Determinants of Plasma Factor VIIa Levels in Humans

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Several enzymes can activate factor VII in vitro, but the protease responsible for generating factor VIIa in vivo has not been determined. Using recombinant tissue factor that has undergone a COOH-terminal truncation, a sensitive functional assay has been established for measuring plasma factor VIIa levels. To evaluate the mechanism responsible for the generation of factor VIIa in vivo, we measured the levels of this enzyme after administering purified concentrates of factor IX and factor VIII to patients with severe deficiencies of these clotting factors. In patients with hemophilia B, factor VIIa levels were initially reduced to 0.5 ± 0.1 ng/mL and gradually increased to normal after infusing 100 U/kg of body weight (BW) of factor IX. Despite these increases, there were no significant changes in the generation of factor Xa or thrombin. In patients with hemophilia A, only a slight reduction in factor VIIa levels (2.5 ± 1.3 ng/mL) was observed as compared with controls (3.3 ± 1.1 ng/mL) and no significant changes were observed after factor VIII levels were normalized. The administration of recombinant factor VIIa (10 μg/kg BW) to patients with factor VII deficiency increased the mean circulating level of the enzyme to 118 ng/mL, but this only resulted in normalization of the levels of the activation peptides of factor IX and factor X. The above data indicate that factor IXa is primarily responsible for the basal levels of free factor VIIa generated in vivo (i.e., in the absence of thrombosis or provocative stimuli) and that changes in the plasma concentrations of free factor VIIa in the blood do not necessarily lead to alterations in the extent of factor X activation.

HUMAN FACTOR VII IS A plasma glycoprotein whose enzymatic activity is greatly increased after limited proteolysis to factor VIIa. A number of enzymes are capable of activating factor VII in vitro, but the protease responsible for mediating this transformation in vivo has not yet been determined. Thrombin and factor XIa can activate factor VII in the absence of cofactors, whereas factor Xa and factor IXa require the presence of phospholipid and calcium. The binding of factor VII to tissue factor increases the catalytic efficiency of factor Xa in mediating this transformation and also permits factor VII to be autoactivated to factor VIIa.

Evidence for reductions in factor VII activity in patients with deficiencies of factor IX and factor VIII was previously provided by Miller et al. using a factor VII coagulant activity assay with increased sensitivity to factor VIIa. The levels of free factor VIIa can now be measured directly in human plasma with a clotting assay that uses recombinant tissue factor that is truncated to remove its cytoplasmic and transmembrane domains. This protein is unable to activate factor VII in the presence of phospholipid and calcium, but can support factor VIIa-mediated activation of factor X. Wildgoose et al. provided data that basal factor VIIa levels are markedly reduced in patients with severe factor IX deficiency, whereas patients with severe factor VIII deficiency exhibit only a moderate reduction of the enzyme as compared with normal individuals. However, factor VIIa levels are known to increase with advancing age and it was not reported whether the hemophilic patients were compared with an appropriate age-matched control group.

To more fully evaluate the mechanisms responsible for the activation of factor VII in vivo, we performed a series of infusion studies in patients with severe factor deficiencies of factor IX, factor VIII, and factor VII and monitored the levels of free factor VIIa in their blood.

The findings of these investigations indicate that, under basal conditions (i.e., in the absence of thrombosis or provocative stimuli), factor IXa is primarily responsible for the generation of free factor VIIa in vivo.

MATERIALS AND METHODS

Collection and processing of blood samples. Venipunctures were performed atramatically with 19- or 21-gauge butterfly infusions sets. Blood samples for factor VIIa levels were drawn into 3.8% (wt/vol) sodium citrate and the ratio of anticoagulant to blood used was 0.1:0.9 (vol/vol). For assays of factor IX activation peptide (FIXP), factor X activation peptide (FXP), and prothrombin fragment F1 + 2, an anticoagulant containing ACD (38 mmol/L citric acid, 75 mmol/L sodium citrate, 136 mmol/L dextrose), 6 mmol/L EDTA, 6 mmol/L adenosine, and 25 U/mL heparin was used; the ratio of anticoagulant to blood used was 0.2:1.0 (vol/vol). Platelet-poor plasma fractions were obtained by centrifugation at room temperature for 20 minutes at 1,600g and stored at −80°C before analysis.

