII from normal human plasma, activated it and successfully administered it to two hemophiliac patients with inhibitors against Factor VIII. [26] She recognized it was impractical to purify Factor VIIa in quantity from plasma; moreover such a product would carry a high risk of viral contamination. Soon after she focused her efforts develop a recombinant form of factor VIIa, in collaboration with the Danish company, Novo Nordisk. The project began in 1985, and three years later the first patient was treated with recombinant Factor VIIa (rVIIa). [27] At that time rVIIa was concurrently in intensive research trials, first in animals, [28-30] and in humans, studies which are continuing.

Production of recombinant VIIa [31]

The gene for Factor VII, known to be on chromosome 13, has been isolated from a liver gene library, amplified and transferred to a baby hamster kidney cell line. This transfected cell line secretes Factor VII in its usual single chain form into the supernatant. Filtration is used to remove viral particles, then immunoaffinity chromatography to isolate Factor VII. Ion exchange chromatography is used to activate Factor VII to VIIa. After analysis to confirm purity of rVIIa, and to rule out viral and *Mycoplasma* contamination, the product is lyophilized.

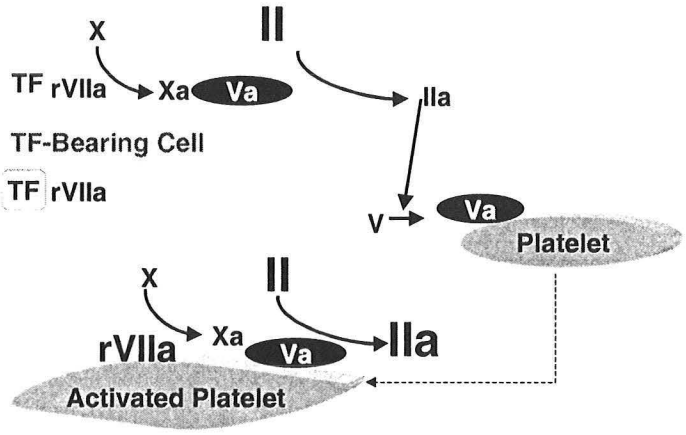
Analysis shows rVIIa is identical to naturally occurring Factor VIIa in terms of amino acid sequence and composition. The γ-carboxylation is nearly identical: natural factor VII has 10 γ-carboxylated Gla residues, whereas the

recombinant product has 9 fully γ-carboxylated residues, and one partially γ-carboxylated. There are minor differences in carbohydrate composition, but no difference *in vitro* in terms of enzymatic activity.[32]

Mode of action of recombinant factor VIIa in Hemophilia

In hemophilia A or B there is inadequate Thrombin production. The deficient binding of Factor VIIIa or Factor IXa on the platelet surface interferes with the production of the Factor VIIIa/Factor IXa complex, known as "tenase," needed to convert Factor X to Xa and generate the major Thrombin burst.

Action of pharmacologic doses of factor VIIa in hemophilia



Adapted from (14)

Recombinant VIIa in pharmacologic doses (10nM) can bind directly to phospholipid surfaces, without first binding to tissue factor [14, 33, 34]. It binds to the phosphatidylserine residues of the activated platelet membrane, and activates circulating factor X directly, so that factor Xa is bound to the platelet membrane in quantities sufficient to generate the Thrombin burst.

Use of recombinant Factor VIIa in Hemophilia

The first patient was successfully treated with rVIIa in 1988. [27] He was a hemophiliac with inhibitors who underwent an open synovectomy; he received what we would now regard as a relatively low dose, 54mcg/Kg pre-operatively, another dose 2 hours after surgery, then 4 hourly infusions for 2 days and 6 hourly for 10 days. This was before much pharmacokinetic data was available.

Subsequently numerous trials have been undertaken. One of the earliest and largest of these was by Lusher *et al*, who in a randomized, double blind, dose-finding study treated 179 bleeding episodes in 78 hemophilia patients at rVIIa doses of 35mcg/Kg or 70mcg/Kg. [35] Treatment began within 9 hours of a bleeding episode, and doses were repeated 2 hourly, up to 6 doses. The treatment was very effective in 60% and moderately effective in a further 12%. There was no difference in efficacy or in the number of doses needed between these two dose levels. More recent studies suggest that better efficacy is obtained from somewhat higher dosing; the current recommended dose is 90mcg/Kg, given every 2 hours, with dosing intervals extended as symptoms abate. [36] Children clear rVIIa more rapidly, and may need higher doses than 90mcg/Kg. [37] Since VIIa only produces its effect at sites of tissue damage, there seems a significant safety margin in terms of thrombotic risk, particularly in children, and doses up to 300 mcg/Kg have been administered without problems. [38] Home treatment with bolus dosing has been found safe and efficacious; logically, the earlier treatment is initiated the fewer doses are needed. [39]

In addition to regular treatment of joint and other bleeding, rVIIa has been used safely in a number of surgical procedures. These include minor procedures such as line placement, dental surgery, and skin biopsy, but recently more major procedures, including synovectomy, liver biopsy, joint fusion and arthroplasty have been successfully undertaken. One teenager successfully underwent cardiac transplant using factor VIIa, after he sustained severe myocardial damage from an infarct in the setting of the thrombogenic prothrombin complex concentrate. [40]

