Human Plasma Extrinsic Pathway Inhibitor Activity: II. Plasma Levels in Disseminated Intravascular Coagulation and Hepatocellular Disease

By Thomas A. Warr, L. Vijaya Mohan Rao, and Samuel I. Rapaport

Plasma or serum extrinsic pathway inhibitor (EPI) activity was measured in 24 patients with disseminated intravascular coagulation (DIC) and in 23 patients with severe hepatocellular disease. EPI activity was measured as activity in a test sample that inhibited factor VIIa/tissue factor (TF)-catalyzed activation of $^3$H-factor IX (activation peptide release) in the presence of factor X. Of the 24 patients with DIC, 13 had sepsis and five had metastatic carcinoma, disorders in which tissue factor is believed to initiate DIC. EPI activity ranged from 68% to 300% (mean 134% ± 50%). Serial measurements in nine patients failed to show depletion of EPI activity coincident with worsening DIC. DIC induced by tissue factor or other activating materials may progress despite normal EPI levels. In the patients with liver disease, of whom 15 had decompensated chronic hepatocellular disease (two fatal cases) and eight had acute fulminant liver failure (seven fatal cases), plasma or serum EPI activity varied from <20% to 194%. Values were distributed in a bimodal fashion. EPI activity could not be correlated with either the etiology of the liver disease or the degree of prolongation of the prothrombin time. Patients with chronic hepatocellular disease who survived had normal or elevated EPI activity. Patients with fatal hepatic dysfunction had low, normal, or high values for EPI activity. This must mean that secretion of EPI from cells other than hepatocytes can maintain normal plasma EPI levels.

ALTHOUGH THE EXISTENCE of a material in plasma and serum that can inhibit the catalytic activity of the factor VIIa/tissue factor (TF) complex was demonstrated >30 years ago, its requirement for factor Xa as a cofactor, association with lipoproteins, purification, and characterization as a Kunitz-type proteinase inhibitor by translation of its cDNA have been delineated only during the past 5 years. This inhibitor is referred to by different investigators as either the extrinsic pathway inhibitor (EPI), the term used herein, or the lipoprotein-associated coagulation inhibitor (LACI). A physiologic role for the inhibitor has yet to be established experimentally. Nevertheless, its activity in vivo could account for the abnormal bleeding of hemophiliac patients. Inhibition of factor VIIa/TF by a mechanism triggered by the factor Xa generated initially at a wound site would explain the need for a factor IXa/factor VIIIa/phospholipid complex as a continuing activator of factor X.

Assays for plasma EPI activity are based on EPI’s ability in a test sample to inhibit factor VIIa/TF in the presence but not in the absence of factor Xa. We recently described the standardization of such an assay and its use to measure physiologic variation in EPI activity in normal subjects. Limited and somewhat conflicting data on plasma EPI activity in pathologic states were recently published by two laboratories. Both reported finding normal plasma EPI levels in patients with chronic liver disease. However, one group reported finding low levels of plasma EPI in patients with disseminated intravascular coagulation (DIC), whereas the other group reported finding levels that varied from 60% to 190%. We report additional data on plasma EPI levels in patients with DIC of multiple causes and in patients with liver disease of different degrees of severity.

MATERIALS AND METHODS

Platelet-poor plasma (PPP) specimens were obtained from normal persons and from patients at UCSD Medical Center, San Diego VA Medical Center or Los Angeles County/University of Southern California Medical Center by the standard techniques used to prepare such plasma for clinical coagulation studies. Additional serum samples from patients with terminal liver failure were obtained from the University of Southern California Liver Service at Rancho Los Amigos Medical Center. Specimens were stored frozen until assayed for EPI activity. (Freezing and thawing does not affect plasma EPI activity.) A human plasma standard, prepared by pooling plasma from 23 fasting healthy young adults, was assigned a value of 100% EPI activity.

All coagulation assays other than measurement of EPI activity were performed in the clinical coagulation laboratories of the hospitals cited above. The EPI assay has been described in detail elsewhere. In this assay, the concentration of TF in the reaction mixture determines the concentration of factor VIIa/TF available to react with the EPI of a test sample. Residual factor VIIa/TF is measured by its ability to activate factor IX as monitored by release of activation peptide from sialyl $^3$H-factor IX. All reagents are purified human materials. Final concentrations of reactants are: 0.25 µg/mL factor VII, 10 ng/mL reconstituted purified TF, 2.5 µg/mL factor X, a dilution between 2% and 5% of the test plasma (vol/vol), 5 µg/mL $^3$H-factor IX, and 10 mmol/L CaCl$_2$ in a final volume of 120 µL. Each plasma specimen is assayed in duplicate at two concentrations, and trichloroacetic acid (TCA) soluble radioactivity is converted to percentage of EPI activity from a reference curve prepared with dilutions of the standard plasma, which is arbitrarily assigned a value of 100% EPI activity. The mean value of the four determinations is used as the EPI activity of a given specimen.

