



Mechanism of Action of Recombinant Activated Factor VII: An Update

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Bleeding episodes in patients with hemophilia and inhibitors must be managed using agents that are hemostatically active in the absence of factor VIII or IX. Activated prothrombin complex concentrates have long been used in this context. However, the search for safer and more effective agents has led to the development of recombinant activated factor VII (rFVIIa; NovoSeven[®], Novo Nordisk, Bagsvaerd, Denmark). This paper presents an update on the mechanism of action of rFVIIa, and describes how pharmacologic doses of this agent enhance thrombin production and thus contribute to the development of a stable, lysis-resistant fibrin plug at the site of vessel damage. This mechanism explains the reported efficacy of rFVIIa in a range of clinical situations characterized by impaired thrombin generation.

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In patients with hemophilia complicated by inhibitory antibodies, hemostasis must be achieved with agents that are hemostatically active in the absence of factor VIII (FVIII) or IX (FIX). Activated prothrombin complex concentrates (aPCCs) containing both activated coagulation proteins and zymogens are still widely used for this purpose. To date, only two controlled studies have compared an aPCC with a non-activated PCC in this context, and both were published in the early 1980s.^{1,2} The trials demonstrated an efficacy rate of approximately 50% to 65% for aPCCs. In addition, the reported association of these concentrates with thromboembolic events means that their efficacy and safety are far from optimal.³

In the search for (pharmacologic) agents with greater efficacy and more robust safety profiles, activated factor VII (FVIIa) was identified as an attractive candidate. In healthy individuals and hemophilia patients, FVII circulates in its activated form (approximately 1% of the total FVII protein mass), highlighting the fact that FVIIa is not enzymatically active by itself. It must form a complex with the tissue factor (TF) that is exposed as a result of an injury to the vessel wall before it is capable of activating factor X (FX) into activated factor X (FXa). Activated FVII is not immediately inhibited by circulating antithrombin,⁴ and is therefore able to find its way

to the TF at the site of injury without its activity being neutralized.

Preliminary investigations showed that purified FVIIa from human plasma could induce hemostasis in two hemophilia patients with inhibitors.⁵ Based on these encouraging findings, recombinant FVIIa (rFVIIa; NovoSeven[®], Novo Nordisk, Bagsvaerd, Denmark) was developed for the treatment of such patients,^{6,7} and subsequent studies confirmed the utility of the agent in this indication. One report demonstrated an efficacy rate of 80% to 90% when rFVIIa was used to manage serious bleeds in hemophilic inhibitor patients⁸; furthermore, the efficacy rate during major surgery, including hip and knee arthroplasty, was found to be 90% to 100%.^{9,10} Today, rFVIIa is well established as an effective hemostatic treatment for hemophilia patients who develop inhibitors to FVIII or FIX.

Mechanism of Action of rFVIIa

Normal hemostasis is initiated by the formation of a complex between TF exposed as a result of vascular injury, and activated FVII already present in the circulation. Tissue factor is expressed by a number of different cells, all of which are located in the deeper layers of the vessel wall. It is a true receptor protein, and has one intramembranous and one intracellular component. The complex formed between TF and FVIIa is tight, and enzymatically activates FX on the surface of the TF-expressing cell. As a result of these initial reactions, a limited amount of thrombin is generated, and this amount is sufficient to activate the cofactors FVIII and FV. The initial

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thrombin generation also activates platelets, leading to the exposure of negatively charged phospholipids on their surfaces.¹¹ The negatively charged platelet surface forms a perfect template for the formation of the tenase and prothrombinase complexes, which in turn leads to the generation of the full thrombin burst necessary for the formation of a tight and stable hemostatic fibrin plug. The tenase complex is formed when activated FIX (FIXa), generated by the TF-FVIIa complex, binds tightly to the activated platelet surface. It then complexes with activated FVIII (FVIIIa) generated by the initially formed thrombin, leading to the activation of FX and the binding of FXa to FVa on the activated platelet surface, thus forming the prothrombinase complex.

The full thrombin burst is highly important for the fibrin structure of the hemostatic plug, as demonstrated by studies suggesting that the structure and porosity of fibrin vary according to the amount of added thrombin.^{12,13} Thrombin is also required for activation of factor XIII (FXIII), the fibrin-stabilizing factor necessary for the cross-linking of fibrin monomers that makes the hemostatic plug more resistant to premature lysis. Furthermore, high thrombin concentrations are required for the full activation of thrombin-activatable fibrinolytic inhibitor (TAFI).¹⁴ These observations suggest that a full thrombin burst is essential for the formation of a stable fibrin hemostatic plug that is resistant to premature fibrinolysis, thus providing a reliable and maintained hemostasis.

The Role of rFVIIa

The administration of pharmacologic doses of rFVIIa that reach plasma concentrations of ≥ 25 nmol/L induces hemostasis. This probably occurs via the enhancement of thrombin production on the surface of platelets activated by the initial thrombin generation. Ultimately, this leads to the formation of a tight, stable, hemostatic fibrin plug.

In a cell-based *in vitro* model, it was shown that rFVIIa binds to thrombin-activated platelet surfaces with a low affinity, requiring higher concentrations of rFVIIa than those found naturally in circulating blood.¹⁵ In the same model, thrombin generation in the absence of FIX was substantially improved following addition of rFVIIa in concentrations of 50 to 100 nmol/L, although it never completely normalized to the levels observed with physiological concentrations of factor XI.¹⁶

Optimal hemostatic efficacy requires not only an effective initial hemostasis, but also a maintained hemostasis that depends on the formation of a tight, stable, hemostatic fibrin plug resistant to premature lysis. In addition to efficient thrombin generation, which is characterized by rapid onset and a high rate and sufficient peak of production, a successfully maintained hemostasis requires activation of TAFI and FXIII. Optimal thrombin generation and polymerization of fibrin play an important role in these activation processes. Indeed, in one study, a dose-dependent normalization of fibrin permeability was achieved by adding rFVIIa to hemophilia plasma containing platelets—an effect that was reflected in a tighter fibrin structure.¹⁷

Conclusion

The hemostatic effect of exogenous rFVIIa, when added in pharmacologic doses, seems to be mediated by an enhanced thrombin generation rate on thrombin-activated platelet surfaces. This results in further activation of platelets at the site of injury, leading to increased exposure of phospholipids on the platelet surfaces, increased platelet adhesion, and aggregation involving glycoprotein Ib.¹⁸ Furthermore, this thrombin generation facilitates full activation of TAFI and FXIII and subsequent formation of a hemostatic plug that has a tight fibrin structure resistant to premature lysis. As a result of these processes, the fibrinolytic potential is downregulated.

To date, rFVIIa has demonstrated considerable efficacy not only in hemophilia, but also in platelet disorders such as Glanzmann's thrombasthenia (GT).^{19–21} Indeed, the agent has recently been approved in Europe for use in patients with GT who have antibodies to glycoprotein IIb–IIIa and/or human leukocyte antigen, with past or present refractoriness to platelet transfusions. Recombinant FVIIa has also been successfully used in trauma- and surgery-related hemorrhages, which may trigger severe coagulopathy.^{22–25} Based on the mechanism of action of rFVIIa described in this article, the hemostatic benefits provided by this agent in various situations characterized by impaired thrombin generation can be explained and understood.

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