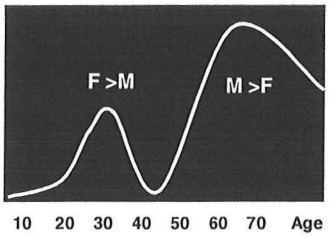
Most of these surgical procedures would have been unthinkable in the days of prothrombin complex concentrates. Even with rVIIa, surgical procedures are not undertaken lightly. Ingerslev recommends a pre-operative pharmacokinetic trial, and the use of all available adjuncts including fibrin glue, topical thrombin and fibrinolytic inhibitors, unless the latter are contra-indicated by liver disease. [41-43]

Acquired coagulation inhibitors

Once the efficacy of rVIIa in bypassing coagulation inhibitors had been demonstrated in congenital hemophilia, it was a logical step to use it in acquired hemophilia. In this condition patients who have previously had normal hemostasis, develop autoantibodies to coagulation factors; these are usually, but not exclusively, to factor VIII. [44] This autoimmune disorder occurs in a variety of situations: about half the time there is some associated condition: other autoimmune disorders, pregnancy, lymphoproliferative disorders or solid tumors. There is a bimodal age distribution with pregnant women and autoimmune diseases in the younger age peak, and the older patients with malignancy, or with the idiopathic variant in the larger peak seen in the older age group. Fortunately, this condition is relatively rare, occurring about 1:1,000,000 persons per year.

Patients with acquired hemophilia often present with dramatic bleeding. Despite the fact that the antibodies are usually directed at Factor VIII, these patients have a very different bleeding pattern from congenital hemophilia; joint bleeds are rare and the patients often have striking skin or muscle bleeds or bleeding from the GI tract. Green reported that 87% patients with acquired inhibitors present with bleeding. [45] There is a much higher mortality than in hemophilia, of the order of 15 to 20%.

Incidence of acquired coagulation inhibitors



As with patients with congenital hemophilia the goal of treatment in this group of disorders is two-fold. Usually patients present with severe bleeding, so the first call of business is to stop the bleeding and the long-term strategy is to evaluate for underlying disorders and try to eradicate the inhibitor. The same management strategies for congenital hemophilia are employed: the affected factor is identified and the titer of its inhibitor. With low level inhibitors, (less common in this situation) high doses of human factor VIII can be used; porcine factor VIII can be used if there is no cross reactivity, [46] or the inhibitor can be bypassed with prothrombin complex concentrates. [24, 25] None of these solutions is very effective.

Recombinant factor VIIa has been used for this group of patients with some success. Data on its use in this group of patients was collected from the compassionate use protocols. This was not prospective data, and the study required that patients had already failed other therapies before being enrolled. Despite this stipulation, rVIIa was used as first line therapy in 14 bleeding episodes in 6 patients, with 100% reporting "good" efficacy. Of 60 episodes in the remaining 29 patients, for whom rVIIa was salvage therapy, 75% were judged as "good", 17% as "partial" and 8% as "poor." Overall mortality was 7.9%, which compares favorably with reported mortality of 15-20%. [47]

Hereditary Factor VII Deficiency

Inherited deficiency of Factor VII is an uncommon autosomal recessive disorder, with an incidence of 1:500,000. Thirty-four different mutations have

been reported to cause factor VII deficiency: 30 single base pair substitutions and 4 short deletions. [48-51] The effect of these mutations, combined with the known polymorphic variations in the gene, results in highly variable Factor VII coagulant activities reported in these patients, even those with the same mutations. Some mutations appear to cause more severe clinical symptoms, including the two-thirds that affect the serine protease domains. Most symptomatic patients are homozygotes, occasionally double heterozygotes. Heterozygotes usually have a very mild clinical syndrome, or are hemostatically normal. The clinical phenotypes are also quite variable; moreover they correlate poorly with Factor VII coagulant levels. Severe bleeding is usually encountered if the Factor VII coagulant levels are <1%. This bleeding can include hemarthroses, epistaxis, menorrhagia, and gastro-intestinal bleeding. [52]

In the past, congenital Factor VII deficiency has primarily been treated with fresh frozen plasma and occasionally with the prothrombin complex concentrates containing the Vitamin K-dependent Factors, because they include Factor VII/VIIa. The level of Factor VII needed for adequate hemostasis is not certain, but levels of 15 – 25% of normal are thought to be adequate for achieving normal hemostasis. Because the half-life of Factor VII is so short, it is difficult to administer plasma in sufficient quantities for prolonged bleeding or surgery without causing volume overload. For this reason, rVIIa appears to be a very good option for treatment of these uncommon patients. [53-55]

The doses needed to provide the hemostatic levels in the plasma are significantly less than those needed to treat patients with hemophilia and inhibitors, and recombinant Factor VIIa is usually given at a dose of 10 – 30 mcg/Kg. Small series of patients have been reported: one reported 10 patients treated with rVIIa for 21 bleeding episodes, including orthopedic surgery and cesarean section, with 95% efficacy. Doses of 15-30mcg/Kg were used, administered every 4-6 hours. Two patients in a series of 13 with congenital factor VII deficiency developed antibodies, which reacted with both plasma-

derived and recombinant factor VII. As in hemophilia, the patients who developed inhibitors were those with very low levels of their own factor. [56]