Supplemental tests were performed on several specimens chosen at random to confirm that the inhibition of factor VIIa/TF observed in the assay was dependent on factor Xa. The reaction mixture of the assay was modified by omission of supplemental factor X, by substitution of factor VIIa for factor VII, and by addition of a
polyclonal antifactor X antibody to neutralize the factor X activity of the test sample. No test sample assayed under these conditions contained measurable factor VIIa/TF inhibitory activity.

Twenty-four patients met all of the following criteria for the diagnosis of DIC: an underlying disease known to predispose to DIC (Table 1), a prolonged prothrombin time, thrombocytopenia, either hypofibrinogenemia or a fibrinogen level rapidly decreasing from a previously elevated level, and fibrin degradation products >10 μg/mL as measured by the Thrombo-Wellic test. Most patients had clinical evidence of thrombosis, abnormal bleeding, or both, and seven patients had histologic confirmation of intravascular deposits of fibrin.

Fifteen patients with chronic liver disease had a history of cirrhosis due to alcohol abuse (11 patients) or chronic active hepatitis (four patients) dating back months or years. The patients were jaundiced, had ascites, or both, and exhibited other stigmata of chronic liver disease. Eight patients with acute liver disease had a recent onset of symptoms, signs, and laboratory features of acute hepatitis. Etiologies included fulminant hepatitis B in six patients, acute fatty liver of pregnancy in one patient (the only nonfatal case), and acute fatty liver of unknown etiology in one patient.

RESULTS

Plasma EPI levels in DIC. The conditions of the 24 patients with DIC are shown in Table 1. In 15 patients, EPI was measured in a single plasma specimen; in nine patients EPI was measured in serial samples. Figure 1 summarizes the data for EPI activity in a single plasma specimen from each of the 24 patients. In the nine patients in whom EPI was assayed serially, the single value used in Fig 1 corresponds to the time at which the clinical and laboratory evidence of DIC was most pronounced. Mean plasma EPI activity for a group of 43 normal adults was 105% (range 74% to 172%), whereas mean plasma EPI activity for the 24 patients with DIC was 134% (range 67% to 300%).

Clinical or laboratory evidence of worsening DIC was not associated with a substantial decrease in plasma EPI activity below the lower level of the range for normal adults in any of the nine patients in whom EPI levels were measured serially. Indeed, in several patients, plasma EPI activity appeared to increase as the clinical condition deteriorated (eg, at a time when one patient was developing gangrenous changes in fingers and toes). Brief clinical histories and serial laboratory values are presented for two patients with DIC secondary to different underlying disorders.

Patient 1: DIC secondary to metastatic gastric carcinoma. A 36-year-old woman in the second trimester of pregnancy was admitted to the hospital because of persistent bleeding from the site of biopsy of a supraclavicular mass and from a bone marrow (BM) biopsy site. The biopsies demonstrated poorly differentiated adenocarcinoma. Laboratory data included the following: hemoglobin 10.7 g/dL, platelets 85,000/μL, prothrombin time 15.4 seconds (control 11.5 seconds), fibrinogen 70 mg/dL, and fibrin degradation products 80 μg/mL. Results of specific assays of hemostatic factors were as follows: factor VII 101%, factor X 40%, factor V 39%, factor VIII 100%, antithrombin III 101%, and α2-antiplasmin 16%. Liver function tests were abnormal: AST (SGOT) 134 IU/L, ATL (SGPT), 191 IU/L, LDH 374 IU/L, and alkaline phosphatase 237 IU/L (normal <130 IU/L).

An intravenous (IV) infusion of a combination of heparin, 500 IU/hour, and ε-aminocaproic acid, 1 g/h was stopped
after ten minutes because the patient became acutely short of breath. Chemotherapy with 5-fluorouracil, Adriamycin, and cis-platinum was administered on the fifth hospital day. The patient died on the sixth hospital day after a second episode of acute shortness of breath. Autopsy demonstrated widely metastatic gastric adenocarcinoma. Many microscopic tumor emboli associated with deposits of fibrin were noted in the pulmonary microvasculature. An arterial embolus was present in the right kidney.

