FVIIA as therapeutic agent in hemophilia and beyond

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1. ABSTRACT

Around 20% of the patients with severe hemophilia develop inhibitory antibodies against the factor they lack. In these patients the administration of FVIII/FIX-concentrates are not hemostatically effective. Since FVIIa is not enzymatically active unless complexed with tissue factor (TF) exposed following an injury to the vessel wall, it was considered an attractive candidate for improved treatment of patients with inhibitors. Plasma-derived FVIIa was purified and shown to induce hemostasis in two hemophilia A patients with inhibitors. Later recombinant FVIIa (rFVIIa) was developed and pharmacological doses have an efficacy rate of around 90% in serious bleedings and permit major orthopaedic surgery. These findings were a breakthrough in understanding the FVII-TF pathway in hemostasis. The initially formed FVIIa-TF complexes provide a limited amount of thrombin, activating FVIII, FV, FXI as well as platelets. On the activated platelet surface the full burst of thrombin necessary for generating a firm fibrin hemostatic plug occurs. In case of impaired thrombin generation, loose fibrin plugs easily dissolved are formed. Extra rFVIIa enhances thrombin generation and generates tight fibrin plugs.

2. INTRODUCTION

The most serious bleeding disorder is severe hemophilia and patients with hemophilia suffer from repeated joint bleedings resulting in the development of a chronic athropathy if not optimally treated. They also require immediate effective treatment of bleedings associated with trauma or essential surgery. Hemophilia A patients, lacking functionally active coagulation protein FVIII, are treated with FVIII-concentrates while hemophilia B patients, lacking functionally active FIX, are given FIX-concentrates. Around 20% of patients with severe hemophilia A, and a slightly lower percentage of patients with severe hemophilia B develop inhibitory antibodies against FVIII. In these patients the administration of FVIII/FIX-concentrates are not hemostatically effective, since the respective coagulation factor is neutralized by the antibodies. These patients present with a major challenge in the clinical practice. Much effort has, therefore, been focused on finding a treatment which is hemostatically effective independent of the presence of FVIII/FIX. In the beginning of the 1970s activated prothrombin complex concentrates (aPCC) were suggested for treating hemophilia A patients with inhibitors

(1, 2). The prothrombin complex concentrates (PCC) were originally developed for treating hemophilia B patients and contained especially high amounts of FIX. However, all vitamin K-dependent coagulation proteins are present in high concentrations. The originally PCCs contained varying amounts of both the zymogen forms and the activated forms of these coagulation factors. With the aPCCs efforts were made to make sure more of the proteins were available in already activated forms with the idea to increase the FVIII-by-passing effect (1, 2). Such aPCCs were reported to be successful in six patients, four with congenital hemophilia A with inhibitors and two with acquired hemophilia (2). On the other hand thrombo-embolic complications were reported (3, 4, 5, 6) attributed to the presence of activated coagulation proteins especially FIXa and FXa (7, 8, 9) although also other factors such as prothrombin and FXIa were discussed. Studies in normal dogs showed that injection of an activated PCC induced a systemic activation of the coagulation system (decrease in platelet counts, fibrinogen, increase in fibrinogen degradation products, FDP, positive ethanol gelation test). Pretreatment with antithrombin III (AT) together with heparin neutralized the laboratory changes (10, 11) indicating that the factors responsible for the systemic activation of the coagulation system were inhibited by a combination of AT and heparin. Neither has any consensus been achieved on what factor(s) might contribute to the potential hemostatic effect. In this context FVIIa as well as a "modified FXa-complex" in addition to FIXa and FXa were discussed (12, 13, 14, 15). The hemostatic effect of the aPCCs was not very dramatic and was found to vary substantially between different batches (16, 17). An efficacy rate of 50-60% of aPCC was later confirmed in the only controlled studies having been performed (18, 19, 20). The combination of an efficacy rate not exceeding 65%, in controlled studies, and reports of thromboembolic events, made treatment with these concentrates less attractive, especially as, like all plasma-derived blood products, aPCCs carry a potential risk of transfusion-transmitted infection.