Use of Recombinant factor VIIa in Cirrhosis

In patients with advanced liver disease and cirrhosis, particularly those in Child-Pugh B and C classes, a coagulopathy is both integral to the classification and reflects a major clinical problem. [57-59] The hemostatic defect is often multi-factorial. Most coagulation factors are low, since all, except factor VIII, are synthesized solely by the liver. Thrombocytopenia associated with hypersplenism and low thrombopoietin levels also contributes, as does hyperfibrinolysis. [60] The deficiency of factor VII is usually more severe than other clotting factors, primarily because of its short half-life.

In the past most patients with this type of coagulopathy needed treatment with fresh frozen plasma (FFP), should they bleed or need a surgical procedure. The volumes of FFP needed are large and must be repeated frequently because of the short half-life of Factor VII. This can produce very problematic volume overload in such patients; this may worsen portal hypertension and paradoxically lead to further bleeding. Because the amount of Factor VII in FFP is quite variable, it is necessary to monitor the PT frequently to ensure adequate treatment. [60]

Thus, hepatologists were amongst the early physicians outside the hemophilia field to use recombinant Factor VIIa. Jeffers *et al* used it as an adjunct to laparoscopic liver biopsy, a potentially hazardous procedure in this patient group, using the low dose of 5mcg/Kg. [61] None of the five patients receiving the factor had bleeding complications during or after their procedure. Bernstein *et al* have used it to correct the prolonged prothrombin time in patients with Child's Class B or C cirrhosis, with PTs prolonged 7-18 seconds above normal. [62, 63] The efficacy was dose dependent, as measured by the prothrombin time. A dose of 5 mcg/Kg normalized the PT for up to two hours,

20 mcg/Kg normalized the dose for four hours; at 80 mcg/Kg the mean PT remained normal for more than 12 hours.

Rapaport points out that the *in vitro* correction of the PT by recombinant Factor VIIa may not reflect the *in vivo* events, because the PT testing system requires only very modest thrombin generation to convert enough fibrinogen to fibrin to be detected by with the fibrinometer. [64] Even if factor VII is boosted there may still not be enough factor X, Prothrombin and fibrinogen for clinical hemostasis, so caution is warranted in interpreting Bernstein's laboratory studies.

In this particular patient group, the issue of initiating or worsening disseminated intravascular coagulation (DIC) is particularly germane, as patients with liver disease have a reduced capacity to clear activated coagulation factors, and often have elevated plasma levels of D-dimers and prothrombin fragment 1+2, suggesting a pre-existing background of low grade DIC. Bernstein found no significant change in DIC parameters including fibrinogen levels, D-dimers, fibrinopeptide A, prothrombin 1+2, and APTTs. Moreover, none of his patients demonstrated antibodies to Factor VII.

Other uses of rVIIa have been described in patients with liver disease, to reduce blood loss in major liver resection and even orthotopic liver transplantation. Patient and graft survival after liver transplant are significantly lower in patients who are transfused more than 10 units of red blood cells; one pilot study has shown definite reduction in transfusion requirements in patients who received perioperative rVIIa. [65]

Recombinant VIIa in Treatment of Bleeding Secondary to Warfarin Overdose.

Patients receiving Warfarin type anticoagulation are at significant risk of bleeding. [66] Even with PT-INRs in the therapeutic range, bleeding can be a problem, with an estimated 3% major bleeding episodes per year, and an exponentially increasing risk when the PT-INR is supra-therapeutic. In patients who need ongoing anticoagulation, but have a markedly supra-therapeutic PT-

INR, there is always a therapeutic dilemma: to what degree to correct the coagulopathy, whether to use low dose Vitamin K repletion or plasma replacement. Correction by vitamin K does not usually begin for 8-24 hours; moreover, it can make subsequent anticoagulation difficult. If there are bleeding problems, or the PT-INR is dangerously high, clotting factors must be replaced. Traditionally, fresh frozen plasma has been used as the source of these depleted clotting factors, with the few patients where volume was crucial receiving prothombin complex concentrates. The latter are risky in this clinical situation, because they are potentially thrombogenic in a patient population who need the opposite effect.

Factor VII levels are likely to be the lowest of all the vitamin K-dependent factors, because of its short T½. A number of both animal and human studies have been carried out in this clinical scenario using rVIIa. The length of time that the PT-INR is corrected is related to the actual dosing, but again there are concerns, as with patients with liver disease, who also have low levels of multiple coagulation factors, that PT-INR is too insensitive in this setting. This is definitely an area where more study is needed.

