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Recombinant activated factor VII: 30 years of research and innovation[1]

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ABSTRACT

Recombinant activated factor VII (rFVIIa) was initially developed to treat bleeding episodes in patients with congenital haemophilia and inhibitors. The story of its development began in the 1970s, when FVIIa was identified as one of the activated coagulation factors that has minimal potential for inducing thromboembolic side-effects. Extensive research over the last 30 years has greatly increased our knowledge of the characteristics of FVII, its activation, and the mechanisms by which rFVIIa restores haemostasis. In haemophilia, the haemostatic effect of rFVIIa is mediated via binding to thrombin-activated platelets at the site of injury, thereby enhancing thrombin generation also in the absence of factor (F) VIII or FIX. The mechanism of action of rFVIIa has also allowed its successful use in other clinical scenarios characterised by impaired thrombin generation, and its licensed uses have now been extended to acquired haemophilia, congenital FVII deficiency and Glanzmann's thrombasthenia.

1. Introduction

Haemophilia is the most serious known congenital bleeding disorder. Without proper treatment, patients with severe forms of the disease have a life expectancy of around 16 years [1]. In the 1970s, factor (F) VIII and FIX concentrates became available for the treatment of haemophilia A and B, respectively. Prophylaxis (regular administration of FVIII or FIX concentrates several times per week) was introduced as early as the late 1950s [2,3]. However, up to 30% of patients develop inhibitors against the missing coagulation factor [4]; in these patients, treatment with FVIII and FIX concentrates is ineffective.

Previously, there were essentially two options available for treating haemophilia patients with inhibitors:

1) Large amounts of FVIII or FIX concentrates can be used to neutralise the inhibitors and increase the plasma concentration of the missing factor to a haemostatic level. In most patients, extracorporeal adsorption of the antibodies will also be required [5]. In addition, cytotoxic drugs may be needed to postpone, or at least mitigate, the booster effect of the administered FVIII/FIX protein. Such treatment can be used in elective, necessary surgery, but is not optimal for the treatment of mild to moderate joint or muscle bleeds.
2) The use of 'FVIII-bypassing' products such as plasma-derived activated prothrombin complex concentrates (pd-aPCCs), including Factor Eight Inhibitor Bypassing Activity (FEIBA®, Baxter, Deerfield, IL, USA). These concentrates were originally produced as prothrombin complex concentrates (PCCs) for the treatment of haemophilia B patients lacking FIX. The PCCs are produced from pooled plasma after the fractions containing FVIII have been removed; they contain all the vitamin K-dependent coagulation proteins (FII, FIX, FX, FVII, Proteins C and S, and other plasma proteins) as well as small amounts of FVIII protein.

The first report of a haemostatic effect produced by pd-PCCs/aPCCs in haemophilia with inhibitors was presented in 1972 [6], and pd-aPCCs have been available on the market since the mid-1970s. In three published studies, pd-PCCs and pd-aPCCs produced efficacy rates of approximately 50–65% [7–9].

Thromboembolic side-effects have repeatedly been observed in association with the use of pd-aPCCs [10–17], and a booster effect of pd-aPCCs on the titre of anti-FVIII inhibitors has been observed [18–20]. In addition, plasma-derived concentrates are known to have a potential risk of transferring blood-borne pathogens [21–25].

In summary, the treatment of haemophilia patients with inhibitors in the mid-1970s was clearly suboptimal. Patients were often hospitalised for several weeks as a result of joint bleeds, and most developed severe haemophilic arthropathy.

2. Development of an alternative treatment modality for haemophilia with inhibitors

During the 1970s, work began on exploiting the haemostatic effect of pharmacological doses of FVIIa. The idea behind, and the proof of principle for, the administration of purified plasma-derived FVIIa (pd-FVIIa), and the development of recombinant activated factor VII (rFVIIa, eptacog alfa activated, NovoSeven®, Novo Nordisk, Bagsværd, Denmark) are discussed below. The ultimate goal was to make treatment for haemophilia patients with inhibitors similar to the treatment for patients without inhibitors.

