Recombinant Factor VIIA, its Clinical Properties, and the Tissue Factor Pathway of Coagulation

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Abstract: Recombinant factor VIIa (rFVIIa) is a synthetic coagulation protease that is structurally similar to human-derived plasma FVIIa. Pharmacologic doses of rFVIIa have been shown to enhance thrombin generation and assist in forming stable fibrin plugs at the site of injury. Recombinant factor VIIa appears to emerge as a valuable treatment alternative for the treatment of bleeding episodes and for achieving hemostasis post surgery in those with bleeding disorders.

Key Words: Recombinant factor VIIa, tissue factor, hemostasis, coagulation.

INTRODUCTION

The coagulation of blood is a delicately balanced interaction between proteins and cells. When functioning correctly, blood is maintained in a fluid state, but is able to rapidly form a clot and reduce blood loss in the event of vascular disruption. Clot formation is a complex series of protein-cell interactions, initiated by vascular disruption. Ideally this results in a hemostatic plug composed of fibrin and platelets only at the site of injury. The coagulation protein, Factor VII (FVII) is vital during the initiating events of coagulation. In the absence or decreased amounts of factor IX or factor VIII as in hemophilia, the ability to form a stabile fibrin clot diminished as thrombin formation is slowed dramatically. The introduction of rFVIIa has provided effective therapy to those afflicted with this disorder. Soon it became apparent that rFVIIa is effective in controlling bleeding in variety of clinical situations, and the list of rFVIIa application to clinical hemostatic disorders has never ceased to expand. A detailed analysis of the clinical experience with rFVIIa has been the focus of several review articles [1-3]. This mini review summarizes the classic coagulation cascade and the modern tissue factor pathway, the activity of plasma and recombinant Factor VIIa and its clinical properties.

THE COAGULATION CASCADE

The original models of coagulation were constructed as a cascade, with the coagulation factors existing as proenzymes and their activation pathways classified into the Extrinsic, Intrinsic or Common pathway (Fig. **1**) [4, 5]. Subsequent observations exposed the cascade model's inability to explain hemostatic abnormalities or the importance of other components, such as platelets. Such abnormalities that are not well explained are seen in the existence of factor deficient or absent individuals. Hemophilia is one such example that pertains to the absence or severe deficiency of coagulation factors VIII or IX (also known as hemophilia A or B, respectively). Individuals with hemophilia have a decreased ability to coagulate on their own. These individuals frequently experience bleeding episodes in muscles and joints, resulting in severe pain and joint destruction over time. Individuals with a deficiency of factor VII are also at increased risk for bleeding and can exhibit symptoms from mild to severe [6]. However, abnormalities with other coagulation proteins may not manifest with such significance. Factor XII deficiency, for example, results in prolongation of the activated partial thromboplastin time (aPTT), but does not result in clinically significant bleeding. Hence, use of the classic cascade model of coagulation fails to adequately describe the mechanism of how blood is able to clot, in the setting of these disorders.

Subsequent observations noted the importance of tissue factor and activated factor VII complex at the site of injury and its role as the initiating step in the formation of a clot [7-9]. This led to the cell based model of coagulation, incorporating the role of tissue factor bearing cells, platelets, and coagulation proteins at the site of vascular injury for the formation of a clot [10]. Upon vascular disruption, tissue factor, normally sequestered from the circulation, is exposed to circulating blood. Circulating factor VII interacts with the exposed tissue factor, is activated, and forms a complex with tissue factor on the surface of the tissue factor bearing cell. The tissue factor / activated factor VII complex (TF/FVIIa) is able to act as a catalyst for the activation of platelets accumulated at the site of the injury and the coagulation factors X and IX (Xa and IXa, respectively). Fators IXa and Xa, in turn, are able to act with their respective cofactors and activate their substrates. Fig. (**2**) summarizes the cell based model of hemostasis.