As having identified FVIIa as one of those factors that are not readily inactivated by AT in the circulation (21), as well as the relatively high levels of FVII/FVIIa found in patients following treatment with aPCCs, suggested that FVIIa should not be inducing the thrombogenicity in such concentrates. On the contrary, in case extra FVIIa administered induces hemostasis in hemophilia patients with inhibitors, it would be an ideal candidate for use as a FVIII by-passing activity. In collaboration with Dr Walter Kisiel at the time part of Prof Earl W Davie´s research group at the University of Washington, Seattle, human FVIIa was purified from normal human plasma and after having been through required tests according to the Swedish Health Authorities, administered to two hemophilia A patients with inhibitors (22). The highly purified FVIIa (pdFVIIa) initiated no signs of a systemically activated coagulation system in the same dog model as used previously (10, 22) confirming the hypothesis that activated FVII in the circulation should not be thrombogenic. The specific goal of this study was to evaluate the hemostatic effect, if any, of exogenous plasma-derived FVIIa. The results were encouraging and further studies confirmed a hemostatic effect of purified FVIIa from human plasma in joint bleeds in hemophilia patients with inhibitors (23). It was, however, clear that if FVIIa ever would be available to hemophilia patients on a wider basis, recombinant technique had to be used. Furthermore, at the beginning of the 1980s it became obvious that the HIV was transferred through contaminated donor blood making hemophilia patients treated with plasma-derived coagulation concentrates obtained from large plasma pools one of the target groups of acquired immune-deficient disease. A similar epidemiology had been observed in regard to various types of hepatitis previously. Recombinant technique in the area of coagulation proteins was initiated at the biotech company, ZymoGenetics in Seattle headed up by Earl Davie and his co-workers.

3. DEVELOPMENT OF RECOMBINANT FVIIa (rFVIIa)

The development of rFVIIa for use in hemophilia patients with inhibitors was initiated at Novo Nordisk A/S in June 1985. Since FVII is a complicated protein with necessary posttranslational modifications for full activity, mammalian cells had to be used. Thus a baby hamster cell line (BHK) expressing human FVII obtained from ZymoGenetics was used (24). The FVII secreted was the single-chain form of the zymogen, which was spontaneously converted into the active two-chain molecule during the purification process (25). The rFVIIa molecule was characterized with regard to Gla residues (9 out of 10 possible Gla-residues were fully gamma-carboxylated and one partially, approximately 50%) (26). Both of the two O-glycosylated sites of plasma-derived FVIIa were similarly O-glycosylated in rFVIIa, but the relative amounts of the three O-glycosylated structures differed slightly between plasma-derived and rFVIIa (27). Minor quantitative differences were seen in the carbohydrate composition of the two FVIIa molecules (26) and a full characterization of the N-linked carbohydrate structures of rFVIIa was later performed (28). The detailed production procedure was described in Jurlander *et al* (29).

4. PRECLINICAL DEVELOPMENT

The rFVIIa was found to normalize the prolonged APTT of both hemophilia A and B plasma in vitro at a concentration of 3.8 ug/ml (30), which roughly corresponds to between 250 and 300 ug/kg of rFVIIa i.v. depending on the individual recovery of injected rFVIIa. Also, the prolonged APTT in plasma from non-hemophilic patients with an acquired inhibitor against FVIII normalized on the addition of rFVIIa. The hemostatic effect of rFVIIa was demonstrated in hemophilia dogs (31) and the shortening of the PTT was confirmed. The recovery of rFVIIa in the dogs varied between 34 and 44%. Also in warfarin treated rats rFVIIa was demonstrated to be hemostatic (32). No systemic activation of the coagulation was found following the injection of rFVIIa into rabbits using a somewhat modified stasis model originally developed as a thrombosis model (33) demonstrating that rFVIIa promotes the initiation of hemostasis at the site of injury (in this model

initiated by complete venous stasis) without inducing systemic activation of the coagulation system. The same study in the modified stasis model showed on the contrary decreased levels of platelets and fibrinogen following injection of an aPCC (FEIBA) (34).