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1. **Recombinant activated factor VII: 30 years of research and innovation**
Anchor Name: More than 30 years of clinical experience *[Agency FCB Halesway Olga Kooi]*

2.1. Plasma-derived FVIIa in haemophilia patients with inhibitors

Initial experiments demonstrated that the laboratory parameters indicative of systemic activation of coagulation, and therefore potentially responsible for the thromboembolic side-effects associated with the use of aPCCs, were almost entirely eliminated by the addition of heparin and antithrombin [26,27]. FVIIa was then identified as one of the activated coagulation factors that had minimal potential for inducing thromboembolic side-effects [28]. The haemostatic effect of exogenous FVIIa was observed in two haemophilia patients with inhibitors – one had received purified pd-FVIIa to treat a muscle bleed and the other had received it for bleeding caused by loss of a primary molar [28]. A similar haemostatic effect was later confirmed in more haemophilia patients with inhibitors [29].

2.2. Development of rFVIIa

From the experience of purifying FVII from human plasma, it was obvious that such a procedure would never be suitable for large-scale purification. However, in the early 1980s, it was demonstrated that human FVII could be expressed by baby hamster kidney (BHK) cells [30,31]. Soon afterwards, in 1983/4, with this finding in mind, the idea to use recombinant technology to produce FVIIa for the treatment of haemophilia patients with inhibitors was introduced at Novo Nordisk; a project to develop recombinant human FVIIa (rFVIIa) for this purpose was approved by Novo Nordisk A/S on 30 June 1985. By this time, a proof of principle for the haemostatic effect of FVIIa had already been achieved [28,29].

The development of rFVIIa included extensive research into the characteristics of FVII and its activation [32–35]. The pharmacodynamics and pharmacokinetics of rFVIIa were also studied during the 1980s and 1990s [36–41]. Most of the research was performed by scientists in the haemostasis research group at Novo Nordisk that existed between 1985 and 1995, although much of the research was also performed outside of Novo Nordisk in collaboration with a host of well-known research groups within the area of haemostasis.

The first haemophilia patient with inhibitors was successfully treated with rFVIIa on 9 March, 1988 to cover open synovectomy of a knee joint at the Karolinska Hospital, Stockholm, Sweden [42]. The second patient was treated in May 1988 at Chapel Hill, NC, USA in association with a life-threatening bleed in the larynx region [43]. This case was followed by treatment of a series of seriously ill haemophilia patients with inhibitors who received rFVIIa with a high success rate [44]. The clinical development of rFVIIa was then undertaken by the clinical department at Novo Nordisk during the first half of the 1990s, and NovoSeven® was approved in Europe (February 1996), the United States (1999) and Japan (2000).

2.3. Mechanism of action of rFVIIa

It soon became apparent that more research should be devoted to the mechanism of action of pharmacological doses of rFVIIa. Mainly due to lack of resources for this part of the research at Novo Nordisk in the early 1990s, an extensive collaboration was initiated between Novo Nordisk and the Thrombosis and Hemostasis Group at the University of North Carolina at Chapel Hill, NC, USA, headed by Dr Harold Roberts. This collaboration produced a cell-based model for studying the mechanism of haemostasis on cell surfaces. It had been known since the mid-1980s that rFVIIa did not bind only to tissue factor (TF), as had been assumed previously, because it was able to normalise the activated partial thromboplastin time (aPTT) in the presence of artificial phospholipids in haemophilic plasma with inhibitors [45]. A similar shortening of the aPTT by rFVIIa in the absence of TF was reported by Telgt et al. [46], and it was suggested that rFVIIa may have the ability to bind to both TF and phospholipids in the cell membrane, and to be the mechanism for the control of bleeding in patients with haemophilia [44,47]. During the early 1990s, studies using the cell-based model, which included TF-expressing monocytes and platelets, led to increased recognition of the cell surface as an important factor in haemostasis, and a new model of the haemostatic mechanism was thus proposed [48–50]. In this model, the initial phase of coagulation occurs on the surface of a TF-expressing cell, following the exposure of blood to TF through a damaged vessel wall. At this stage, a complex is formed between TF and already-activated FVII present in the circulating blood. This complex provides the first limited amount of thrombin by activating FX on the cell surface. This small amount of thrombin is not sufficient to form the final fibrin haemostatic plug, but it does activate the platelets at the site of injury. FVIII–FIX complexes are formed on the activated platelet surface, and these complexes provide the most effective activation of FX, resulting in a full thrombin burst [48–50]. The amount and rate of thrombin generation correlate with the formation of a well-structured and tight fibrin plug, which is resistant to premature lysis [51,52].

In haemophilia, the initial step of the haemostatic process, including formation of the FVIIa–TF complex and the initial thrombin generation, is essentially normal [50]. However, as haemophilia patients lack FVIII or FIX, full FX activation on the thrombin-activated platelet surface does not occur, and there is no full thrombin burst. The resulting fibrin plugs are therefore loose and easily dissolved by normal fibrinolytic activity. This explains why the bleeding pattern in haemophilia patients is characterised by an initial weak haemostasis with the formation of loose, sloppy fibrin clots that are easily dissolved.

