Development and applications of recombinant activated factor VII

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Abstract

Recombinant activated factor VII (rFVIIa) was initially developed for treatment and prevention of bleeding during surgery and invasive procedures in congenital hemophilia with inhibitors against coagulation factors. Extensive research over the last few decades has contributed to the development of rFVIIa. These thorough studies not only helped to improve the biological activity and half-life of rFVIIa but also to enhance the knowledge regarding the mechanisms of action of rFVIIa to re-establish normal hemostasis. Since rFVIIa has been successfully in use for hemophilia treatment, it has been extended to other coagulopathies which characterized by the impairment of thrombin generation, including acquired hemophilia, Glanzmann's thrombasthenia, and congenital FVII deficiency. The development, the mechanism of action, and the clinical applications of rFVIIa are reviewed in this article.

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Introduction

Hemophilia is a most serious congenital bleeding disorder accounted by deficiency of coagulation factor VIII (FVIII; hemophilia A) and coagulation factor IX (FIX; hemophilia B). Without appropriate treatment, patients with severe hemophilia have a life expectancy of around 16 years.[1] The causes of development of factor inhibitors in hemophilia are multifactorial including genetic mutation, ethnicities, infection/inflammation and related genetics such as TNF-alpha, type of factor concentrate and the regimen of factor concentrate administration (prophylaxis versus on-demand). Large deletions that involve multiple domains have the highest proportion of inhibitor formation of 88%[2]. An inhibitor incidence of the most common severe FVIII mutation, intron 22 mutation, is 21%.[2] Patients of African or Hispanic descent have an increased risk of inhibitor formation3, however, the mechanisms that involved in these racial/ethnic differences remain unclear. Polymorphisms of TNF-α gene and IL-10 are associated with an increased risk of inhibitor formation.[4, 5] Intensive administration of FVIII or FIX concentrates for treatment of hemophilia A or B could induce inhibitors or antibodies against FVIII or FIX.[6] Thereafter, treatment with FVIII or FIX concentrates is ineffective. Previously, the other treatment for hemophilia A or B with inhibitors was plasma-derived activated prothrombin complex concentrates (aPCCs) and prothrombin complex concentrates (PCCs). Both products have the same efficacy. The aPCCs contains activated forms of all vitamin K-dependent coagulation factors, FII, FVII, FIX, and FX, and small amounts of coagulation factor VIII.[7] However, thromboembolic side-effects have been found in patients who were treated with overdosing and rapid infusion of aPCCs.[8] Activated coagulation factor VII (FVIIa) has been identified as one

of the activated coagulation factors that contains minimal potential for inducing thromboembolic side-effects.[9]

Nonetheless, the plasma-derived products are associated with the potential risk of transfusion-transmitted infections[8, 10, 11] though the risk is quite low in the era of viral inactivating method during the process of aPCC production. The requirement of newer products for improving the treatment of hemophilia with inhibitors, along with the progress in recombinant DNA technology, led to the development of recombinant activated factor VII (rFVIIa).[1, 12-14] Recombinant activated factor VII (rFVIIa, eptacog alfa [activated], NovoSeven®, Novo Nordisk A/S, Bagsværd, Denmark) was firstly approved for use in Europe in 1996, in the United States in 1999, and in Japan in 2000. rFVIIa has been commercially available since then. rFVIIa is currently licensed in Europe and in the United States for treatment and prevention of bleeding during surgery or invasive procedures in both congenital hemophilia with inhibitors against coagulation factors and acquired hemophilia.[15] In Europe, it is also approved for prevention of bleeding during surgery or invasive procedures in patients with congenital FVII deficiency, Glanzmann's thrombasthenia (GT), or antibodies to glycoprotein IIb-IIIa and/or human leukocyte antigen (HLA), with history or current refractoriness to platelet transfusions.[15]

