Protein C Deficiency in Liver Disease*

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ABSTRACT

Protein C is a vitamin K-dependent zymogen of a serine protease that is found in blood plasma. The active form, activated protein C, can inhibit blood coagulation and stimulate fibrinolysis. Protein C is synthesized in the liver as a single chain protein. Its synthesis requires several post-translational modifications including carboxylation of glutamic acid residues, hydroxylation of aspartic acid residues, and glycosylation. Plasma protein C levels are sensitive to liver function. Protein C levels fall more rapidly than other vitamin K-dependent proteins when synthesis is altered by the administration of oral anticoagulants. In addition, low protein C levels are highly indicative of abnormal liver function. In one case, homozygous protein C deficiency has been corrected by liver transplantation. In liver transplantation for end-stage liver failure, plasma protein C levels may be a good indicator of the success of the transplantation.

Introduction

Protein C is a vitamin K-dependent protein found in blood plasma. Protein C can be converted to a serine protease that is able to inhibit blood coagulation and stimulate fibrinolysis. Protein C was originally discovered as a vitamin K-dependent protein that had no known function. Work by several groups led to the discovery of its effects on coagulation and fibrinolysis, raising the possibility that protein C may be an essential regulator of blood coagulation along with antithrombin III, a plasma protease inhibitor that can be potentiated by heparin. A substantial amount of work has been carried out on the molecular basis of the anticoagulant and profibrinolytic activities of activated protein C, as well as the clinical consequences of inherited and acquired defects in the protein. The mechanism of the inhibition of blood coagulation by activated protein C has been shown to be through the proteolytic inactivation of factors Va and VII. The activity of both of these proteins is lost through the cleavage of their heavy chains.

A number of studies have also indicated that activated protein C can potentiate the fibrinolytic activity of tissue plasminogen activator. Some have suggested that this is through a mechanism by which plasminogen activator
inhibitor (PAI) is inactivated by activated protein C;7,9 but, it is still unclear as to the actual mechanism by which fibrinolysis is enhanced.21 Recently, a third effect of activated protein C, inhibition of endotoxin induced septic shock in baboons, has been observed. High doses of activated protein C have been observed to block the effects of lethal doses of endotoxin.46 The mechanism of this effect is unclear. These observations and a growing list of clinical studies, indicate that protein C is an important regulator of hemostasis and also plays a role in a number of immunologic phenomena.

Like other vitamin K-dependent proteins, protein C is synthesized only in the liver.24 Hepatocytes have been observed to synthesize protein C,18 secreting it as a single-chain protein. This suggests that there is a protease that can cleave an asp-lys bond and convert the single chain into the two-chain protein.18 In plasma, most of the circulating protein C is the two-chain variety, although some single chain has also been observed.18 The gene for protein C has been cloned and sequenced by two groups.24,35 It was cloned from a human liver mRNA library and identified either by the synthesis of protein C24 or by use of synthetic oligonucleotides as probes.35 Expression systems for protein C, as well as other vitamin K-dependent proteins, require mammalian cells which, unlike prokaryotic cells, contain the enzymes needed for the post-translational modifications required for the formation of an active enzyme.

There are at least three post-translational modifications made to protein C. First is the carboxylation of 10 glutamic acid residues near the amino terminus by a vitamin K-dependent liver carboxylase.14 The resulting residues, gamma-carboxyglutamic acids, are essential for the calcium-dependent, lipid binding properties of protein C, responsible for its anticoagulant activity.14 In addition, protein C contains a modified aspartic acid, beta-hydroxy aspartic acid, and is also glycosylated. The beta-hydroxyaspartic acid appears to be important in the interaction between protein S and protein C.39 The liver enzyme involved in the hydroxylation has not been described. No definite function has been described for the glycosylated regions of activated protein C, but they may alter its rate of clearance in the hepatic system.

The primary structure of protein C resembles that of other vitamin K-dependent proteins.22 The amino-terminal region contains the gamma-carboxyglutamic acid residues. This is generally considered to be the membrane binding domain of the protein. The second domain in the sequence is the epidermal growth factor domain. This region has a two-fold repeat characterized by a large number of disulfide bonds and probably involved in the interaction with protein S.39 The third domain is the protease domain. This region contains significant sequence homology with trypsin and includes the active site serine that is involved in proteolysis.23 Protein C is converted to its active form, activated protein C, by the removal of a dodecapeptide from the amino-terminus of the heavy chain.34 The cleavage of this peptide is catalyzed by thrombin.34 Thrombin, alone, is a poor activator of protein C and is inhibited by physiological levels of calcium ion.15 However, when thrombin is bound to the surface of endothelial cells, it becomes a potent activator of protein C that requires the presence of calcium ions.15 This effect is due to the presence, on the surface of endothelial cells, of thrombomodulin, an intrinsic membrane protein,12 which has a high affinity for thrombin. When thrombin is in complex with thrombomodulin, its substrate specificity changes such that protein C
becomes a better substrate than fibrinogen, platelets, or factor V. Hence, the partitioning of thrombin onto the endothelial surface provides a mechanism by which the clotting process can be limited. By this mechanism, thrombin, the final protease formed in the coagulation cascade, appears to be able to limit its own production.

The anticoagulant activity of activated protein C is dependent upon the presence of a membrane surface where it can form a complex with a second vitamin K-dependent protein, protein S. Protein S is a cofactor for activated protein C. It is required for the maximal expression of the anticoagulant activity of activated protein C on the surface of platelets, endothelial cells, or membrane vesicles. Protein S is also synthesized in the liver, but it is also found in endothelial cells and megakaryocytes. In plasma, it is found free and in complex with C4b-binding protein. Only free protein S is active as a cofactor for activated protein C. The anticoagulant activity of activated protein C can be altered by acute phase reactants, of which C4b-binding protein is one, through changes in plasma levels of free protein S.