5. CLINICAL DEVELOPMENT

The first clinical use of rFVIIa was pushed by an urgent need of surgery in a patient with a high inhibitor titer against FVIII, and took place in March 1988 at the Karolinska Hospital, Stockholm, Sweden. An open synovectomy was performed without any complication and no per- or postoperative abnormal bleeding (35). The successful treatment of this patient spurred a demand of rFVIIa for complicated hemophilia patients with inhibitors resulting in the establishment of a "Compassionate Use Program" making rFVIIa available for patients with inhibitors against coagulation factors suffering from "life- and limb-threatening bleedings in which available treatment has failed" (36, 37). In this material including almost 500 patients treated at around 1900 bleeding episodes, an efficacy rate between 76 and 84% was achieved (36, 38). In parallel with the Compassionate Use Program, a dose-finding study including 35 or 70 ug/kg was run (39). Following a couple of uncontrolled studies of rFVIIa in major surgery showing an excellent hemostatic efficacy (40), a controlled study in major surgery including major orthopaedic surgery confirmed an efficacy rate of 90-100% (41). Another study using rFVIIa in a home-treatment setting using bolus dosing of 90 ug/kg with a maximum of three doses allowed for achieving hemostasis in mild to moderate joint and muscle bleedings. The efficacy rate in this study was 92% but a mean of 2.2 doses of 90 ug/kg each were used, meaning that some patients required more than 90 ug/kg to stop a mild to moderate bleeding (42). The optimal dosing in this setting should induce hemostasis by one single injection.

6. MECHANISM OF ACTION

The finding that pharmacological doses of rFVIIa provided hemostasis in patients with severe hemophilia in the absence of FVIII or FIX was a breakthrough in the understanding of the importance of FVII and tissue factor (TF) in hemostasis, and stimulated research into the TF- and FVII-dependent pathway of hemostasis. 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 (43). For the first time the separation of the hemostatic process into the extrinsic and the intrinsic was questioned and suggested to be obsolete (44).

Current concept of hemostasis suggests that the hemostatic process is localized to cell surfaces, mainly the TF-bearing cells and the thrombin-activated platelets (45). TF-expressing cells are present in the deeper layers of the vessel wall (44). 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. TF is a receptor protein anchored to cells by a transmembrane domain and therefore 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 (46). Previous observation in transfusion experiments of a distribution pool for factor VII considerably larger than the intravascular plasma volume suggests that K-dependent clotting factors including FVII are expected to be present in interstitial fluid (47). Vitamin K-dependent coagulation proteins were later demonstrated in rabbit limb lymph (48) as well as in human peripheral afferent lymph (49). Thus, FVIIa-TF complexes may always be present on at least some tissue cells that constitutively express TF (46). Furthermore, functional FVIIa-TF complexes were demonstrated in an umbilical vein model system (50) and recently, perivascular TF in mice bound FVII or FVIIa in the absence of a clear injury (51). The physiological presence of FIX-activation peptide in circulating blood also support the hypothesis that FVIIa-TF-complexes are normally present extravascularly and that they catalyze a limited, continuous, physiological extravascular activation of FIX. Tissue injury or inflammation increase the vascular permeability and thus may enhance this basal level of FVIIa-TF catalytic activity by increasing the concentration of FVII at TF expressing sites as well as facilitating the extravasation of other coagulation proteins such as FVIII, vWillebrand factor and fibrinogen. Furthermore, platelets may diffuse out into the extravascular space enhancing the formation of small fibrin hemostatic plugs in the microvasculature. The observation of cross-linked fibrin in guinea pig skin after increased vessel wall permeability (52) supports the importance of extravascular coagulation.

