Prolonged in vivo half-life of FVIIa by fusion to albumin

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For the treatment of hemophilia patients with inhibitors, recombinant Factor VIIa (rFVIIa) is available as a therapeutic option to control bleeding episodes with a good balance of safety and efficacy. The short in vivo half-life of approximately 2.5 h requires multiple injections, which is inconvenient for patients and potentially places an increased burden of life-long prophylactic FVIIa treatment in genetic factor VII deficiency. In this study the prolongation of the plasma half-life of rFVIIa was investigated.

Background

Coagulation Factor VIIa (FVIIa) is a prothrombin factor with a molecular weight of approximately 50 kDa, synthesized in the liver and secreted as an inactive pro-enzyme into the bloodstream (1). In 1996, recombinant FVIIa (NovoSeven®) became available as a therapeutic option for hemophilia A and B patients with decreased FVII activity. In treating bleeding episodes in hemophilia patients with inhibitors (2), the short in vivo half-life of approximately 2.5 h makes multiple injections necessary. Thus, improvements in extending the half-life of FVIIa would facilitate the long-term therapy of human patients suffering from hemophilia A or B. Fusion of protein to albumin is a well-recognized strategy to extend the halflife of biopharmaceuticals (3). In this study, the expression and characterization of recombinant FVIIa fusion proteins was investigated. The results demonstrate that the in vivo half-life, the recovery, and the AUC of rVIIa-FP are significantly prolonged compared to rFVIIa.

Methods

Expression and purification: HEK-293 and CHO cells were transfected with FVII wild-type and rVII-FP cDNAs using the Lipofectamine 2000 reagent (Invitrogen), and grown in serum-free medium (293 Express, Invitrogen). In the presence of 25 µg/ml chloramphenicol, recombinant protein sera were collected after a fusion factor activity chromato-affinity. The recombinant Factor VIIa (rFVII) was secreted to cell culture medium, rFVII-FP activity was determined using a commercially available chromogenic test kit (Chromogenix Coaset FVII) using standard human plasma. By using these parameters, the concentrations of test samples were calculated using standard human plasma as reference. For pharmacokinetic analyses the concentration versus time data. The area under the curve (AUC) was calculated using the linear trapezoidal rule (5). SDS-PAGE analysis was performed on 8-16% Tris-glycine gradient gels using SeeBlue Plus 2 molecular size marker (Invitrogen).

Results and discussion: By genetic engineering human albumin cDNA was fused to the 3´-end of human FVII cDNA. In control, a human FVII cDNA was transfected. Purification of the FVII-FP and rFVIIa was accomplished through ion-exchange and size exclusion chromatography followed by activation of FVIIa. The specific activity of rFVIIa-FP was calculated using the specific activity of FVIIa in plasma (6) and assuming that the recovery factor of rFVIIa was 100%. The specific activity of rFVIIa-FP was determined and found comparable to commercially available rFVIIa (NovoSeven®). The specific activity of rFVIIa-FP was calculated as 104.8 IU/nmol (rFVIIa) and 72.8 IU/nmol (rFVIIa-FP). Thus, after conversion to the corresponding activated forms, rFVIIa was approximately 1.4-fold more potent in this assay than rFVIIa-FP.

Pharmacokinetic studies: In order to evaluate the half-life of FVIIa fusion proteins animal studies were performed. The pharmacodynamics of rFVIIa-FP was compared to rFVIIa and NovoSeven® in rats. First, the time course of the plasma levels for all three FVII preparations and human albumin is shown in Fig. 2. To analyze the data quantitatively, half-life and area under the curve (AUC) were calculated for each protein as shown in Table 2. Coagulation Factor VIIa (FVIIa) is a prothrombin factor with a molecular weight of approximately 50 kDa, synthesized in the liver and secreted as an inactive pro-enzyme into the bloodstream (1). In 1996, recombinant FVIIa (NovoSeven®) became available as a therapeutic option for hemophilia A and B patients with decreased FVII activity. In treating bleeding episodes in hemophilia patients with inhibitors (2), the short in vivo half-life of approximately 2.5 h makes multiple injections necessary. Thus, improvements in extending the half-life of FVIIa would facilitate the long-term therapy of human patients suffering from hemophilia A or B. Fusion of protein to albumin is a well-recognized strategy to extend the halflife of biopharmaceuticals (3). In this study, the expression and characterization of recombinant FVIIa fusion proteins was investigated. The results demonstrate that the in vivo half-life, the recovery, and the AUC of rVIIa-FP are significantly prolonged compared to rFVIIa.

For functional characterization the FVII coagulation activity was determined. The specific activity of rVIIa-FP was calculated using the specific activity of FVIIa in plasma (6) and assuming that the recovery factor of rFVIIa was 100%. The specific activity of rFVIIa-FP was determined and found comparable to commercially available rFVIIa (NovoSeven®). The specific activity of rFVIIa-FP was calculated as 104.8 IU/nmol (rFVIIa) and 72.8 IU/nmol (rFVIIa-FP). Thus, after conversion to the corresponding activated forms, rFVIIa was approximately 1.4-fold more potent in this assay than rFVIIa-FP. In this study, a fusion protein of FVIIa and albumin was generated to extend the half-life of FVIIa after intravenous injection. An rVII-FP was generated in which albumin was linked via a flexible glycine-serine linker. Pharmacokinetic studies in rats demonstrated the extended half-life of the rVIIa-FP. Therefore, our data demonstrate that it is possible to extend the half-life of a complex protein by fusion to albumin.

Conclusion

The superior pharmacological properties of the rVIIa-FP could facilitate a single dosing regimen of one injection rather than the current multiple injection strategy. Thus, our data demonstrate that it is possible to extend the half-life of FVIIa by fusion to albumin. The short in vivo half-life of approximately 2.5 h makes multiple injections necessary. Thus, improvements in extending the half-life of FVIIa would facilitate the long-term therapy of human patients suffering from hemophilia A or B. Therefore, our data demonstrate that it is possible to extend the half-life of a complex protein by fusion to albumin.

References