INITIAL CLINICAL USE OF FACTOR VIIA

The first clinical use of factor VIIa to treat hemophilia patients is described in a paper published in 1983 by Hedner [11]. Patients with hemophilia may typically be treated by administering the factor in which they are deficient (hemophilia A: VIII or hemophilia B: IX). Over time, administration of these factors can induce an immunologic reaction which elicit the formation of antibodies against the foreign coagulation proteins. Treatment of such individuals by replacing the deficient clotting factor is rendered useless by the

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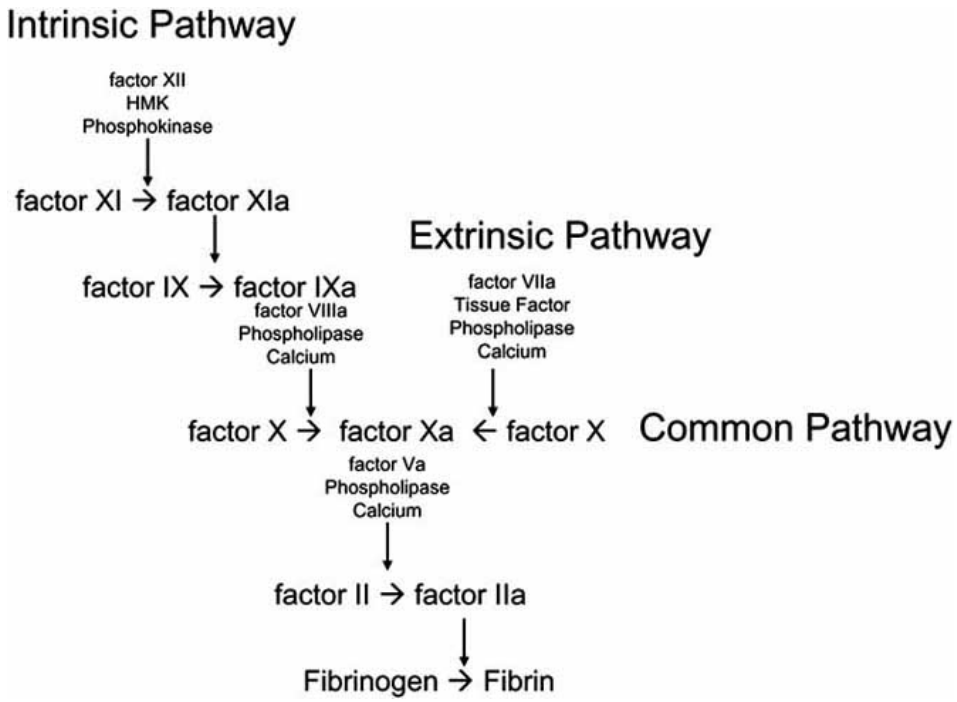

Fig. (1). The original model of coagulation constructed as a cascade, incorporating three activation pathways. Adapted from [4, 5].