Coagulation studies. Quantitation of plasma factor VIIa levels was performed with a recombinant tissue factor protease that does not promote factor VII activation according to the procedure of Wildgoose et al. This soluble form of tissue factor contains the first 218 amino acids of tissue factor (sTF218) and was kindly provided by Dr. Yale Nemerson (Mount Sinai School of Medicine, New York, NY). It was purified from the culture media of transfected BL-21 (DE3) Escherichia coli cells by ammonium sulfate precipitation followed by SP Sepharose and Q Sepharose (Pharmacia Biotech Inc., Piscataway, NJ) ion exchange chromatography. Plasma factor VIIa levels were measured in duplicate in an one-stage clotting assay using an ACL 3000-plus automated coagulometer (Instrumentation Laboratories, Lexington, MA) in the research mode. The standard curve contained recombinant factor VIIa (Novo Nordisk, Gentofte, Denmark) at concentrations of 0.01, 0.1, 1.0, and 100 ng/mL in

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performed using standard laboratory methods. The coagulant activity in plasma samples was determined from the clotting times by factor VIIa assay was constructed from 148 healthy individuals (7 male patients; median age, 35 years; age range, 21 to 84 years). Patients and clotting factor infusions. Infusions of factor IX and factor VIII were performed in patients with hemophilia B and hemophilia A, respectively, at the A. Bianchi Bonomi Hemophilia and Thrombosis Center (Milan, Italy). These infusions provided treatment for mild bleeding episodes (eg, simple hemarthroses, hematomas, epistaxis, and hematuria). Blood samples were obtained before the administration of the purified concentrate and after the infusion at 5 different time points from 15 minutes to 6 hours. For hemophilia B patients, a highly purified nanofiltered factor IX concentrate produced by Laboratoire Francais du Fractionnement et des Biotechnologies (LFB; Lille, France) and a monoclonal antibody (MoAb)-purified factor IX concentrate (Mononine; Armour Pharmaceutical Company, Blue Bell, PA) were used. These concentrates do not contain detectable amounts of prothrombin, factor VII, or factor X. An MoAb-purified factor VIII concentrate (Hemophil-M) supplied by the Hyland Division of Baxter Healthcare Corp (Glendale, CA) was administered to patients with hemophilia A. None of the patients with hemophilia B or hemophilia A had received concentrate during the week preceding the infusion. One unit is defined as the amount of factor in 1 mL of pooled normal plasma. Twelve of the hemophilia patients were human immunodeficiency virus (HIV) positive, but without symptoms of HIV disease, and none had clinical evidence of severe liver or kidney disease. Patients with factor VII deficiency were not experiencing bleeding when recombinant factor VIIa (Novo Nordisk) was administered to patients with hemophilia B. Informed consent. The experimental protocols were approved by the Institutional Review Boards at the centers where the studies were performed. Written informed consent was obtained from all patients receiving infusions of purified clotting factor concentrates in accordance with the precepts established by the Helsinki Doctrine.

RESULTS

We first investigated the effect of factor IX concentrates on plasma factor VIIa levels in hemophilia B patients. To this end, 6 individuals (median age, 33 years; age range, 20 to 54 years) were infused with 100 U/kg of body weight (BW) of a highly purified factor IX preparation (Factor IX LFB). All had baseline plasma factor IX levels that were less than 1% of normal and their mean factor VIIa concentration was significantly suppressed as compared with that of normal individuals (0.5 ± 0.1 [SD] ng/mL vs 3.3 ± 1.1 [SD] ng/mL; P < .0001). The plasma levels of factor IX and factor VIIa before and after the infusions are shown in Fig 1. The highest factor IX measurements were attained at 15 minutes postinfusion, and the measurements remained within the normal range throughout the 6-hour study period. However, the mean factor VIIa concentration increased gradually after the infusion and reached its highest level at the 6-hour time point (3.2 ± 1.2 [SD] ng/mL).