Serial values for EPI, fibrinogen, and platelets are shown in Fig 2. In this patient with DIC secondary to metastatic adenocarcinoma, repeated assays of plasma EPI activity remained essentially unchanged at the lower level of the normal range despite a persisting hypofibrinogenemia suggesting a continuing defibrination for at least 6 days and autopsy evidence of intravascular deposits of fibrin.

Patient 2: DIC secondary to a mediastinal abscess caused by a gram-negative organism. A 59-year-old man shot himself in the chest in an attempted suicide. An emergency thoracotomy was performed, and a perforated left ventricle was repaired with a pericardial patch. A damaged left lower lobe of the lung was also removed. Twenty-five units of packed RBCs, 20 U fresh frozen plasma, and 30 U platelets were administered in the first hospital day. The patient improved and was extubated 36 hours after admission. However, on the fourth hospital day he developed fever, intermittent hypotension, and respiratory insufficiency requiring reintubation. The WBC count was 23,800/µL, the plasma protamine paracoagulation test for fibrin monomer was positive, and fibrin degradation products were 80 µg/mL. During the ensuing 48 hours, his urinary output decreased and he developed oliguric renal failure requiring dialysis. Because his condition worsened despite systemic antibiotics, an exploratory laparotomy with cholecystectomy was performed on the seventh hospital day. An intraabdominal source of infection could not be found. On the eighth hospital day, the thoracotomy wound dehisced and a pocket of purulent fluid was discovered in the chest cavity. Acinetobacter was grown from the wound, abscess fluid, and sputum. After drainage and a change of antibiotics, the patient began to improve. He became afebrile and slowly recovered adequate respiratory function. However, oliguric renal failure has persisted, and the patient requires chronic hemodialysis therapy.

Serial fibrinogen levels, platelet counts, and plasma EPI levels are plotted in Fig 3. The initial increase in fibrinogen level, which reached 850 mg/dL on the fifth hospital day, reflects increased fibrinogen synthesis secondary to the inflammatory stimuli of trauma, surgery, and infection. The subsequent decrease in fibrinogen, reflecting its consumption in intravascular coagulation triggered by gram-negative endotoxemia, was associated with an increasing level of plasma EPI activity. The intrathoracic abscess was drained on day 8. Abbreviations as in legend to Fig. 2.
inflammatory stimuli of trauma and infection. The decrease in fibrinogen between hospital days 5 and 8 is believed to reflect gram-negative endotoxemia of sufficient degree to initiate intravascular coagulation extensive enough to decrease fibrinogen levels substantially despite a presumed continuing increased fibrinogen synthesis. The persisting oliguric renal failure probably stems from renal cortical necrosis secondary to deposition of fibrin in the glomerular capillary bed (ie, the generalized Shwartzman reaction). EPI levels did not decrease, but instead increased, during the period of DIC.

**Plasma EPI levels in liver disease.** Plasma or serum was obtained for measurement of EPI activity from 15 patients with chronic liver disease at a time of decompensation of the liver disease requiring hospitalization. Two of the patients were terminally ill. In 13 of these patients, the prothrombin time ranged from 13.1 to 19.3 seconds (control ~12 seconds). In the two terminally ill patients, the prothrombin time, performed at another institution by the Prothrombin and Proconvertin (P and P) method, was <8%. Plasma or serum was also available for assay from eight patients with severe acute hepatitis, seven of whom died of fulminant liver failure. Prothrombin times were 19 seconds in two patients, 58 seconds in a third patient, and <8% by the P and P method in the remaining five patients.

These patients had a wide range of EPI activity (from <20% to 194%). Seven (30%) had EPI activity <70%, which is the lower limit of normal for EPI activity. As shown in Fig 1, the values appeared to segregate into two groups: a group of values clustering around 170% and a second group clustering around 70%. A goodness-of-fit analysis confirmed the visual impression (Fig 1) that the EPI activities for the entire group of liver disease patients were not part of a single normally distributed population. Values for EPI activity from patients with acute fatal liver disease and from patients with decompensated chronic liver disease were distributed approximately evenly between the high and low groups. Moreover, there was no correlation between a patient's prothrombin time and plasma (or serum) EPI activity. A Mann-Whitney test demonstrated no difference between the EPI activities from patients with acute and chronic liver disease.

**DISCUSSION**

DIC could decrease plasma EPI levels by two possible mechanisms, either through formation of EPI/factor Xa complexes2,3 and their subsequent accelerated intravascular clearance or through the stoichiometric formation of putative quaternary EPI/factor Xa/factor VIIa/TF complexes. Therefore, testing the hypothesis that DIC from any cause decreases plasma EPI activity appeared reasonable. Moreover, we believed it important to examine whether a decrease in plasma EPI activity resulting from an initial episode of DIC could worsen subsequent DIC when exposure of blood to TF was the pathogenetic mechanism.