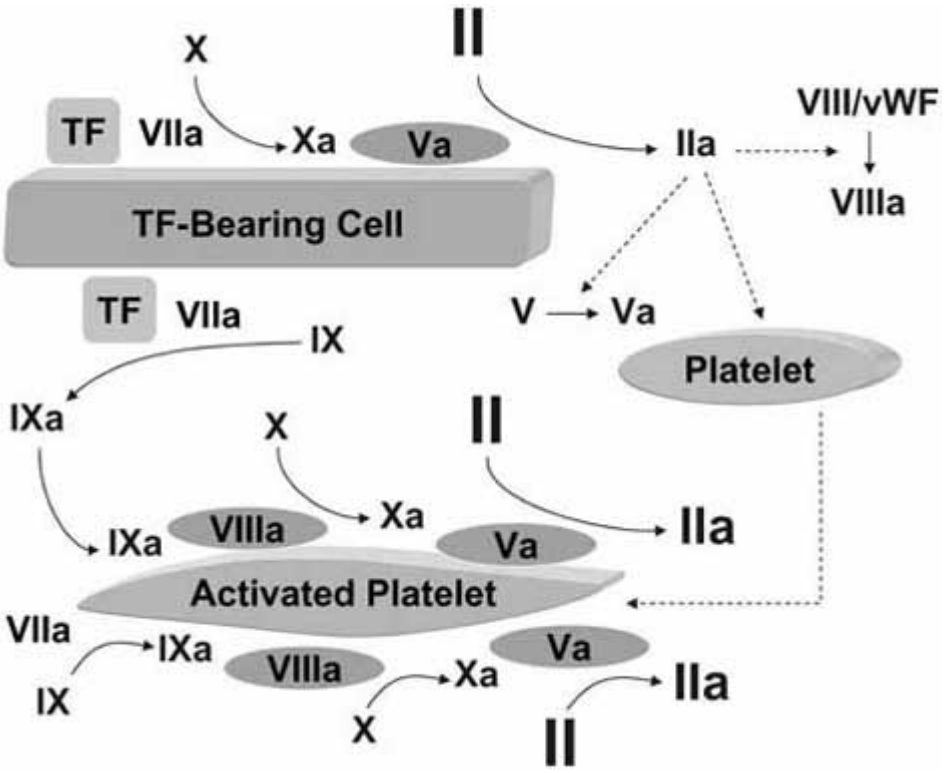

Fig. (2). The coagulation reaction is considered to be comprised of three major components: Initiation, Amplification, and Propagation. The initiation step is characterized by the formation of the TF/VIIa complex, the formation of Xa and IXa, and the generation of a small amount of thrombin (IIa). This initial amount of IIa is able to activate local platelets, activate factor V, dissociate factor VIII from vonWillibrands factor, and activate factor XI. This is the hallmark of the amplification phase. During propagation larger amounts of thrombin is generated, sufficient for the formation of a stable clot. Interaction of factor IXa with its cofactor VIIIa catalyze the activation of X. Xa and its cofactor, Va are able to generate thrombin in sufficient quantities for coagulation. Adapted from [10].

patient's antibody. In Hedner's report, two patients, each with hemophilia A and high-titer alloantibodies were treated with FVIIa for a gastrocnemius muscle bleed and a tooth extraction. Hemostasis was restored in both patients without deleterious side effects. Factor VII was purified for treatment from human citrated fresh-frozen plasma through a method described by Bronze and Majerus [12], activated to FVIIa and sterilized prior to administration. In Hedner's prior studies, the thrombogenic potential of FVIIa was examined in healthy dogs and found to not exhibit adverse effects [13].

It was not until 1988 that a Danish company, Novo Nordisk, was able to produce recombinant Factor VIIa (rFVIIa). In 1996 and 1999 rFVIIa was approved for use in the European Union and United States, respectively, for the treatment of patients with hemophilia with inhibitors. The medical journal, The Lancet, published a case in 1999 in which rFVIIa was use to control bleeding in a non-hemophilia patient, after a high velocity rifle injury [14]. This was the first published case of use of rFVIIa in traumatic injuries. Since that time, investigational uses of rFVIIa have been described in the medical literature for non-hemophilia patients, involving many areas of general hemostasis, including the treatment of intracranial hemorrhage, post-cardiac surgery, liver surgery and transplant, and traumatic injuries [15-19]. In Europe, rFVIIa has been approved also for the use in patients with factor VII deficiency and in those patients with Glanzmann thrombasthenia who have become unresponsive to platelet transfusions because of the development of alloantibodies to glycoproteins (GPIIb/IIIa).

STRUCTURE OF HUMAN FACTOR VII, VIIA AND RECOMBINANT FACTOR VIIA

Factor VII is coded by the gene on band 13q34, closely located to the gene for factor X (FX). It exists in circulation as a single chain zymogen of 406 residues. It has a molecular weight of 50,000 daltons [20] and is a vitamin K dependant factor that requires proteolytic activation to express its activity. As a mature vitamin K dependent clotting factor, factor VII contains an amino-terminal gamma-carboxyglutamic acid (Gla) domain. This Gla domain is necessary for the clotting factor interaction with phospholipid membranes, and hence, its function as a clotting factor requiring a cell surface to elicit its activity on its substrates. The formation of the Gla domain can be inhibited with the addition of warfarin. Warfarin acts as a vitamin K epoxide reductase inhibitor, limiting the recycling of oxidized vitamin K to its reduced form for subsequent coagulation factor activation. A diagrammatic summary of the carboxylation reaction for the formation of the Gla domain can be seen in Fig. (**3**).

Zymogen FVII, in common with other trypsin-like molecules, consists of two distinct β-barrel domains (Fig. **4**): a serine protease domain and an amino terminal Gla domain. There are also two epidermal growth factor-like domains [21]. The liver is the primary site for synthesis and post-translational modification. Modifications made for the active form of factor VII include the gamma-carboxylation of 10 glutamic residues, N-glycosylation of asparagines residues at positions 145 and 322, and O-glycosylation of serine residues 52 and 60. Endogenous FVII circulates in the blood as a single chain zymogen of 406 residues. Upon the association of factor VII and tissue factor, factor VII is activated through cleavage of the peptide bond between Arg 152 and Ile 153. The TF/FVII association results in conformational changes in a contiguous collection of 4 peptide segments collectively termed the activation domain [22]. Principal among these peptide segments is the new N-terminus itself, which becomes buried with its nonpolar side chain in a hydrophobic environment and its charged α-amino nitrogen atom compensated by a salt bridge to a key Asp side chain from the catalytic active site. Three associated loop segments undergo changes that create the substrate binding cleft. For a large number of other homologous proteins, this activation scenario provides full enzymatic competence. For FVIIa, however, the activated form exhibits poor amidolytic activity, is essentially devoid