Platelet Disorders and Recombinant VIIa

The platelet is integral to hemostasis, and provides one of the vital cellular surfaces for assembly of the coagulation system, especially for the generation of "tenase." The effect of recombinant factor VIIa has been evaluated in both platelet dysfunction and in thrombocytopenic states. Glanzmann's thrombasthenia is a rare autosomal recessive disorder where platelet dysfunction is caused by the absence of glycoprotein IIb/IIIa on the platelet membrane. This causes defective platelet aggregation because of inadequate binding to fibrinogen and von Willebrand factor. Patients with this disorder have mild to severe mucocutaneous bleeding, a long bleeding time and absent aggregation in vitro to all the usual platelet agonists. Although day-to-day symptoms are mild in most patients with Glanzmann's thrombasthenia, bleeding can be severe in the setting

of trauma or surgery. Such patients often need platelet transfusion, with attendant risks of viral transmission and alloimmunization.[67]

This rather rare disease now has an International Registry, which includes 44 Glanzmann's thrombasthenia patients and 4 with the equally rare form of platelet dysfunction, Bernard Soulier syndrome. [68] Recombinant VIIa was used for 21 invasive procedures and 76 bleeding episodes in these patients. Treatment was effective in 89% of the invasive procedures, but only 69% of the bleeding episodes, some of the latter probably received inadequate dosing.

It is of interest that two patients in this group treated by continuous infusion of factor had thrombotic complications: one developed a clot in the renal pelvis after gynecological surgery and the other developed bilateral DVT and pulmonary embolism six days after fairly high dose continuous infusion. Notwithstanding, Koon believes rVIIa, especially bolus doses, is a safe and effective alternative form of treatment for this uncommon form of platelet dysfunction, especially in patients alloimmunized to platelets transfusion.

In vitro experiments have shown that rVIIa can increase the initial thrombin generation seen in thrombocytopenic model systems although it does not normalize the amount of Thrombin produced overall. [69, 70] A single study has looked at administration of rVIIa in thrombocytopenic subjects: 55/105 (52%) had shortened bleeding times after treatment. The reduction in the bleeding time was more pronounced in patients who were more severely thrombocytopenic (<20,000/μl.) All 8 bleeding thrombocytopenic subjects had a reduction in the amount of bleeding, and in 6 it stopped altogether.[71]

Measurement and Pharmacokinetics of Recombinant Factor VIIa

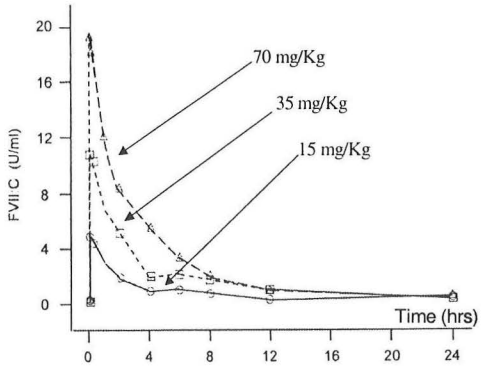
Most assessment of the pharmacologic effect of factor VIIa employs the same assays of factor VII coagulant activity used to assess levels in deficient plasma. The PT is a very unreliable test for following VIIa effect; at pharmacologic doses the PT will be dramatically shortened, because it reflects only the initial trace of thrombin generated *in vivo.*

There are specific assays for activated factor VIIa, such as ELISA methods to measure VIIa antigenically, but they are available in research laboratories only. Others are evaluating the amount of factor Xa or thrombin generated. The search is on for a simple whole blood clotting assay, which will reflect the total generation of Thrombin, and hopefully better reflect *in vivo* events.

The pharmacokinetic profile of rVIIa has been evaluated in a number of different scenarios.[72-74] These include normal healthy adult volunteers pretreated with warfarin anticoagulation, adult and pediatric hemophilia patients with and without inhibitors and patients with advanced cirrhosis. Almost all pharmacokinetic assessment has involved IV bolus administration.

Mean plasma concentration of factor VII after infusion at various doses

Sem Thromb Hemostasis 2000 26:385



A number of different dose-ranging studies have been used in all of these clinical settings, but regardless of the dose, the T½ appears to be the same, in the vicityof 2½ hours. Patients who are actively bleeding demonstrated a slightly shorter half-life during bleeding episodes. Pediatric patients also have a shorter half-life and higher clearance than the adult patients and adult volunteers.[37]

The data on continuous infusion of rVIIa are preliminary, but this method of administration offers theoretical advantages, including simplicity, a steady state of the VIIa activity and lower overall dosing and cost. Schulman [75, 76],

who has reported the data from an international group studying this issue, suggests an initial bolus of rVIIa, prior to infusion, and continuous infusion rates based on an individual patient's pre-assessed clearance rate of the factor. He draws attention to the risk of thrombophlebitis at the infusion site, a complication often seen between 1-3 days, and to the risk of bleeding if pumps malfunction.