Overview of FVII

Coagulation factor VII (FVII; UniProt: P08709) is also known as proconvertin and serum protein conversion accelerator (SPCA). FVII is a vitamin K-dependent serine protease with a molecular weight of 55 kDa. Gene encoding FVII is located on chromosome 13 (13q34)[16] and contains 9 exons and spans about 12.8 kb.[17] FVII is synthesized in the liver, and circulates in the blood as inactive zymogen. Circulating FVII is a single-chain polypeptide containing 406 amino acids. It is composed of four discrete domains including a γ-carboxyglutamic acid (Gla)-containing domain, two epidermal growth factor (EGF)-like domains, and a serine protease (catalytic) domain. After the serine protease cleavage of the bond between Arg152 and Ile153 by the coagulation factor IXa, factor Xa, factor XIIa, thrombin or minor proteolysis, this molecule is converted into an active form, activated factor VII (FVIIa). FVIIa consists of a 20-kDa light chain with γ-carboxyglutamic acid residues, and a 30-kDa heavy chain, which contains the catalytic domain. These two chains are held together by a disulfide bond.[18] FVII contains complex post-translational modification including γ-carboxylation, N- and O-linked glycosylation, and β-hydroxylation.[19] These modifications are necessary for secretion to blood circulation,[20] interaction with tissue factor (TF or coagulation factor III),[21] and platelet surface interaction between FVIIa/TF and its substrate coagulation factor X.[22, 23] FVIIa forms a complex with the tissue factor to activate the coagulation factors IX, X, and (autocatalytically) FVII.[16, 17]

Development of rFVIIa

Improvement of the rFVIIa activity has been extensively studied. The following is a review about the progress in the research on rFVIIa. Commercially available rFVIIa, NovoSeven®, is produced in the baby hamster kidney (BHK) cell line. rFVIIa produced from BHK contains a low degree of sialylation and a low degree of γ-carboxylation on the eleventh Gla residue than plasma-derived FVII.[24] O-glycans on Ser52 and Ser60 have been found to be different on plasma-derived FVII when compared with rFVII produced from BHK.[25] In addition, terminal N-acetyl galactosamines (GalNAc) were detected only on rFVII produced from BHK.[24, 26, 27] However, the biological activities of this rFVIIa are not affected, and are comparable to those of plasma-derived FVII.[28] There are reports of differences in N-glycosylation of rFVII derived from BHK, Chinese hamster ovary (CHO), and human embryonic kidney (HEK) 293 cells.[29] CHO-derived rFVII has been found to contain the highest degree of sialylation and no terminal GalNAc, with all other high-quality protein components at high productivity. The higher activity of CHO-derived rFVII in comparison to BHK-derived rFVII may have resulted from the different glycosylation patterns and sialylation content.[30]

Met306 in FVIIa appears to be involved in the communication between TF and the catalytic center of FVIIa responsible for the allosteric enhancement of FVIIa's activity.[31, 32] Substitution of Asp for Met306 prevents TF-induced allosteric changes which normally result in

extremely increased FVIIa activity.[33] In addition, substitution of Val for Leu305 increases the enzymatic activity of FVIIa.[34] Furthermore, the most active FVIIa variants carry concurrent substitution at positions 158, 296, and 298.[35] Substitution of Val for Glu296 and Gln for Met298 has been observed to increase the intrinsic amidolytic activity in comparison with wild-type FVIIa.[36] An additive effect was observed upon their combination. Substitution of Gln for Met298 is required for increased factor X activation, and the simultaneous substitutions of Asp for Val158, Val for Glu296, and Gln for Met298 (Mutant V158D/E296V/M298Q-FVIIa) resulted in the most profound effect on intrinsic amidolytic activity.[36] The rFVIIa analog DVQ (V158D/E296V/M298Q mutations; also called NN1731 and Vatreptacog alfa) was found to have higher proteolytic activity than rFVIIa in the tissue factor (TF)-independent activity on the surface of activated platelets, but was found to retain the same activity as rFVIIa in the presence of TF.[37] The DVQ analog has been shown to have increased procoagulant and antifibrinolytic activities in in vitro models of hemophilia at up to 50-fold lower concentration when compared to rFVIIa.[38, 39] In a later study, a new FVIIa variant with high intrinsic activity, called FVIIaVEAY or L305V/S314E/K337A/F374Y-FVIIa, was reported.[40] FVIIaVEAY was found to possess 22 times higher catalytic efficiency than wild-type FVIIa. Activation of factor X in solution occurred about 10 times faster with FVIIaVEAY than with wild-type FVIIa.[40] Recently, several rFVIIa analogs have been developed to have substantially higher tissue factor (TF)-independent activity than rFVIIa. Disulfide locked variants of factor VIIa with a restricted β-strand conformation were constructed to enhance the enzymatic activity.[41] These variants do not require TF as a cofactor for maximal activity in amidolytic assays.