Recently, the bio-distribution of rFVIIa following *i.v.* injection into normal mice showed that rFVIIa immediately after the administration associated with the endothelium lining of large blood vessels. Within 1 hr this rFVIIa was transferred to the perivascular tissue surrounding the blood vessels and thereafter diffused throughout the tissue. In general, the rFVIIa in extravascular spaces was mostly co-localized with TF and is retained for extended time periods. In the synovial and the mineralized bone regions, specifically in the zone of calcified cartilage within the growth plate region of the joints rFVIIa was found decreasing after day 3, but still clearly visible in tissue specimens collected on day 7. Interestingly, FIX was found to localize to the same region of calcified cartilage, but contrary to rFVIIa it had disappeared from the region by 24 h whereas FVIIa persisted for a much longer time. Furthermore, FIX did not associate with the endothelium lining blood vessels. In addition to the zone of calcified cartilage, rFVIIa also accumulated in the bone marrow. The staining intensity peaked at 10 min to 24 h after injection and thereafter slowly decreased, but was clearly visible even on day 7. Both FVIIa and FIX may initially bind through their Gla residues, but FVIIa binding to TF in mineralized bone may slow down its dissociation from the bone. In the heart intense staining of FVIIa was observed in endothelial cells as well as in fibroblasts and pericytes at 10 min after

administration. Diffuse staining deep into the heart tissue although weak persisted even at 7 days after rFVIIa injection (53). In a later study it was demonstrated that the rFVIIa transported from blood stream into tissues is functionally active. While the rFVIIa in the first study was labelled with Alex fluor-488 and identified by antibodies specific to AF488, the FVII/FVIIa activity in tissue homogenates was measured in a FX activation assay and was higher in tissues derived from mice receiving rFVIIa compared to mice receiving saline. These data suggest that rFVIIa administered to mice entered into tissues and this rFVIIa is remained functionally active. Furthermore, it was found that exogenously added FVIIa is capable of binding to extravascular TF suggesting that TF sites are not saturated with endogenous FVII (54). Binding of FVIIa to the endothelial cell protein C receptor (EPCR) was found to facilitate the FVIIa endocytosis, while it did not accelerate FVIIa activation of FX . The binding was reported to be independent of both TF and negatively charged phospholipids. Based on these data it was concluded that EPCR serves as a cellular binding site for FVII/FVIIa (55).

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). Stressing the importance of the cell membrane composition for TF function, depletion of cholesterol from cell membranes was shown to impair the functional TF expression in fibroblasts (56). Protein disulfide isomerase (PDI) released from platelets has been suggested to enhance the formation of disulfide bonds claimed to be important for the coagulant activity of TF (57). However, no consensus on this topic has been achieved so far (58). Recently, a study claiming that inhibition of cell surface PDI induces an increase in TF procoagulant function, whereas exogenous addition of PDI inhibits TF decryption. Thus, PDI is suggested to act as a negative regulator of coagulation by contributing to the regulation of PS exposure (59). However, PDI has been shown to be localized in the intracellular stores of the dense tubular system in platelets neither being released nor targeted to the cell surface (60). 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 canicular system (OCS). TF was identified in the OCS and occasionally in the alpha-granulae of the platelets (61). Whether platelet-related TF is constituitively present in platelets (62) or transferred from other cells (62, 63) is a matter 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, while 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 tissue factor pathway inhibitor (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 (64). 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 (65). Thrombin enhances platelet activation leading to release of FV as well as activation of FV and FVIII (66). 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 activated coagulation proteins to the platelet surface. FIXa initially activated 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 takes place (45). Although FIXa binds to pre-activated platelets, this binding is much tighter in the presence of FVIIIa . Neither FIX nor FIXa bind to resting platelets (67). Results from the same study suggested that pharmacological doses of rFVIIa activated FIX into FIXa on activated platelets in the absence of TF. Furthermore, it was speculated that FIX bound to activated platelet surface could provide a binding site for rFVIIa thereby facilitating the FX activation occurring on the activated platelet surface (68). The binding of coagulation proteins on the platelet surface is furthermore, 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 (69, 70). These "collagen and thrombin activated platelets" (COAT-platelets) constitute part of the activated platelets and support a robust prothrombinase activity. Furthermore, they retain high levels of several procoagulant proteins on the cell surface (71). Recently, it has been found that the potential to produce these cells varies greatly among individuals (72). This may at least partly, explain the individual variation in capacity of thrombin generation on the thrombin surface demonstrated previously (73). The FIXa-FVIIIa complex ("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 hemostatic plug. Only small amounts of FX are necessary for a saturated formation of FXa according to results obtained in a cell-based in-vitro model (74). 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 (74). 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 (75). A tight fibrin network makes the hemostatic plug more resistant against premature proteolysis thereby helping to maintain hemostasis (76). 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. hemophilia, a loose fibrin plug is formed, which is less resistant towards proteolysis. As a result, these patients are characterized by a defectively maintained hemostasis and repeated rebleedings. A full thrombin burst is also dependent on the number of platelets, which leads to the formation of loose fibrin plugs in thrombocytopenia. Patients with low platelet counts thus, form fibrin plugs that are easily dissolved by fibrinolytic enzymes, which results in the characteristic bleedings from tissues rich in fibrinolytic enzymes such as the gastric mucosa, the ear-nose-throat region and the urinary tract.