Use of recombinant factor VIIa in Trauma and Surgery

Critically ill trauma patients are often profoundly coagulopathy. Their coagulopathy is usually multi-factorial with contributions from DIC, fibrinolysis from release of tissue plasminogen activator (TPA), massive transfusion with dilution of coagulation factors and platelets and "shock liver". After successfully treated an exsanguinating Israeli solder with rVIIa, [77] Martinowitz and colleagues began a prospective randomized study of this agent in swine with grade V liver injury and hypothermia. [78] With promising results from this animal study, they began a compassionate use protocol for patients suffering massive, life-threatening bleeding as a result of trauma or surgery, who had failed all conventional modalities, and where a fatal outcome seemed inevitable. They excluded patients with recent thromboembolism and extensive atherosclerosis. They write "...In all patients diffuse bleeding "dried out" within 5 to 15 minutes after rVIIa administration." There was reduction in transfusion requirements, and dramatic shortening of PT and aPTT. Four of the 7 patients survived; three died, but not specifically from bleeding. [79] Acknowledging the preliminary nature of this uncontrolled study, they have called for controlled animal and clinical trials of the use of rVIIa in this setting.

There are numerous case reports and small case series of the use of rVIIa to control bleeding in other types of surgery. [80-82]

Cost of recombinant VIIa

Recombinant VIIa is an enormously expensive product, an issue of great concern. Not only are the treating physicians concerned, but this is becoming an issue for society at large, especially as the indications for rVIIa use are expanding. The case presented, where treatment appeared dramatically life-saving, used 544mg of rVIIa, which would have cost $440,000, had it not been supplied on a compassionate use base prior to the product's licensure. The case of a patient with acquired hemophilia at Duke received front page coverage in the Wall Street Journal last year when his hospital bill topped $5.2 million, 95% of which was for clotting factor concentrates; daily costs of rVIIa were $40,000 to $50,000. [83] Most coagulation concentrates, recombinant or plasma-derived, are also very expensive, but this product, together with porcine factor VIII, are in a realm all their own.

Safety of Recombinant Factor VIIa

Recombinant factor VIIa appears to cause very little immune reactivity, probably because it is nearly identical to the naturally occurring factor. There are isolated reports of infusion- related reactions. Several patients with severe factor VII deficiency have developed antibodies to factor VII, which precluded further treatment.

Given its powerful effect on thrombin generation, there has been a lot of concern that rVIIa might cause thrombosis [84] , however, reports of this complication have been modest. Continuous infusion administration has been associated with localized thrombophlebitis in the infusing vein [76] and one study in cerebral aneurysm rebleeding was halted when cerebral arterial thrombosis, adjacent to the aneurysm occurred in one patient. [85]

Recombinant factor VIIa was first used clinically in 1988. It was licensed for use in Europe in 1996 and in the U.S. in 1999, and prior to licensure there were several compassionate use trials. Novo Nordisk has recently published safety data from 1988 through May 2001. These show a low rate of thrombotic

complications in the 6500 patients who received more than 180,000 standard doses of the factor. [86] There were 17 episodes of reported thrombosis, 11 patients with arterial thrombosis and 6 with venous thrombosis. Four patients died of thrombotic complications. A number of the patients who had thrombosis were older than 70 years of age, with 4 arterial thrombotic events occurring in the setting of prostatectomy. Roberts concluded that:

> *...rVIIa is safe and effective in hemophilia patients, with or without inhibitors, and in patients with a variety of other bleeding disorders. Thrombotic events occurred rarely and most could be attributed to improvements in the clotting mechanism rather than a direct effect of the rVIIa itself.*

Conclusion

In the fourteen years since it was first used, rVIIa has become recognized as a remarkable hemostatic agent, not quite the "universal hemostatic agent" Hedner claims [1], but one with wide applicability in coagulation disorders. We still need an assay to measure its action and reflects *in vivo* events and a pricing structure that will allow us to use it for its rapidly expanding list of indications. Research into its mode of action has driven the development of the cell-based model of coagulation and dramatically advanced our basic knowledge of many phases of hemostasis.

REFERENCES

1. Hedner, U., *NovoSeven as a universal haemostatic agent.* Blood Coagul Fibrinolysis, 2000. 11 Suppl 1: p. S107-11.
2. Roberts, H.R., D.M. Monroe, and M. Hoffman, *Molecular Biology and Biochemistry of the Coagulation Factors and Pathways of Hemostasis*, in *Williams Hematology*, E. Beutler, Editor. 2001, The McGraw-Hill Companies. p. 1409 - 1434.
3. Sakai, T., et al., *The gamma-carboxyglutamic acid domain of human factor VIIa is essential for its interaction with cell surface tissue factor.* J Biol Chem, 1990. 265(4): p. 1890-4.
4. Banner, D.W., et al., *The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor.* Nature, 1996. 380(6569): p. 41-6.
5. Toomey, J.R., K.J. Smith, and D.W. Stafford, *Localization of the human tissue factor recognition determinant of human factor VIIa.* J Biol Chem, 1991. 266(29): p. 19198-202.