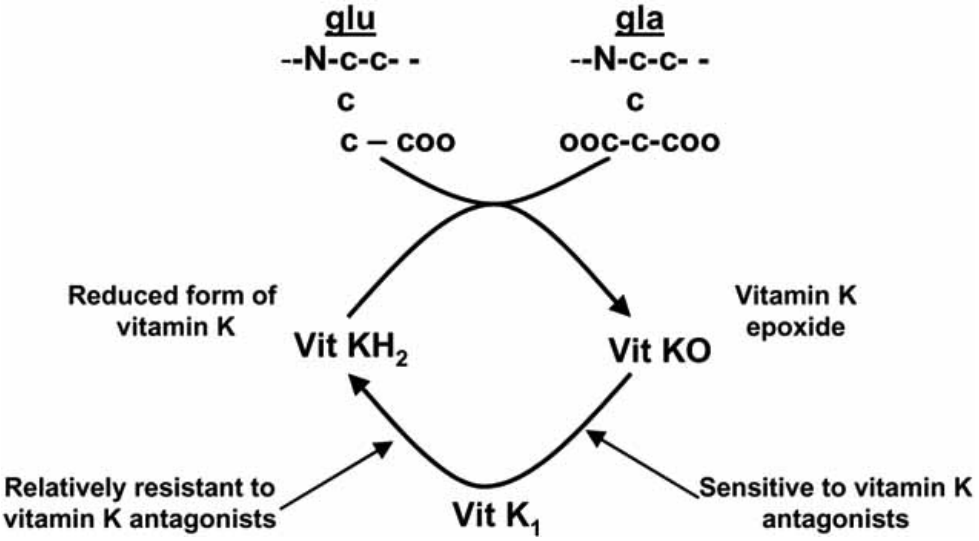

Fig. (3). citation: Warfarin interferes with the carboxylation of vitamin K dependant clotting factors. Vitamin K epoxide is reduced in two steps for use in carboxylating the dependant clotting factors. The first step is sensitive to vitamin K antagonists, while the second step is relatively resistant. Adapted from [67].