7. FVIIA AS A HEMOSTATIC AGENT

In the 1970s FVIIa as a hemostatic agent was a new concept. The vision at the time was to develop a therapy for hemophilia patients with inhibitors, that would be as easily available and convenient to use as existing treatments for hemophilia 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 hemophilia patients with inhibitors because of the risk of uncontrollable bleeding. With this background, elective essential surgery was the first target for demonstrating efficacy of rFVIIa. An efficacy rate of 90-100% was achieved in major surgery including major orthopaedic surgery (35, 77, 41). A similar efficacy rate was found in serious bleedings using essentially the same dosing schedule as that recommended in surgery (36). As part of the vision of providing a treatment for hemophilia 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 hemostasis was 2.2 meaning that several patients required more than one dose of 90 ug/kg for hemostasis (Key et al 1998). An optimal treatment of mild to moderate joint bleedings administered immediately after the first symptoms should include one single dose/injection. Thus, it was obvious that a dose of 90 ug/kg was not the optimal dose for everyone.

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 (68). This TF-independent reaction confirmed previous findings (78, 79, 80). Recent studies have suggested that The GPIb-IX-V complex on the activated platelet surface may contribute to the TF-independent thrombin generation by rFVIIa (81). However, the interaction between GPIb and rFVIIa, if any, is still not completely clarified. 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 (82, 83). 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 ug/kg, and 75-80 nM of FVIIa following the dose of 270 ug/kg) (83). Thus, it does not seem to be necessary to achieve a fully normalized thrombin peak at least as measured in the cell-based model, in order to get hemostasis. However, a normalized lag phase or even a quicker onset of thrombin generation may be the most important, which in fact, is supported by previous findings that the rate of thrombin generation is important for the formation of a tight, well-structured fibrin net-work of the hemostatic plug (75). A firm and tight fibrin net-work was also demonstrated to form from hemophilia plasma in the presence of rFVIIa and preactivated platelets (84). Furthermore, a tight fibrin net-work makes the hemostatic plug more resistant against premature lysis (76, 85, 86).
The hemostatic 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 GPIb as well as other mechanisms (87, 45). The enhanced thrombin generation ensures the formation of a tight fibrin structure of the hemostatic plug, as well as full activation of TAFI and FXIII (both activated by thrombin) necessary for maintaining hemostasis (88).

As demonstrated by other workers a shortening of the clotting time is TF-dependent in FIX-deficient plasma (89). The same group found an inverse dependency between the TF concentration and the concentration of added FVIIa. It is obvious that TF is necessary for the initiation of hemostasis resulting in a limited thrombin formation mediated by the TF-FVIIa complexes formed at the site of injury. However, a full hemostatic effect requires a burst of thrombin generation on the surface of the platelets activated by the thrombin formed initially. In the absence of FVIII/FIX concentrations of rFVIIa of up to 75 nM or higher are required for hemostasis as demonstrated in a cell-based model containing platelets instead of phospholipids (74). It is emphasized that specific binding sites on the platelets may regulate the formation of the coagulation complexes supporting the concept that factors other than phosphatidylserine are necessary for platelet-dependent thrombin generation (45). The importance of platelet accumulation at the site of a vascular lesion for the FVIIa-dependent hemostasis was also later underlined by Butenas *et al* (90).