6. Chang, J.Y., D.W. Stafford, and D.L. Straight, *The roles of factor VII's structural domains in tissue factor binding.* Biochemistry, 1995. 34(38): p. 12227-32.
7. van't Veer, C. and K.G. Mann, *The regulation of the factor VII-dependent coagulation pathway: rationale for the effectiveness of recombinant factor VIIa in refractory bleeding disorders.* Semin Thromb Hemost, 2000. 26(4): p. 367-72.
8. Sandset, P.M., *Tissue factor pathway inhibitor (TFPI)--an update.* Haemostasis, 1996. 26 Suppl 4: p. 154-65.
9. Macfarlane, R.G., *An enzyme cascade in the blood clotting mechanism.* Nature, 1964. 202: p. 498-499.
10. Davie, E.W. and Ratnoff. O.D., *Waterfall sequence for intrinsic blood clotting.* Science, 1964. 145: p. 1310-1312.
11. Nemerson, Y., *Regulation of the initiation of coagulation by factor VII.* Haemostasis, 1983. 13(3): p. 150-5.
12. Nemerson, Y., *The tissue factor pathway of blood coagulation.* Semin Hematol, 1992. 29: p. 170.
13. Butenas, S. and K.G. Mann, *Blood coagulation.* Biochemistry (Mosc), 2002. 67(1): p. 3-12.
14. Hoffman, M., D.M. Monroe, 3rd, and H.R. Roberts, *Activated factor VII activates factors IX and X on the surface of activated platelets: thoughts on the mechanism of action of high-dose activated factor VII.* Blood Coagul Fibrinolysis, 1998. 9 Suppl 1: p. S61-5.
15. Edgington, T.S., et al., *Cellular immune and cytokine pathways resulting in tissue factor expression and relevance to septic shock.* Nouv Rev Fr Hematol, 1992. 34 Suppl: p. S15-27.
16. Mackman, N., et al., *Complete sequence of the human tissue factor gene, a highly regulated cellular receptor that initiates the coagulation protease cascade.* Biochemistry, 1989. 28: p. 1755.
17. Eddleston, M., et al., *Astrocytes are the primary source of tissue factor in the murine central nervous sustem.* J. Clin. Invest., 1993. 92: p. 349.
18. Schonbeck, U., et al., *CD40 ligation induces tissue factor expression in human vascular smooth muscle cells.* Am J Pathol, 2000. 156(1): p. 7-14.
19. Aledort, L., *Inhibitors in hemophilia patients: current status and management.* Am J Hematol, 1994. 47(3): p. 208-17.
20. Kulkarni, R., et al., *Therapeutic choices for patients with hemophilia and high-titer inhibitors.* Am J Hematol, 2001. 67(4): p. 240-6.
21. Lusher, J.M., *Inhibitor antibodies to factor VIII and factor IX: management.* Semin Thromb Hemost, 2000. 26(2): p. 179-88.
22. Brackmann, H.H., J. Oldenburg, and R. Schwaab, *Immune tolerance for treatment of factor VIII inhibitors - twenty years: 'Bonn protocol'.* Vox Sang, 1996. 70(Suppl 1): p. 30-35.
23. Di Michele, D.M., *Immune tolerance: a synopsis of the international experience.* Hemophilia, 1998. 4: p. 568-573.
24. Penner, J.A., *Management of haemophilia in patients with high-titre inhibitors: focus on the evolution of activated prothrombin complex concentrate AUTOPLEX T.* Haemophilia, 1999. 5 Suppl 3: p. 1-9.
25. Negrier, C. and e. al, *Multicenter retrospective study on the utilization of FEIBA in Frnace in patient with factor VIII and IX inhibitors.* Thromb Haemost, 1997. 77: p. 1113-1119.
26. Hedner, U., et al., *Clinical experience with human plasma-derived factor VIIa in patients with hemophilia A and high titer inhibitors.* Haemostasis, 1989. 19(6): p. 335-43.
27. Hedner, U., et al., *Successful use of recombinant factor VIIa in patient with severe haemophilia A during synovectomy.* Lancet, 1988. 2(8621): p. 1193.
28. Brinkhous, K.M., et al., *Effect of recombinant factor VIIa on the hemostatic defect in dogs with hemophilia A, hemophilia B, and von Willebrand disease.* Proc Natl Acad Sci U S A, 1989. 86(4): p. 1382-6.
29. Diness, V., T. Lund-Hansen, and U. Hedner, *Effect of recombinant human FVIIA on warfarin-induced bleeding in rats.* Thromb Res, 1990. 59(6): p. 921-9.
30. Hedner, U., J. Ljundberg, and T. Lund-Hansen, *Comparison of the effect of plasma-derived and recombinant human FVIIa in vitro and in a rabbit model.* Blood Coagul Fibrinolysis, 1990. 1(2): p. 145-51.
31. Thim, L., et al., *Amino acid sequence and posttranslational modifications of human factor VIIa from plasma and transfected baby hamster kidney cells.* Biochemistry, 1988. 27(20): p. 7785-93.
32. Bjoern, S., et al., *Human plasma and recombinant factor VII. Characterization of O-glycosylations at serine residues 52 and 60 and effects of site-directed mutagenesis of serine 52 to alanine.* J Biol Chem, 1991. 266(17): p. 11051-7.
33. Monroe, D.M., et al., *A possible mechanism of action of activated factor VII independent of tissue factor.* Blood Coagul Fibrinolysis, 1998. 9 Suppl 1: p. S15-20.
34. Monroe, D.M., et al., *Platelet activity of high-dose factor VIIa is independent of tissue factor.* Br J Haematol, 1997. 99(3): p. 542-7.