Since the commercially available rFVIIa, NovoSeven®, has a very short half-life of approximately 2.4 hours, albumin fusion technology was introduced to overcome this problem. Using this approach, albumin is genetically fused to the C-terminus of rFVIIa *via* a glycine serine linker.[42, 43] The half-life of the rFVIIa fusion protein (rFVIIa-FP) was extended to 6- to 7-fold compared with wild-type rFVIIa, and its hemostatic properties were comparable to wild-type rFVIIa. PEGylation is an alternative approach to prolong the half-life of rFVIIa (N7-GP). The half-life on the N7-GP was also extended to 4- to 5-fold compared with wild-type rFVIIa,[44] and its enzymatic activity was fully retained.[45]

NovoSeven®, the rFVIIa, is secreted as an inactive, single chain rFVII into the culture medium. The single chain rFVII is autoactivated *in vitro* in the presence of a positively charged surface during purification.[46] The secreted rFVIIa is successfully obtained by co-transfection of human factor VII and hepsin genes to the Chinese hamster ovary (CHO) cell line.[47] The rFVIIa derived from the hepsin activation was sufficient to initiate the coagulation pathway and lead to thrombin formation.

The limitations of the mammalian expression system are low level of expression and high cost; a variety of recombinant protein expression systems have been developed as a resource of FVII gene expression. In 2010, the insect expression system which is considered as a higher eukaryotic expression system was tested in an attempt to produce rFVII in combination with the baculovirus expression vector system.[48] Due to the lack of endogenous vitamin K-dependent carboxylase, simultaneous expressions of human γ-carboxylase and human FVII genes were generated to achieve the functional rFVII.[48] rFVII production by the Lizard Leishmania expression system has also been reported.[49] However, functional rFVII obtained from this system was only 9%. This may be related to its post-translational modifications like γ-carboxylation. Thus, more investigations are required in order to determine post-translational γ-carboxylation of glutamic acid residues in the Gla domain of this product in Leishmania cells. In addition, an efficient protocol to enhance the expression of the recombinant coagulation factor VII (rFVII) in CHO cells by optimizing the signal peptides in the fed-batch culture was successfully established.[50]

Mechanism of action of rFVIIa

Based on the information from cell-based models of hemostasis, hemostasis occurs on the cell surfaces of TF-bearing cells and thrombin-activated platelets.[51, 52] The cell-based model of hemostasis is composed of three phases: initiation, amplification, and propagation. The initial phase of coagulation occurs when the damaged vessel wall brings plasma into contact with TF-bearing cells. This

leads to the formation of the TF-FVIIa complex on the cell surface at the injury site. This complex activates factor X (FX) and generates small amounts of thrombin.[51] This limited amount of thrombin is not enough to form the fibrin clot, but it activates the platelets at the site of injury. In the amplification phase, thrombin accelerates the platelet activation, and this results in the activation of FV, FVIII, and FXI.[51] The assembly of FVIII-IXa and FXa-FV complexes on activated platelets initiates the third stage of hemostasis, the propagation phase, and results in a burst of thrombin generation. This large amount of thrombin enhances the recruitment and adherence of additional platelets and the cleaving of fibrinogen into fibrin. This polymerization leads to the strengthening of the initial platelet plug into a stable fibrin clot.[53]