We also measured plasma factor VIIa levels in 4 patients (median age, 36 years; age range, 31 to 54 years) who received 100 U/kg BW of another highly purified factor IX concentrate (Mononine). The concentrations of FIXP and FXP had previously been determined in these individuals (patients no. 3, 4, 5, and 6 in Bauer et al19), thus affording us the opportunity to examine the relationship between changes in plasma factor VIIa levels and in vivo indices of factor IX and factor X activation (Fig 2). In these patients, the factor IX infusions were followed by a gradual normalization of factor VIIa levels over several hours, similar to that observed in the hemophilia B cohort receiving Factor IX LFB, and a gradual normalization of FIXP levels. A highly significant correlation was observed between the plasma levels of factor VIIa levels and FIXP in these patients (r = .72). Despite the increases in these measurements, a significant increase in the levels of FXP (Fig 2) and prothrombin activation fragment F1+2 levels was not observed.16

We next examined the effect of factor VIII replacement on factor VIIa levels in 6 patients (median age, 28 years; age range, 17 to 52 years) with severe factor VIII deficiency (factor VIII levels <1% of normal). The infusion of 100 U/kg BW of a highly purified factor VIII concentrate (Hemofil M) resulted in a rapid normalization of factor VIII levels (Fig 3). The mean baseline factor VIIa level in the hemophilia A patients was modestly reduced compared with that of normal individuals (2.5 ± 1.3 [SD] ng/mL vs 3.3 ± 1.1 [SD] ng/mL; P = .0028), but a significant increase was not observed during the 6-hour observation period after the infusions.

To further investigate the relationship between plasma factor VIIa levels and the activation of factor IX and factor X, we measured the concentrations of free enzyme in plasmas of 2 unrelated patients with factor VII coagulant activity levels of less than 1% of normal and 3%, respectively, who had previously been infused with recombinant factor VIIa.19 Before infusion, no factor VIIa was detectable in their plasmas. After administering 10 μg/kg BW of recombinant factor VIIa, the levels of factor VIIa peaked at 134 ng/mL and 103 ng/mL at 15 minutes, which are about 25-fold higher than in normal individuals (Fig 4). The levels of FIXP and FXP in the 2 patients were markedly reduced at baseline, but only increased into the normal range after the infusions of recombinant factor VIIa (Fig 4).

DISCUSSION

Several assays are available that measure activation peptides in human blood that are liberated when coagulation zymogens are converted to serine proteases in vivo. These assays include sensitive radioimmunoassays for FIXP, FXP,
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Fig 1. Plasma levels of factor IX coagulant activity and factor VIIa in 6 patients with severe factor IX deficiency in response to intravenous infusions of a highly purified factor IX concentrate at a dose of 100 U/kg BW. The bars denote the mean ± SEM.

and F1+2. The presence of these species, albeit at an extremely low level, in healthy individuals indicates that the coagulation system is normally active. The application of these techniques to the study of patients with hereditary coagulation factor deficiencies has also generated information regarding the pathways responsible for coagulation activation in vivo under basal conditions (ie, in the absence of thrombosis or provocative stimuli). Patients with factor VII deficiency, but not factor XI deficiency, have reduced levels of F1XP, FXP, and F1+2, and administration of relatively small doses of recombinant factor VIIa (ie, 10 μg/kg BW) to these patients increases the levels of these markers into the normal range. Patients with severe deficiencies of factor IX or factor VIII have normal levels of F1XP and F1+2, and these levels are unchanged after infusions of factor IX or factor VIII, respectively. Thus, under basal conditions, coagulation system activation is mainly attributable to factor VII-tissue factor pathway function, which is responsible for the generation of factor IXa, factor Xa, and thrombin. However, the factor IXa that is formed is unable to convert factor X to factor Xa.