35. Lusher, J.M., et al., *A randomized, double-blind comparison of two dosage levels of recombinant factor VIIa in the treatment of joint, muscle and mucocutaneous haemorrhages in persons with haemophilia A and B, with and without inhibitors. rFVIIa Study Group.* Haemophilia, 1998. 4(6): p. 790-8.
36. Key, N.S., et al., *Home treatment of mild to moderate bleeding episodes using recombinant factor VIIa (Novoseven) in haemophiliacs with inhibitors.* Thromb Haemost, 1998. 80(6): p. 912-8.
37. Shapiro, A.D., *Recombinant factor VIIa in the treatment of bleeding in hemophilic children with inhibitors.* Semin Thromb Hemost, 2000. 26(4): p. 413-9.
38. Kenet, G., et al., *Treatment of bleeding episodes in patients with hemophilia and an inhibitor: comparison of two treatment protocols with recombinant activated factor VII.* Blood Coagul Fibrinolysis, 2000. 11 Suppl 1: p. S35-8.
39. Santagostino, E., A. Gringeri, and P.M. Mannucci, *Home treatment with recombinant activated factor VII in patients with factor VIII inhibitors: the advantages of early intervention.* Br J Haematol, 1999. 104: p. 22-26.
40. Sheth, S., et al., *Heart transplant in a factor VIII-deficient patient with a high-titre inhibitor: perioperative management using high-dose continuous infusion factor VIII and recombinant factor VIIa.* Haemophilia, 2001. 7(2): p. 227-32.
41. Schulman, S., et al., *Recombinant factor VIIa in multiple surgery.* Thromb Haemost, 1994. 71(1): p. 154.
42. O'Connell, N., et al., *Recombinant factor VIIa in the management of surgery and acute bleeding episodes in children with haemophilia and high responding inhibitors.* Br J Haematol, 2002. 116(3): p. 632-5.
43. Ingerslev, J., et al., *Major surgery in haemophilic patients with inhibitors using recombinant factor VIIa.* Haemostasis, 1996. 26 Suppl 1: p. 118-23.
44. Boggio, L.N. and D. Green, *Acquired hemophilia.* Rev Clin Exp Hematol, 2001. 5(4): p. 389-404.
45. Green, D. and K. Lechner, *A survey of 215 non-hemophiliac patients with inhibitors to factor VIII.* Thromb Haemost, 1981. 45: p. 200-203.
46. Morrison, A.E., C.A. Ludlam, and C.M. Kessler, *Use of porcine facotr VIII in the treatment of patients with acquired hemophilia.* Blood, 1993. 81: p. 1513-1520.
47. Hay, C.R., C. Negrier, and C.A. Ludlam, *The treatment of bleeding in acquired haemophilia with recombinant factor VIIa: a multicentre study.* Thromb Haemost, 1997. 78(6): p. 1463-7.
48. Carew, J.A., et al., *A new mutation in the HNF4 binding region of the factor VII promoter in a patient with severe factor VII deficiency.* Blood, 2000. 96(13): p. 4370-2.
49. Bharadwaj, D., et al., *Factor VII central. A novel mutation in the catalytic domain that reduces tissue factor binding, impairs activation by factor Xa, and abolishes amidolytic and coagulant activity.* J Biol Chem, 1996. 271(48): p. 30685-91.
50. Chaing, S., et al., *Severe factor VII deficiency caused by mutations abolishing the cleavage site for activation and altering binding to tissue factor.* Blood, 1994. 83(12): p. 3524-35.
51. Herrmann, F.H., et al., *Molecular biology and clinical manifestations of hereditary Factor VII deficiency.* Semin Thromb Hemost, 2000. 26: p. 393-400.
52. Ingerslev, J. and H.L. Kristensen, *Clinical picture and treatment strategies in factor VII deficiency.* Haemophilia, 1998. 4(4): p. 689-96.
53. Bauer, K.A., *Treatment of factor VII deficiency with recombinant factor VIIa.* Haemostasis, 1996. 26 Suppl 1: p. 155-8.
54. Berrettini, M., et al., *Pharmacokinetic evaluation of recombinant, activated factor VII in patients with inherited factor VII deficiency.* Haematologica, 2001. 86(6): p. 640-5.
55. Billio, A., et al., *Successful short-term oral surgery prophylaxis with rFVIIa in severe congenital factor VII deficiency.* Blood Coagul Fibrinolysis, 1997. 8(4): p. 249-50.
56. Nicolaisen, E.M., *Antigenicity of activated recombinant factor VII followed through nine years of clinical experience.* Blood Coagul Fibrinolysis, 1998. 9 Suppl 1: p. S119-23.
57. Craxi, A., C. Camma, and M. Giunta, *Clinical aspects of bleeding complications in cirrhotic patients.* Blood Coagul Fibrinolysis, 2000. 11 Suppl 1: p. S75-9.
58. Kaul, V.V. and S.J. Munoz, *Coagulopathy of Liver Disease.* Curr Treat Options Gastroenterol, 2000. 3(6): p. 433-438.
59. McCormick, P.A. and K.M. Murphy, *Splenomegaly, hypersplenism and coagulation abnormalities in liver disease.* Baillieres Best Pract Res Clin Gastroenterol, 2000. 14(6): p. 1009-31.
60. Rutherford, C.J. and E.P. Frenkel, *Hemostatic Disorders in Liver Disease,* in *Diseases of the Liver,* E.R. Schiff, M.F. Sorrell, and W.C. Maddrey, Editors. 1999, Lippincott-Raven. p. 583-598.
61. Jeffers, L., et al., *The use of recombinant factor VIIa in laparoscopy liver biopsy: A pilot trial.* Gastroenterology, 1998. 114: p. L0275 (abstract).
62. Bernstein, D.E., et al., *Recombinant factor VIIa corrects prothrombin time in cirrhotic patients: a preliminary study.* Gastroenterology, 1997. 113(6): p. 1930-7.