8. DOSING OF RFVIIA

By increasing the physiological level of FVIIa, the binding of rFVIIa to activated platelets is exploited. This is a new concept in treatment of bleedings, and the exact relationship between the plasma concentration of FVII:C and the thrombin generation at the site of injury is not known. Unfortunately, the assays for thrombin generation recently described, measure thrombin formation in circulating blood or plasma rather than the thrombin generated at the site of injury where rFVIIa is active. Furthermore, the interindividual response to rFVIIa has been found to vary widely as supported by the findings in the home-treatment study (42). Thus, the recovery measured as the FVII:C concentration in plasma at 10 min after injection varies between 40 and 80% (median 43%; mean 46%) (91, 92). The recommended dose in hemophilia would then correspond approximately to 25 nM to 35 nM of rFVIIa in plasma. Not only the recovery varies between individuals, but also the clearance rate and the capacity of thrombin generation on the activated platelet surface (88). Accordingly, the optimal dose might show a great individual variation. Furthermore, the clearance rate in children below 15 years of age may be as much as three times the normal rate for adults (93), which has lead to the recommendation of a higher dose (270 ug/kg) especially in children and in hemophilia patients with repeated bleedings (94, 20), approved in some markets, but not in the US. However, not only the dose is important in hemophilia treatment, but also an immediate initiation of therapy. In a recent paper registry data showing a lower percentage of rebleeds when the treatment was initiated within less than 2 hrs after debut of symptoms (5.7% vs 15.8% when treatment was initiated later than 2 hrs after debut of symptoms) were presented. Furthermore, data demonstrated that in case treatment was initiated more than 2 hrs after symptoms were notified, a dose of >250 ug/kg decreased the percentage of rebleeds from 15.8% to 0, indicating that a higher dose was even more important in delayed treatments (95). In the same population 79 out of 128 bleeding episodes treated with a mean dose of 153 ug/kg required only one injection for hemostasis, while episodes needing 5 or more injections were given a mean dose of 99 ug/kg, indicating that starting with a higher dose resulted in hemostasis by one single dose while starting with a lower initial dose (99 ug/kg) often required more injections to achieve hemostasis (95).

The feasibility of administration of rFVIIa in a continuous infusion (CI) was explored by Schulman et al (96) in two hemophilia patients with inhibitors. They initiated treatment with a bolus of 90 ug/kg and continued with a CI dosing adjusted by the pharmacokinetic of each patient. The experience from this initial study of rFVIIa administered as a CI pointed out the importance of the individual pharmacokinetics of each patient and recommended the dose schedule to be accordingly adjusted (96). Later varying schedules for CI rFVIIa therapy have been reported and were recently reviewed (97). Both successes (98) and failures were reported and may reflect the experience in hemophilia 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. Any success may also depend on the use of adjunct therapy like antifibrinolytics (97, 99). Extra bolus doses seem to be required in some cases to keep hemostasis which requires extra attention from the medical staff. The most extensive study using CI administration of rFVIIa demonstrated that a very high continuous dosing of rFVIIa (50 ug/kg/hr) was required to keep hemostasis in major surgery (100), which makes any cost saving uncertain.