3. The role of rFVIIa in haemostasis and in haemophilia patients with inhibitors

Recombinant FVIIa binds to platelets with low affinity, and this low-affinity binding is exploited when using pharmacological doses of rFVIIa to initiate haemostasis in haemophilia patients [49]. The cell-based model demonstrated that adding increasing amounts of rFVIIa resulted in a dose-dependent increase of the thrombin burst, which at least partially normalised the impaired thrombin generation seen in haemophilia blood lacking FVIII or FIX [53]. Platelets from different individuals were found to vary widely in procoagulant function; and it has also been suggested that platelets contribute more than simply a surface for the generation of thrombin [50,53]. The increased generation of thrombin provided by the added rFVIIa results in the formation of a well-structured fibrin plug that is more resistant to premature lysis [54], which is probably essential for the haemostatic effect of pharmacological doses of rFVIIa seen in haemophilia patients [52].

A recent study has confirmed the importance of the platelet binding of rFVIIa (previously suggested by several authors [47,49,50]) by titrating rFVIIa into platelet-rich haemophilia A plasma and triggering coagulation with either TF or direct platelet activators [55]. When coagulation was initiated with TF, addition of rFVIIa up to 6 nM had hardly any effect on thrombin generation, while higher rFVIIa concentrations produced successively higher thrombin peaks. When coagulation was triggered with direct platelet activators, the effect of rFVIIa on thrombin generation was qualitatively very similar, with a measurable increase in peak thrombin generation at an rFVIIa concentration of 6 nM. This suggests that TF-independent, platelet-surface FX activation by rFVIIa begins to manifest itself at 6 nM or just below, when

1. **However, in the early 1980s, it was demonstrated that human FVII could be expressed by baby hamster...**
Anchor Name: Established in 30 years of research and experience *[Agency FCB Halesway Olga Kooi]*

competition with zymogen FVII for TF no longer occurs. This, in turn, suggests that the dose-dependent effect of rFVIIa in haemophilia works by a TF-independent mechanism of action [55]. These data support those from a mechanistic study in which a mutant murine FVIIa lacking any TF-dependent activity was as effective as normal murine FVIIa in controlling bleeding in haemophilia B mice [55,56].

Clinical data suggesting a prolonged haemostatic effect of rFVIIa in haemophilia patients with inhibitors [57] have raised the question of how this extended effect is brought about, considering the short biological half-life of rFVIIa in the circulation [41]. Studies in murine models suggest that this finding can be attributed to the localisation of rFVIIa to extravascular tissues [58,59]. These studies suggest that rFVIIa rapidly disappears from the blood following intravenous administration and associates with endothelium in an endothelial cell protein C receptor-dependent manner [58,59]. Once bound to endothelial cells, rFVIIa is thought to be transferred to the perivascular tissue surrounding the blood vessels and then diffused throughout the tissue, leading to retention of functionally active rFVIIa in tissues for an extended period [58,59]. Localisation of rFVIIa to these extravascular spaces, which occurs predominantly in regions that contain TF-expressing cells [58–60], may lead to rapid resolution of subclinical micro-bleeds and, as a result, prolonged haemostasis.

4. The role of rFVIIa in non-coagulopathic patients

Successful use of rFVIIa in haemophilia patients with inhibitors has led to its off-label use in a variety of clinical situations characterised by uncontrolled haemorrhage in non-coagulopathic patients. One of these clinical scenarios is trauma, where intractable bleeding is associated with a substantial risk of mortality and morbidity [61]. The first off-label use of rFVIIa was in a trauma patient [62], and based on the favourable results achieved, numerous reports of successful rFVIIa use in trauma have since been published. Most of the published studies are retrospective case series or reports [61,63–65], but two parallel, randomised, placebo-controlled, double-blind clinical trials showed a significant benefit of rFVIIa in blunt trauma [66]. While some guidelines now recommend rFVIIa use in trauma if standard haemostatic therapies fail [64,65], further studies are needed.