63. Bernstein, D., *Effectiveness of the recombinant factor VIIa in patients with the coagulopathy of advanced child's B and C cirrhosis.* Semin Thromb Hemost, 2000. 26(4): p. 437-8.
64. Rapaport, S.I., *Coagulation problems in liver disease.* Blood Coagul Fibrinolysis, 2000. 11 Suppl 1: p. S69-74.
65. Hendriks, H.G., et al., *Reduced transfusion requirements by recombinant factor VIIa in orthotopic liver transplantation: a pilot study.* Transplantation, 2001. 71(3): p. 402-5.
66. Levine, M.N., et al., *Hemorrhagic complications of anticoagulant treamtnet.* Chest, 2001. 119(1 Suppl): p. 108S-121S.
67. Poon, M.C., et al., *Recombinant factor VIIa is effective for bleeding and surgery in patients with Glanzmann thrombasthenia.* Blood, 1999. 94(11): p. 3951-3.
68. Poon, M.C., et al., *Use of recombinant factor VIIa (NovoSeven) in patients with Glanzmann thrombasthenia.* Semin Hematol, 2001. 38(4 Suppl 12): p. 21-5.
69. 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(1): p. 114-20.
70. Kjalke, M., M. Johannessen, and U. Hedner, *Effect of recombinant factor VIIa (Novoseven) on thrombocytopenia-like conditions in vitro.* Semin Hematol, 2001. 38(4 Suppl 12): p. 15-20.
71. Kristensen, J., et al., *Clinical experience with recombinant factor VIIa in patients with thrombocytopenia.* Haemostasis, 1996. 26 Suppl 1: p. 159-64.
72. Erhardtsen, E., *Pharmacokinetics of recombinant activated factor VII (rFVIIa).* Semin Thromb Hemost, 2000. 26(4): p. 385-91.
73. Erhardtsen, E., et al., *Pharmacokinetics and safety of FFR-rFVIIa after single doses in healthy subjects.* J Clin Pharmacol, 2001. 41(8): p. 880-5.
74. Girard, P., et al., *Population pharmacokinetics of recombinant factor VIIa in volunteers anticoagulated with acenocoumarol.* Thromb Haemost, 1998. 80(1): p. 109-13.
75. Schulman, S., *Safety, efficacy and lessons from continuous infusion with rFVIIa. rFVIIa-CI Group.* Haemophilia, 1998. 4(4): p. 564-7.
76. Schulman, S., *Continuous infusion of recombinant factor VIIa in hemophilic patients with inhibitors: safety, monitoring, and cost effectiveness.* Semin Thromb Hemost, 2000. 26(4): p. 421-4.
77. Kenet, G., et al., *Treatment of traumatic bleeding with recombinant factor VIIa.* Lancet, 1999. 354(9193): p. 1879.
78. Martinowitz, U., et al., *Intravenous rFVIIa administered for hemorrhage control in hypothermic coagulopathic swine with grade V liver injuries.* J Trauma, 2001. 50(4): p. 721-9.
79. Martinowitz, U., et al., *Recombinant activated factor VII for adjunctive hemorrhage control in trauma.* J. Trauma, Injury, Infection and Critical Care, 2001. 51: p. 431-439.
80. Hendriks, H.G., et al., *An effective treatment of severe intractable bleeding after valve repair by one single dose of activated recombinant factor VII.* Anesth Analg, 2001. 93(2): p. 287-9 , 2nd contents page.
81. Levy, J.H., *Pharmacologic preservation of the hemostatic system during cardiac surgery.* Ann Thorac Surg, 2001. 72(5): p. S1814-20.
82. Tarantino, M.D. and R. Aberle, *Recombinant factor VIIa (NovoSeven) for post-prostatectomy hemorrhage in a patient with type I von Willebrand disease.* Am J Hematol, 2001. 68(1): p. 62-3.
83. Winslow, R., *Intensive care,* in *Wall Street Journal.*
84. Aledort, L.M., *rFVIIa--its thrombogenicity.* Thromb Haemost, 2000. 84(3): p. 522-3.
85. Pickard, J.D., et al., *Potential role of NovoSeven in the prevention of rebleeding following aneurysmal subarachnoid haemorrhage.* Blood Coagul Fibrinolysis, 2000. 11 Suppl 1: p. S117-20.
86. Roberts, H.R., *Recombinant factor VIIa (Novoseven) and the safety of treatment.* Semin Hematol, 2001. 38(4 Suppl 12): p. 48-50.