9. EFFECT OF RFVIIA AS PROPHYLAXIS

Regular, repeated administration of FVIII/FIX concentrates per week (prophylaxis) in non-inhibitor hemophilia patients has been demonstrated to minimize the development of athropathy, and therefore is the recommended treatment in severe hemophilia patients (101). The first hemophilia patient with inhibitors receiving regular prophylaxis during extensive physiotherapy following a mechanical traction of a knee contracture without any spontaneous break-through bleeding was published in 2001 (102). More recently, several hemophilia patients with inhibitors have been successfully handled with repeated doses of rFVIIa (103). A randomized, prospective clinical trial using once daily administration of rFVIIa in doses of 90 ug/kg or 270 ug/kg decreased the number of bleeds in "heavy bleeders" (more than 5 joint bleeds per month), not only during the three-month treatment period but also during the observation time that followed (three months of no regular treatment) (104). Based on available experience of regular administration of rFVIIa several times per week, prophylaxis with rFVIIa has been approved in Australia, New Zeeland and Argentina. This may mark a step forward toward the goal of making the treatment of hemophilia patients with inhibitors similar to that of non-inhibitor patients. The decrease in number of bleedings in the prospective clinical trial during the treatment period was probably mainly due to amelioration of the inflammatory synovitis. However, it is not fully clear how this effect was achieved by once-daily administration of an agent with a plasma half-life of 2-3 hrs. Neither is it clear why rFVIIa prophylaxis once daily reduces the number of hemorrhagic events in the post-treatment period. Although this 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 (44, 46). 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 (64), 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. In this context the bio-distribution of rFVIIa following *i.v.* injection into normal mice showed that rFVIIa was transferred to the perivascular tissue surrounding the blood vessels within 1 hr. The same study found that the rFVIIa in extravascular spaces mostly co-localized with TF and was retained for extended time periods. Thus, rFVIIa was clearly visible in tissue specimens for up to 7 days in the synovial and the mineralized bone regions, specifically in the zone of calcified cartilage within the growth plate region of the joints (53, 54). 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.

10. CLINICAL EXPERIENCE WITH RFVIIA IN OTHER THAN HEMOPHILIA PATIENTS

The ability of rFVIIa to enhance thrombin generation on the surface of activated platelets makes it a potential hemostatic agent in any situation that requires the formation of a tight hemostatic plug (68, 87, 105). The addition of rFVIIa induced 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 ul^{-1} . Furthermore, a tighter fibrin structure was observed in the presence of rFVIIa and low platelet counts (106). Also, in a flow-chamber model the addition of rFVIIa to whole blood made thrombocytopenic increased the fibrin deposition (107). Provided the events observed *in vitro* also occurs in vivo, these may contribute to the hemostatic effect of rFVIIa in situations characterized by low platelet counts associated with uncontrolled hemorrhage (108). Successful use of rFVIIa in patients with thrombasthenia Glanzmann are reported (109), and its use in such patients, who do not benefit from platelet transfusion, is approved by the European Medicines Agency (EMEA). The dosage recommended is similar to the hemophilia dosage, 70-120 ug/kg every other hour in serious bleeding and surgery (110). Also, in patients with von Willebrand´s disease, type III and II, a successful use of rFVIIa was reported in 96% out of a total of 48 patients with congenital von Willebrand´s disease (111). A patient with mild von Willebrand´s disease with predisposition to allergic problems was successfully treated with rFVIIa in association with an injury bleeding (112).

In any person an impaired thrombin generation may occur as a result of severe trauma or extended surgery. An extensive tissue damage will cause local release of proteolytic enzymes degrading coagulation proteins. In case of profuse bleedings a dilution coagulopathy with lowered levels of most of the coagulation proteins will add to the problem. The impaired thrombin generation in these situations will lead to the generation of porous fibrin deposits, which will be easy targets for premature dissolution by the released enzymes resulting in diffuse bleeding at sites of tissue damage. This process may be mainly localized without signs of generally increased fibrinolytic activity in the circulation. In these situations it may be beneficial to facilitate the generation of tight, well-structured fibrin hemostatic plugs that would be resistant to

premature lysis. Thus, the vicious circle of localizing fibrinolytic activators to half-degraded, loose fibrin resulting in severe oozing from the damaged tissues should be halted. Furthermore, low pH may aggravate the situation by slowing down the release of fibrinopeptide A (FPA) and thereby the formation of fibrin from fibrinogen adding to the generation of defective fibrin plugs. Accordingly, rFVIIa has been used in trauma-related injuries (113, 114, 115, 108, 116). Several studies have reported a decrease in transfusion requirement although an effect on the long-term mortality seems to be more unclear (115, 117). A recently published study exclusively in military casualties indicated that patients treated with rFVIIa (506/2050) were more severely injured, in shock and coagulopathic than those who did not receive rFVIIa (1544/2055) (118). Military patients differ from civilian trauma patients having a higher death rate (>80%) caused by hemorrhage than the civilian patients (26-39%). Furthermore, coagulopathy at admission are more common in the military patients. Although patients who were not treated with rFVIIa had a greater percentage of deaths that occurred in the first 24 hrs than those who were treated (73% vs 58%), propensity scoring including the number of red blood cell units showed no differences of any clinical consequence between those who received rFVIIa and those who did not. Neither were there any significant differences in the rates of mortality nor in complications. The conclusions drawn by the authors are that rFVIIa is used in the most severely injured military casualties, that use of rFVIIa in these patients is not associated with an improvement in survival or an increase in complications and finally that there is a need to identify patients who would potentially benefit from the use of rFVIIa (118).