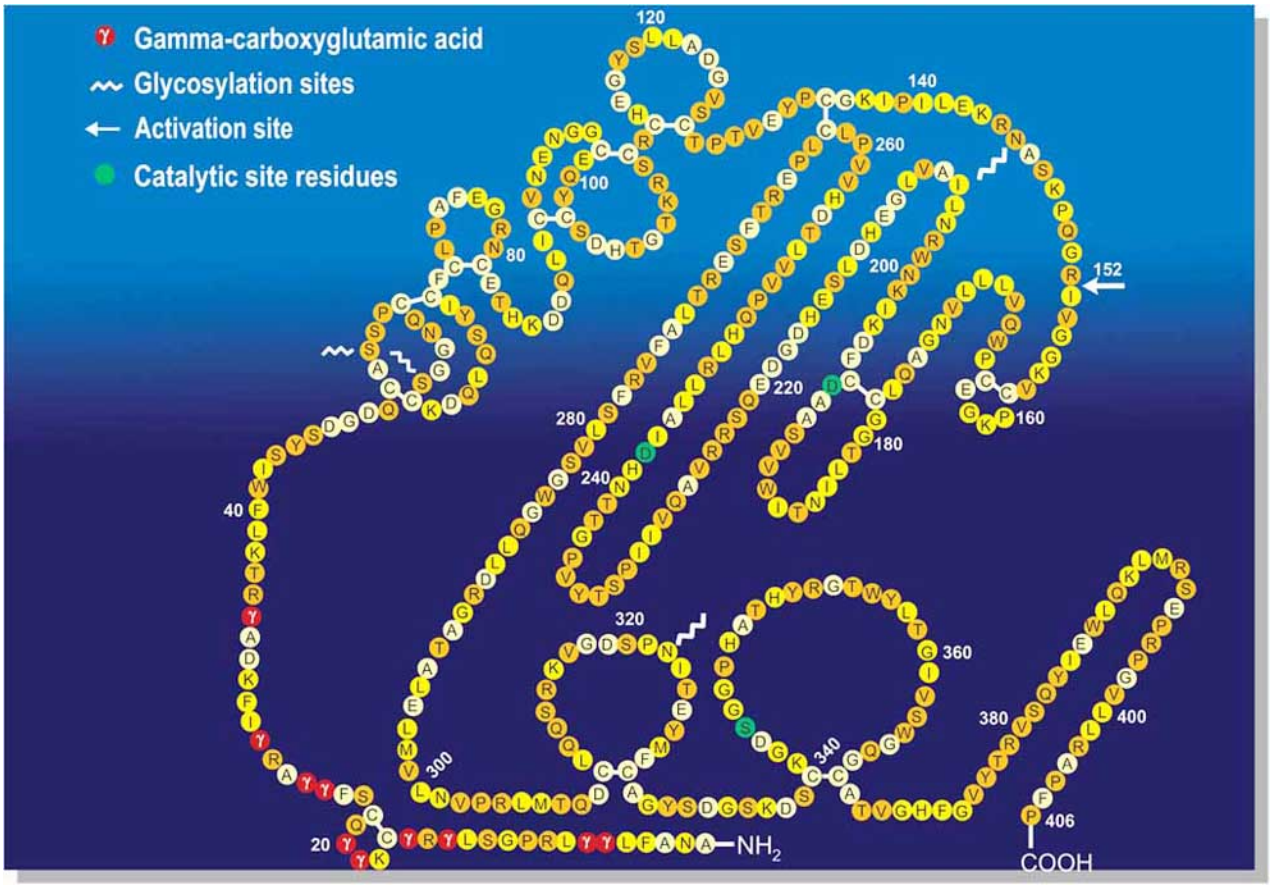

Fig. (4). The structure of Factor VII. Adapted from [21].

of proteolytic activity, but attains full activity only when associated with TF [23]. The low activity of FVIIa has been ascribed to an incomplete conversion of its activation domain from zymogen form to enzyme form [24-26] because the protease domain's N-terminal α-amino nitrogen is more susceptible to chemical modification in the absence of TF than in the presence of TF [27]. Moreover, FVIIa is subject to numerous allosteric influences incurred with the binding of eight Ca^{+2} ions in the γ-carboxyglutamic acid-rich and epidermal growth factor like domains. However the mechanism by which these interactions alter the amidolytic activity at the active site remain unclear [28, 29].

Recombinant factor VIIa by contrast, contains nine fully gamma carboxylated residues and one partially carboxylated residue. However, this partially carboxylated residue appears to have no effect on the function of the recombinant clotting factor [30, 31] The significance of O-glycosylation appears to be important for the association of rFVIIa with tissue factor [32]. Other minor differences have been identified in the carbohydrate structures of human plasma FVIIa and rFVIIa. These are seen at the N-glycosylation sites at asparagine 145 and asparagine 322. Specifically, a higher fructose and lower sialic acid content of the recombinant molecule compared to the zymogen FVII. In general, the same carbohydrate structures are found on both molecules, however, a number of significant quantitative differences have been described [30, 33]. The qualitative effects of these differences appear to be insignificant.

THE PREPARATION OF RECOMBINANT FACTOR VIIA

The human gene for FVII was isolated from a liver gene library, cloned, and transfected into baby hamster kidney cells (BHK). Details on transfection of BHK cells are described by Berkner and colleagues [34]. The BHK cells are fermented and express rFVII into the culture medium for harvesting. A number of purification steps are employed to ensure removal of non-rFVII proteins, viral inactivation, and activation of FVII. A summary of the purification can be found in the paper by Jurlander and coworkers. [35] The complete mechanism of activation is not completely elucidated, but may be due to autoactivation of rFVII by rFVIIa on the ion-exchange column during purification. Sequence analysis demonstrated that the activated two chain form of recombinant factor VII was identical to the plasma derived factor VIIa, in so far as hydrolysis between residues 152 and 153. Subsequent optimization of the process results in nearly 100% yield [30, 36]. The resulting substance is formulated as a solution, dispensed into vials and freeze dried.