Detection of Protein C in Plasma

Over the past few years, there has been considerable evolution in the methods used for the detection of protein C in plasma. The earliest studies, including some of the largest to date, have measured antigenic levels of protein C by radioimmunological assays or enzyme linked immunosorbent assay (ELISA) methodology. For example, in the study of Militech, who determined protein C levels in 4,723 individuals, a double antibody technique was used in which the first antibody captured protein C and the second antibody provided the signal. The majority of recent studies have relied on measurements of protein C using functional assays, all of which require two steps. The first step is the conversion of protein C to its active form, activated protein C. Three methods which have been widely used are (1) activation by thrombin, (2) activation by the thrombin-thrombomodulin complex, and (3) activation by a protein C activator from the venom of the Southern Copperhead snake (Agkistrodon contortrix contortrix).

One problem that arises in the use of either thrombin or the thrombin-thrombomodulin complex is that thrombin will react with other plasma proteins. This means that it is necessary to separate the protein C from some of these components such as fibrinogen prior to activation and to inactivate the thrombin after activation so it will not interfere with the detection of activated protein C. Two methods have been used for the separation of protein C from other plasma components. The first is barium adsorption of plasma with barium citrate. Protein C absorbs to barium citrate which can be washed free of fibrinogen and other plasma proteins, and protein C then eluted with ethylenediamine tetraacetic acid (EDTA). The second method is immunoabsorption using calcium dependent monoclonal antibodies. Protein C is absorbed in the absence of calcium and then eluted from the antibody by the addition of calcium. In the second step, following activation, thrombin is neutralized with antithrombin III, the plasma protease inhibitor which will block thrombin activity but not the activity of activated protein C. Detection of activated protein C is accomplished by using an amidolytic assay, in which one of several synthetic substrates can be converted to colored compounds by activated protein C. The second method is to measure the anticoagulant activity. In this assay, the inhibitory effect of activated protein C on prothrombin time.
(PT) or activated partial thromboplastin time (APTT) is determined. More recently it has been observed that the venom of the Southern Copperhead snake is able to activate protein C. This enzyme appears to be specific for protein C and can be used to activate protein C in plasma. Subsequently, activated protein C can be detected in plasma by using one of the previously mentioned detection methods. This simplification has resulted in a more widespread use of the functional assay for protein C.

NORMAL VALUES

Protein C levels in normal individuals have been measured in a large number of studies. The distribution of concentrations in normals has been found to be log normal with a mean of 4.0 μg per ml. A slight age dependence has been observed. Individuals under 30 years of age have a mean concentration of 3.76 μg per ml while those over 60 have a mean concentration of 4.45 μg per ml. Two standard deviations of the mean encompasses 70 to 140 percent. Heterozygote deficiency is estimated at a frequency of between one in 200 to one in 300, which is in line with the observed rate of homozygosity (approximately one in 160,000 to 360,000). However, upon testing, one in 60 will have levels below 65 percent. Only levels below 55 percent are actually predictive of heterozygosity. The risk factors for individuals with levels between 55 percent and 65 percent are unclear.

PROTEIN C AND LIVER DYSFUNCTION

Patients with poor liver function have significantly depressed plasma levels of protein C. A number of studies have examined protein C levels in patients with cirrhotic livers, acute viral hepatitis, alcoholic hepatitis, or with disseminated tumors with liver metastases, with depressed protein C levels. Protein C levels have also been observed to be low in patients with disseminated intravascular coagulation (DIC). Though several investigators have ascribed these low levels to be due to consumption of protein C, it is more likely indicative of underlying liver disease. In addition, low protein C levels associated with acute leukemia, are due to liver dysfunction rather than DIC. In one study, patients with DIC had no lower levels of protein C than those without DIC. Because low protein C levels are highly correlated with impaired liver function, it is recommended that the observation of low protein C levels should be followed up with liver function studies.

PROTEIN C AND ANTICOAGULANT THERAPY

Since protein C is a vitamin K-dependent protein, its synthesis is sensitive to the oral anticoagulant, coumarin. Coumarin acts to prevent synthesis in the liver of protein C as well as other vitamin K-dependent proteins by the inhibition of the liver vitamin K-dependent, glutamic acid carboxylase. Protein C levels tend to fall more rapidly than do the levels of other procoagulant factors leaving a short period where the patient may be hypercoagulable. The half-life of protein C has been found to be approximately 15 hours, while the half-life of factor X is 24 hours and prothrombin is 42 hours. In some patients with protein C deficiency administration of oral anticoagulants is associated with skin necrosis.

PROTEIN C AND LIVER TRANSPLANTS

Since plasma protein C levels are very sensitive to liver function, it was of interest to follow protein C levels in individuals undergoing liver transplantation. In children, a persistent deficiency of pro-
tein C was observed with enhanced risk of portal vessel thrombosis between four and ten days following transplantation. Plasma concentrations of protein C did not reach normal levels until two weeks following transplantation. Adult liver recipients did not have protein C levels fall out of the normal range and did not appear to have as great a risk for thrombosis as did children. One liver transplant has been performed on a 20-month-old child with homozygous protein C deficiency. This was the first known transplant for the purpose of correcting a specific defect rather than preventing death owing to end-stage liver disease. In this individual, protein C levels were raised to normal levels.

Protein C appears to be an important regulator of blood coagulation which can act either by the inhibition of clot formation or through the enhancement of clot dissolution. Individuals with liver abnormalities tend to exhibit low levels of protein C and have a risk for coagulopathies which emphasize the importance of liver function in the maintenance of hemostasis. In addition, the observation of acquired protein C deficiency is a strong indicator of failing liver function.

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