A special situation characterized by profuse, massive bleeding is the post partum bleedings. Successful use of rFVIIa, often administered as one single dose (90-100 ug/kg), has been reported in such patients (119). Anecdotal reports on the successful use of rFVIIa in cardiac and vascular surgery (120, 121) as well as in uncontrollable postoperative bleedings (122) were published. Furthermore, anecdotal reports of successful use of rFVIIa in patients with increased risk of bleeding due to treatment with anticoagulants are available (110).

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 (123). A single dose of rFVIIa was also reported to limit the growth of an intracerebral hemorrhage. 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 (124, 125, 126).

A couple of extensive reports on 22 (127) and 28 (128) placebo-controlled, randomized trials using rFVIIa in

non-hemophilia patients were recently published. The conclusion from one of the reviews was, that the use of rFVIIa reduced the need for blood transfusion and may reduce mortality (127), while the second one was more cautious and stated that the clinically significant benefits of rFVIIa as a general hemostatic agent in patients without hemophilia remains "unproven" (128). The review of Hsia et al (127) included seven trials in patients with liver disease (2 liver transplantation, 2 variceal bleedings, 2 liver resection), three in cardiac surgery, four in acute cerebral hemorhage, two in trauma, two in stem cell transplantation, one in Dengue hemorrhagic fever, one in spinal fusion surgery, one in prostatectomy, and one in pelvic/acetabular fractures. Furthermore, a consecutive study of 153 off-label uses of rFVIIa in 139 children was recently published showing efficacy in 91% and had with the exception of neonates had an excellent safety (129).

11. SAFETY

No side effects have been observed in healthy volunteers (130, 122). In a thorough review of all adverse events observed among more than 700,000 standard doses (90 ug/kg) of rFVIIa administered between 1996 and 2003, it was concluded that in no case it could be clearly determined that rFVIIa was definitely causally related to the thromboembolic event. It also was stressed that the incidence of thrombotic events with the use of rFVIIa is extremely low (38). Essentially the same conclusions were drawn by Roberts (131) who found that the rate of serious adverse events was less than 1% in spite of administered extensively in many situations that predispose to thrombosis. A safety follow-up of reports on thrombo-embolic and fatal events with use of rFVIIa in congenital and acquired hemophilia A or B between 2003 and 2006 including approximately 800,000 standard doses of rFVIIa was published in 2008 (132). The conclusion drawn was that in spite the use of high doses of rFVIIa (270 ug/kg) rFVIIa appears safe, when used for congenital and acquired hemophilia. The prevalence of thrombo-embolic events is less than 4/100,000. However, the use of rFVIIa for off-label indications was recommended to be monitored closely (132). 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-hemophilia patients with an underlying condition predisposing to thrombosis. The authors of this report pointed out that the spontaneous reporting system data presented does not allow the determination of the frequency of thromboembolic adverse events (133). Most probably the localized effect of rFVIIa through the binding to tissue factor-expressing cells and activated platelets makes the drug safe (134).
No indication of the formation of inhibitory antibodies against rFVIIa was seen in patients with hemophilia or in non-hemophilia patients treated with rFVIIa. However, FVII-deficient patients are at risk for development of antibodies against FVII (110).

12. SUMMARY

The experience of rFVIIa in bleeding-associated pathologies pharmacological dose have been found to be effective to an extent of allowing major orthopedic surgery in severe hemophilia 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 hemostatic plug at the site of injury, it has also been shown to be an active hemostatic agent in other bleeding situations than those occurring in hemophilia patients. Of special interest is the efficacy in stopping postpartum hemorrhages, the most common complication of delivery with a high mortality among young women.

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