CLINICAL PROPERTIES OF RFVIIA

The clinical applications of rFVIIa are consistent with its mechanism of action. As a hemostatic agent, rFVIIa is able to complex with tissue factor and initiate coagulation, as plasma derived factor VIIa can. However, Hoffman and Monroe express doubt that the sole mechanism is through tissue factor alone [10]. As seen in Fig. **2**, the absence of FVIII or FIX would render rFVIIa ineffective but this is not the case. In a cell based *in vitro* model, it has been shown that the addition of increasing amounts of rFVIIa to activated platelets in the presence of factor X produces a linear increase in FXa independent of the presence of TF on platelet surface [37]. This dose response mechanism is able to generate significant amounts of thrombin without the obligatory presence of factors VIII and IX, thus explaining the mechanism of action of rFVIIa in hemophiliacs [38]. The direct activation of FX on activated platelets also explains the mechanism of action of rFVIIa in acquired coagulopathy. In the patient with hemophilia who has developed antibodies to exogenous factor VIII or IX, the administration of rFVIIa has provided an effective therapeutic option for treatment. Additionally, recombinant factor VIIa has also been shown to enhance the inhibition of fibrinolysis by activation of thrombin induced fibrinolysis inhibitor in factor VIII- or factor IX-deficient plasmas [39, 40].

Intravenous recombinant factor VIIa has linear pharmacokinetics. Clearance, steady-state volume of distribution, and mean residence time are all dose independent [41]. The circulating half-life in adults is 2.60 to 2.84 hours, but in the pediatric age group, the half life is shorter at 1.32 hours [38]. Clearance has been shown also to be more rapid in children below the age of 15 years which may have clinical implications in determining the appropriate dosage in this age group [42].

Optimal doses of rFVIIa in the treatment of bleeding disorders have not been well established. Although a dose-response curve have been documented in reversal of coagulopathy [43, 44], this phenomenon was not duplicated in cases of massive bleeding [45]. Effective doses have ranged from 35 to 120 μg/kg [35], but doses of up to 300 μg/kg has been reported in patients with hemophilia A with inhibitors to factor VIII [46]. Although the number of patients included in these reports was limited, the data suggest that the higher doses are not only effective but also safe. *In vitro* experiments corroborate the use of higher doses of rFVIIa and indicate improved hemostasis [47]. The standard recommended dose of rFVIIa is 90 μg/kg given as a bolus and repeated every two to three hours [48] until clinical improvement is observed. Once homeostasis is achieved, the dose interval can be increased to every 4 to 12 hours. The duration of treatment will vary in accordance to the type of surgery, the location, and the degree of coagulation disturbances.

Due to the short half-life of rFVIIa, continuous infusion has been investigated as a mode of administration. Attractive features of continuous infusion include convenience of administration and a potential cost saving associated with the use of less rFVIIa to maintain a target hemostatic level. The reduction in factor clearance associated with continuous infusion contributes to a decrease in factor consumption while maintaining a constant level, thereby minimizing the risk of recurrent bleeding from unpredictable low trough concentrations. The technique has not been perfected, however, and some questions remain. For example, the minimum dosage to achieve hemostasis is not well defined [49, 50]. Satisfactory hemostasis has been described depending on the underlying coagulation disorder, site of bleeding, and the dose used [23, 51, 52]. Santagostino and colleagues [53] report their experience with use of rFVIIa as a continuous infusion (16.5 μg/kg/hr after an initial bolus dose of 90 μg/kg) in 25 patients with hemophilia and high responding inhibitors and 3 patients with nonhemophilic inhibitors. A satisfactory hemostatic response was achieved in 30 of 35 treatment courses. These findings are comparable with those in other published series using bolus administration of rFVIIa [54, 55]. In contrast, the results with continuous infusion of rFVIIa in 8 patients with inhibitors to factor VIII undergoing elective surgery were disappointing. Effective hemostasis was achieved in 1 of 2 minor procedures and in 2 of 6 major operations [56]. The different outcomes observed in the 2 studies might be explained partly by the differences in the treatment intensity, but there might be other patient-specific variables that might affect the clinical responses to rFVIIa [57].

It is not clear at what stage recombinant factor VIIa should be administered. Since the efficacy and safety of recombinant factor VIIa have not been characterized in randomized controlled studies, it may be argued that the safest approach is to administer the agent only if all other treatment has failed. Yet, limited clinical trials have indicated that delay in initiation of rFVIIa in cases of serious bleeding may confer a poor outcome. A retrospective study suggested that using rFVIIa as a last resort to control massive hemorrhage had little impact on improving clinical outcomes [58]. A similar response was observed in patients with hemophilia who had a longer waiting interval between the onset of bleeding and the start of rVIIa administration [23]. Randomized controlled trials are warranted to confirm these findings.