Using recombinant tissue factor that has undergone a COOH-terminal truncation, it is now possible to directly measure factor VIIa levels that are normally present in human plasma. Very low concentrations were reported in patients with hemophilia B as well as moderate reductions in hemophilia A. In the present study, we investigated patients with hereditary deficiencies of factor IX, factor VIII, and factor VII using this new factor VIIa coagulation assay. Our data show that the mean factor VIIa level in patients with severe factor IX deficiency is markedly suppressed as compared with that of age-matched normals. The administration of full replacement doses of factor IX led to a normalization of the factor VIIa levels, thereby showing that factor IXa is primarily responsible for generating the basal levels of free factor VIIa in vivo. We also found that factor VIII-deficient patients exhibit a moderate reduction in factor VIIa levels, but factor VIII infusions did not alter the levels of free factor VIIa. The biologic significance of the reduced factor VIIa levels in hemophilia A patients is thus uncertain.

In patients with hemophilia B, the consequences of changes in plasma factor VIIa levels can be ascertained by correlating the results with the levels of F1XP, FXP, and F1+2 after coagulation factor infusions. After factor IX replacement, the levels of factor VIIa levels increased gradually over a period of several hours and a strong positive correlation was observed with F1XP. These data therefore provide evidence that the cleavage of factor VII by factor IXa is required to accelerate the conversion of additional factor IX to factor IXa. Despite the substantial increments...
in factor VIIa and FIXP, significant alterations were not observed in the generation of factor Xa or prothrombin as measured by assays for FXP and F₁+₂, respectively. This finding contrasts to the situation in factor VII-deficient patients in whom the administration of recombinant factor VIIa at a dose of 10 μg/kg BW increased the levels of FIXP, FXP, and F₁+₂ into the normal range. The peak level of factor VIIa 15 minutes postinfusion in the 2 individuals averaged 118 ng/mL, which is 35-fold higher than that found in normal controls, and remained elevated for the duration of the study.

In attempting to explain the above observations, it is important to consider the role of tissue factor in factor VII activation and in factor VIIa-mediated activation of factor IX and factor X. We previously showed that the administration of a potent MoAb to human tissue factor suppresses basal level activation of factor IX and factor X in normal chimpanzees. Despite the marked reduction in the levels of free factor VIIa in hemophilia B patients, our data suggest that sufficient enzyme must bind or be generated at endogenous tissue factor sites to achieve a normal level of factor X activation. It has been shown that factor VIIa in vitro at picomolar concentrations can activate factor VII bound to tissue factor. After factor IX replacement, the fact that FXP levels remain unchanged as plasma factor VIIa measurements increase into the normal range indicates either that there are no additional tissue factor sites available for enzyme binding or that the sites are occupied by factor VII zymogen, which acts as a competitive inhibitor of the enzyme. However, because FIXP concentrations increase into the normal range with a time course similar to that of factor VIIa, we infer that the endogenous factor VIIa-tissue factor complex activates factor X in a manner that is different than factor IX. In patients with factor VII deficiency receiving recombinant factor VIIa infusions, the presence of a relatively limited number of tissue factor sites also helps reconcile the high factor VIIa concentrations with the levels of FIXP, FXP, and F₁+₂ that only increased to the normal range. We cannot at this time exclude the possibility that the activation of factor VII mediated by factor IXa is independent of tissue factor altogether and that this conversion occurs in the fluid phase or when the zymogen binds to nonspecific phospholipid binding sites.

In summary, these investigations indicate that factor IXa is primarily responsible for the basal levels of free factor VIIa generated in vivo and that changes in plasma concentrations of free factor VIIa in the blood do not necessarily lead to alterations in the extent of factor X activation. Future studies will determine whether plasma factor VIIa levels correlate with indices of coagulation activation such as FIXP, FXP, and F₁+₂ in other clinical and experimental situations.

Fig 3. Plasma levels of factor VIII coagulant activity and factor VIIa in 6 patients with severe factor VIII deficiency in response to intravenous infusions of an MoAb-purified factor VIII concentrate at a dose of 100 U/kg BW. The bars denote the mean ± SEM.

Fig 4. Plasma levels of factor VIIa, FIXP, and FXP in 2 patients, a 17-year-old boy (e) and a 21-year-old woman (C), with factor VII deficiency in response to intravenous infusions of recombinant factor VIIa at a dose of 10 μg/kg BW.
as well as the utility of these assays in predicting thrombotic events.

REFERENCES

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