Another group of non-coagulopathic patients evaluated for treatment with rFVIIa is patients with intracerebral haemorrhage (ICH). In a dose-finding study published in 2005 [67], patients with an ICH showed dose-dependent (40, 80, 160 μg/kg rFVIIa) significantly decreased blood volumes as compared with a placebo group, provided the rFVIIa had been administered within 3 hours after symptom debut. The clinical outcome after 90 days was better in the rFVIIa-treated patients. In a later pivotal phase III study published in 2008 [68], patients showed significantly decreased haemorrhage volume when treated with 80 μg/kg rFVIIa as compared with placebo, provided that rFVIIa treatment was administered within 3 hours of the onset of symptoms. The overall frequency of thromboembolic serious adverse events was similar in the three treatment groups (20 or 80 μg/kg rFVIIa, placebo). While treatment with rFVIIa reduced the progression of the haematoma following ICH, survival or functional outcome were not improved.

It is important to note that rFVIIa is not currently licensed for use in trauma nor ICH patients.

5. Further research into the role of rFVIIa in the treatment of haemophilia patients with inhibitors and rFVIIa mode of action

The concept of using rFVIIa to generate haemostasis and compensate for the lack of FVIII or FIX in haemophilia is a new one. In substitution therapy with FVIII or FIX concentrates in patients without inhibitors, dosing can be adjusted by adding FVIII or FIX until the plasma levels of these factors reach a haemostatic level. However, a similar procedure cannot be applied to rFVIIa, as it is not known exactly how much extra FVIIa is required in the circulation to generate enough local thrombin to provide strong, well-structured fibrin haemostatic plugs at the site of injury. Unfortunately, no method for measuring local thrombin generation on cell surfaces at the site of injury is yet available.

Individual variation in platelet procoagulant function may further add to the difficulties in defining a standard dosing schedule. The demonstration of a higher immediate clearance rate in children <15 years of age [40,69] increases the need to individualise rFVIIa doses. Two controlled studies comparing rFVIIa 3 × 90 μg/kg with a single bolus dose of 270 μg/kg were performed by Novo Nordisk at the beginning of the new millennium [9,70]. Using the cell-based model, we were also able to demonstrate that high doses of rFVIIa also increased initial thrombin generation and mediated faster platelet activation in thrombocytopenia-like conditions [71].

6. Use of rFVIIa in patients with other rare bleeding disorders: Glanzmann's thrombasthenia and congenital FVII deficiency

From early on in its development, rFVII was used to treat heavy bleeding in patients with functionally defective platelets, including various forms of thrombasthenia (e.g., Glanzmann's thrombasthenia) [72,73]. Notably, an international survey in Glanzmann's thrombasthenia patients treated for bleeds and surgical/invasive procedures showed good effectiveness and tolerability for rFVIIa [74], and based on the clinical experience, rFVIIa was approved by the European Medicines Agency (EMA) in 2004 for use in patients with Glanzmann's thrombasthenia with past or present history of platelet refractoriness to platelet transfusions [75,76].

Lastly, an obvious use for rFVIIa is as a treatment for patients with congenital FVII deficiency. In these patients, administration of rFVIIa serves as a substitution treatment for the missing FVII. The dosing of rFVIIa in these FVII-deficient patients should be substantially lower than that used in patients with haemophilia or with platelet defects. In this respect, in FVII-deficient patients successfully treated with rFVIIa in an international registry and a compassionate use programme, the dose used was approximately 13.3–22 μg/kg per injection, with a dose interval of 1–18 hours for cases requiring several doses [77,78]. The EMA-recommended rFVIIa dose range in adults and children for the treatment of bleeds is 15–30 μg/kg, administered every 4–6 hours until haemostasis is achieved, with dose and frequency of injections adapted to each individual patient; for paediatric patients (<12 years old) with congenital FVII deficiency, rFVIIa may also be used for long-term prophylaxis – the dose and frequency of injections should be based on clinical response and adapted for each patient [75].

7. Conclusions

Recombinant FVIIa was initially developed to treat bleeding episodes in patients with congenital haemophilia and inhibitors; in these patients treatment with rFVIIa has been shown to be highly successful for the treatment of bleeds, and also in major surgery. Recombinant FVIIa is also highly effective in treating mild to moderate joint bleeds in a home-treatment setting. Extensive research over the past 30 years has increased our knowledge of the mechanisms of action of rFVIIa to restore haemostasis. Due to its success in haemophilia, rFVIIa use has been successfully extended to other coagulopathies that include

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acquired haemophilia, inherited factor VII deficiency and Glanzmann's thrombasthenia, for which rFVIIa is also licensed.

Conflict of interest statement

U Hedner was previously an employee of Novo Nordisk A/S, Denmark.

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[no notes on this page]