MONITORING THERAPY

Currently, no available laboratory assays have been identified to monitor rFVIIa therapy. Laboratory markers, such as prothrombin, activated partial thromboplastin time, or factor VII level do not necessarily correlate with clinical response [38, 59]. Several monitoring tools including platelet contractile force, thrombin generation over time, and wave form analysis of the partial thromboplastin time have been evaluated for monitoring efficacy of rFVIIa in hemophiliacs but their clinical utility remains to be defined. A modified version of the Staclot VIIa-rTF assay (Diagnostica Stago, Asnieres, France) has been made available for monitoring treatment with rFVIIa at low concentrations [60]. A promising technique based on a modified thrombelastography is being tested in a large population of patients with hemophilia [61]. Thromboelastogram, which can be used to analyze the visco-elastic properties of whole blood, is incorporated on an automated instrument that demonstrates changes occurring during blood coagulation and fibrinolysis. Due to the efficacy of rFVIIa in normalizing the pattern of whole blood from hemophilic patients in a dose-dependent manner, thrombelastography may prove to be helpful in determining the

appropriate dose of rFVIIa. Further studies are under way to validate these scenarios [62].

ANTIGENICITY

The antigenicity of rFVIIa has been a subject of controversy due to conflicting reports of observational studies and clinical trials. The potential for the development of rFVIIa antibodies has been suggested in response to high dose treatment or as a cross reactivity with factor IX [63]. In one cohort, 5 percent of hemophilia A had values for FVII antibodies above the normal range. However, the prior exposure to multiple transfusions and bypassing agents in these patients might have contributed to the production of antibodies to recombinant FVIIa [64]. Similar reports have described antibodies to rFVIIa in patients with factor VII deficiency who have been exposed to extremely high doses [65]. Nonetheless, the hemostatic efficacy appears to be minimally compromised as the bleeding episode was successfully controlled without the need to adjust the frequency or the dosage of administration. Because of the nature of rVIIa preparation in BHK cells, concerns have been raised of antibody formation to trace protein contaminants but none was demonstrated in a randomized trial of patients with hemophilia treated with rFVIIa [23].

ADVERSE EFFECTS

Overview of the literature indicates that rFVIIa is a safe agent for the induction of hemostasis. Relatively few adverse events have been reported with the use of rFVIIa in hemophilia and non hemophilia settings with an estimated incidence between 1% and 2.4% [66]. The primary concern is its potential to induce thrombotic complications given the fact that the pharmacological dose for circulating rFVIIa is approximately 1000 times greater than normal. Since its licensing, isolated reports of myocardial infarction, cerebrovascular accidents, deep vein thrombosis, and disseminated intravascular coagulation have appeared in the literature [38]. Many of the thrombotic events have occurred in patients with a predisposition to thrombotic complications such as diabetes mellitus, obesity, cancer, and atherosclerotic cardiovascular disease, and administration of rFVIIa to such patients should be approached with caution. None of these events were related to the dose or the frequency of administration of recombinant factor VIIa. Furthermore, nearly all randomized controlled trials performed up to date have failed to show significant differences in the incidences of thrombotic events between the treatment and the placebo groups. The exception being demonstrated with the application of rFVIIa in the treatment of intracranial hemorrhage. Arterial thrombotic events were shown to be significantly greater than placebo (5% verses 0%, p=0.01) [15]. Other treatment related side effects (overall incidence <0.6%) reported include angina, acute renal failure, ataxia, and abnormal liver function [51]. Nausea, fever, pain at the injection site, and skin rashes appeared in the postmarketing experience. Despite the safety profile demonstrated thus far, caution should be exerted when rFVIIa is prescribed for "off-label" indication.

CONCLUSION

Recombinant factor VIIa has been shown to induce local hemostasis in a manner consistent with the mechanism described by the tissue factor pathway of coagulation. This has proven to be an effective therapy for the hemophilic patient population as well for those with coagulation disorders. Encouraging data exist for the general hemostasis setting but comparative trials of efficacy and tolerability with other coagulation products are lacking. Pharmacoeconomic analysis is needed to control costs and maximize clinical benefits.

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