Clinical application of rFVIIa

In hemophilia patients with inhibitors, the low affinity binding of FVIIa to platelets leads to the use of pharmacological doses of rFVIIa to trigger hemostasis in hemophilia patients.[54] In addition, platelets from different individuals have been found to vary widely in procoagulant activity.[52, 54] Titrating rFVIIa into platelet-rich hemophilia A plasma and initiating coagulation with either TF or direct platelet activators has confirmed the importance of the platelet binding of rFVIIa.[13, 55] According to findings from cell-based models of hemostatis, increasing the amount of rFVIIa results in an increase in the thrombin burst in a dose-dependent manner.[54] The formation of a well-structured fibrin plug from the increased generation of thrombin is more resistant to premature lysis.[56] In substitution therapy with FVIII or FIX concentrates in hemophilia patients without inhibitors, dosing can be adjusted until the plasma level of these factors reaches the hemostatic level. However, this strategy cannot be applied to rFVIIa because an uncertain dose of rFVIIa is required in blood circulation to trigger enough local thrombin to provide strong and well-structured fibrin plugs at the site of injury.

In Glanzmann's thrombasthenia (GT), the hallmark of the disease is the deficiency or dysfunction of platelet-surface glycoprotein α_{IIb}-β_3 integrin (originally termed glycoprotein IIb-IIIa [gpIIb-IIIa]), and it leads to the impairment of thrombin generation and platelet aggregation.[57] Bleeding in GT is variable, and could include epitaxis, menorrhagia, hematuria, gingival hemorrhage, easy bruising, echymoses, and hemathrosis.[57, 58] Platelet transfusion is the standard treatment for bleeding. However, developing of antibodies to glycoprotein IIb-IIIa and/or HLA may occur.[57] In 2004, rFVIIa was approved by the European Medicines Agency (EMA) for GT patients with a history of platelet refractoriness to platelet transfusion.[59] rFVIIa is effective and relatively safe for the treatment of bleeding and for surgical prophylaxis in patients with GT.[59]

Normal Factor VII plasma concentration is 0.5 µg/mL. Factor VII levels of 15-25% (0.075–0.125 µg/mL) are generally sufficient to achieve normal hemostasis.[60] Congenital FVII deficiency is a rare autosomal bleeding disorder. The clinical phenotypes range from asymptomatic to severe, life-threatening, and disabling bleeding.[61] Treatment in congenital FVII deficiency consists of fresh frozen plasma (FFP), prothrombin complex concentrates (PCCs), or factor VII concentrates.[62] rFVIIa is an excellent alternative treatment and seems to be safe and effective for congenital FVII deficiency.[62] In addition, side effects or evidence of bleeding tendency have rarely been reported. In an estimated 4,500 rFVIIa-treated patients, 7 episodes of myocardial infarction, 5 episodes of DIC, 5 episodes of deep vein thrombosis, 4 incidents of cerebrovascular ischemia or infarction, and 1 episode of intestinal gangrene have been reported. However, most of these cases had apparent comorbid or predisposing factors.[63] rFVIIa seems to be effective in controlling life-threatening bleeding episodes in non-hemophilic patient with uncontrolled hemorrhage, who have not responded to all available standard treatments, including patients with Dengue Shock Syndrome[64, 65,] and in a patient with massive postpartum hemorrhage.[66] One thrombolic event in a nonhemophilic pediatric patient that related to administration of rFVIIa has been reported; however, no other serious adverse effects were published.[67]

Conclusions

From the literature data, it can be understood that several approaches were introduced to improve the biological activity and half-life of rFVIIa. This obviously demonstrates the fact that rFVIIa is a safe and effective treatment option for congenital hemophilia with inhibitors, acquired hemophilia, GT, and congenital factor VII deficiency. This strategy has improved both the treatment outcomes of bleeding patients as well as the quality of their life. However, the dose, timing and efficacy of rFVIIa in non-hemophilic patients with massive bleeding and uncontrolled bleeding should be considered on a case-by-case basis.

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