However, our results show that EPI activity was not depressed in patients with DIC from a variety of causes (Table 1). Levels of plasma EPI activity varied from the lower limit of the normal range to values well above those encountered in normal individuals. Serial measurements from nine patients, as illustrated for two patients in Figs 2 and 3, eliminated the possibility that our failure to detect reduced EPI activity in DIC stemmed from inappropriate sampling times.

In most of our patients, particularly in those with DIC secondary to sepsis or metastatic carcinoma, we have good reason to believe that exposure of the circulating blood to TF triggered the intravascular coagulation.1,2 Therefore, normal levels of plasma EPI probably cannot protect against continuing DIC resulting from a continuing exposure of circulating blood to TF, and variation in plasma EPI activity probably has no modulating influence on DIC from a variety of causes. However, our results differ from those of Bajaj and colleagues,9 who reported plasma EPI levels <60% of normal in single samples from six of ten patients with DIC; they suggested that EPI is consumed in DIC. Nine of the ten patients studied by Bajaj et al had DIC secondary to sepsis, but so did many of our patients (eg, patient 2 whose serial plasma EPI levels are plotted in Fig 3). Therefore, the reason for the difference between our data and those of Bajaj et al is not apparent. Our results are more in accord with the data of Andersson et al,10 who reported plasma EPI levels between 60% and 190% in eight patients with DIC of unspecified etiopathogenesis.

However, a normal level of plasma EPI activity in DIC does not rule out the possibility, indeed the likelihood, of some degree of increased consumption of EPI in DIC. An initial injection of endotoxin into rabbits caused an increased intravascular clearance of fibrinogen which was not reflected by a decrease in fibrinogen level because fibrinogen synthesis simultaneously increased.19 We suggested previously8 that EPI is not an acute-phase reactant because plasma EPI activity failed to increase after surgical procedures that resulted in an increase in fibrinogen level. However, many patients in the present study had elevated levels of EPI activity while seriously ill suggests that EPI levels may increase due to increased synthesis or release in response to severe illness. The increase in plasma EPI level observed in patient 2 during the period of presumed endotoxemia (Fig 3) supports this hypothesis. Increased synthesis or release could well mask a substantially increased consumption of EPI during ongoing DIC.

Why does a normal level of plasma EPI fail to shut off TF-induced DIC when it is so effective in shutting off the continuing factor VIIa/TF activation of factor IX and factor X in vitro? Since plasma EPI activity is not depleted, the answer cannot be that circulating blood is exposed to concentrations of TF exceeding the capability of plasma EPI to neutralize stoichiometrically factor VIIa/TF complexes. Indeed, studies recently completed in this laboratory20 have shown that purified TF can defibrinate rabbits when infused IV in amounts calculated to yield a plasma TF concentration far below the assumed plasma EPI concentration of 100 ng/mL.3 The investigators believe that the delay of at least several minutes before EPI/factor Xa complexes begin to inhibit factor VIIa/TF complexes in plasma systems in vitro best explains why plasma EPI cannot prevent continuing TF-induced DIC either in humans or in experi-
mental animals. Whereas such a period of delay could be advantageous in regulation of blood coagulation at a site of tissue injury, it appears to render plasma EPI useless in preventing pathologic TF-induced blood coagulation in DIC.

Both a hepatoma cell line\(^5,9\) and cultured human umbilical vein endothelial cells\(^9,22\) have been shown to secrete EPI. Therefore, both hepatocytes and vascular endothelial cells could be a major source of plasma EPI. Two groups of researchers have reported that plasma levels of EPI are not reduced in patients with chronic liver disease,\(^6,11,27\) which implies a significant contribution of EPI from vascular endothelium.

In the present study, plasma EPI activity was measured in patients with very severe, often fatal, acute or chronic hepatocellular disease. Levels of plasma EPI activity varied over a wide range and exhibited a bimodal distribution (Fig 1). Whether a particular patient had a high or low EPI level could not be predicted from the etiology of the liver disease or from the degree of prolongation of the prothrombin time. However, all patients with chronic liver disease who survived their hospitalization had a plasma EPI activity >65%. It was of interest that the highest (194%) and the lowest (<20%) values shown in Fig 1 were from two patients with acute, fatal hepatitis. From these studies and the results of other investigators, we believe that the usual hospitalized patient with decompensated chronic liver disease will have a normal or high plasma EPI level whereas patients with fatal hepatic dysfunction of either acute or chronic cause may have a low, normal, or high level. Overall, the data imply that vascular endothelium (and possibly other cells) can provide significant amounts of EPI activity.

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