Fibrinogen and Dysfibrinogenemia

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ABSTRACT

The fibrinogen molecule is becoming increasingly understood. Amino acid sequencing has been undertaken and studies of abnormal fibrinogens are leading to a more functional concept of its structure. Acquired dysfibrinogenemia appears to be a more common problem than previously thought, and may be found in patients with liver disease, cancer, fibrinolysis, and disseminated intravascular coagulation (DIC).

While previous evidence suggested that anti-thrombin activity and fibrin polymerization inhibitors were formed in these conditions, recent studies suggest that slow fibrin formation occurs as a result of structural changes induced in the fibrinogen molecule itself. These relatively minor alterations in structure cause a functional dysfibrinogenemia simulating abnormalities seen in some congenital fibrinogenopathies. A case is presented illustrating such a dysfibrinogenemia in a patient with cirrhosis of the liver and evidence for DIC.

Introduction

The fibrinogen molecule, although large and complex in structure, has been remarkably well described in the last few years. Amino acid sequencing studies by Doolittle et al. have demonstrated in great detail the amino acid structure of the three chains. Locating the disulfide bridges in the chains has led to a better understanding of the folding and reflecting structure within the molecule. Studies of normal and abnormal fibrinogens have produced a better understanding of the striking transformation of the globular fibrinogen into the fibrin monomer, and its subsequent linkage with other monomers to produce the fibrin clot. There is evidence for a carbohydrate moiety, probably sialic acid, but its role is not yet fully clarified.

Congenital Fibrinogenopathies

In recent reviews of inherited fibrinogenopathies, it was pointed out that the single amino acid substitution in fibrinogen Detroit, a replacement of an arginine with a serine at position 19 in the
Amino terminal end of the α chain is associated with a markedly reduced release of fibrinopeptide B from the β chain and slow fibrin formation.

A new fibrinogen (fibrinogen Lille) was discovered in 1978 in France. This fibrinogenopathy was detected in a six year old girl without a history of bleeding. Plasma fibrinogen was unmeasurable by thrombin kinetic measurement, but immunochemical and gravimetric measurement showed normal amounts to be present. Extensive functional work up revealed abnormal fibrin monomer aggregation with delayed onset and decreased rate and extent of aggregation. Fibrin crosslinking and electrophoretic studies were normal as were studies of other coagulation factors in this patient.

Plasmin digestion of fibrinogen Lille revealed an abnormal E fragment, which was found to contain fibrinopeptide A. Thus, it appears that the structural defect of fibrinogen Lille also occurs in the amino terminal region of the molecule, interferes with fibrinopeptide A release by thrombin, and results in slow aggregation of the fibrin monomers. Amino terminal analysis revealed both fibrinogen Lille and normal fibrinogen had amino terminal tyrosine, alanine, and aspartic acid. While the precise amino acid substitution has not yet been defined, it appears to be in the α chain near the junction of the fibrinopeptide A.

Further work on fibrinogen Paris I by Budzinski and Marder has shown that the fibrin formed contains an abnormal γ chain with 20 residues remaining that interfere with γ dimer formation. Without prior γ dimer formation, α chain polymerization of these molecules does not occur. The amino terminal residues of fibrinogen Paris I have been found to be normal. Acquired Dysfibrinogenemias

Renewed interest has been shown in the acquired dysfibrinogenemias associated with liver disease and disseminated intravascular coagulation.

It has been suggested that the abnormal fibrinogen levels found in these diseases, when measured by kinetic techniques, are due to anti-thrombin activity. Alternatively, interference with fibrin monomer polymerization by incomplete fragments of the fibrinogen molecule has been invoked. Recent evidence suggests that more subtle changes in a largely intact fibrinogen molecule may actually be responsible for the slow polymer formation.

Case History

A 60-year old white male was admitted to a hospital affiliated with the University of Connecticut with anorexia, jaundice, dark urine, light stools, abdominal swelling, and ankle edema. Glutamic oxalate transaminase (SGOT) was 87 IU per 1, bilirubin was 42 mg/dl (0.1 to 1.2 mg per dl) with direct fraction of 15 mg per dl. Alkaline phosphatase was 75 IU per 1 (24 to 104 IU per 1). Hepatitis B surface antigen was not present. Ultrasound studies revealed dilated hepatic and cystic ducts to their junction. Surgery revealed benign cholecystochoeycty slightly blocking the ducts and biopsy of the liver showed marked changes of Laennec's cirrhosis. The patient experienced a complicated post operative course six weeks to his demise with hepatic encephalopathy, further ascites, and a coagulopathy consisting of prolonged prothrombin time (PT), prolonged partial thromboplastin time (PTT) and thrombocytopenia with acanthocytes and schistocytes in the peripheral blood smear. At the time his fibrinogen was measured, PT was 27 sec (control 11 sec), PTT was > 150 sec (control 36 sec), platelets were 50,000 per cmm and fibrin degradation products were > 10 but < 40 μg per dl (control < 10 μg per dl) by latex agglutination. Fibrinogen by functional assay was 95 mg per dl but by clot weight method was 145 mg/dl (150 to 400 mg per dl).

Discussion

This patient had ample evidence of liver disease with some element of disseminated intravascular coagulation and a slowly clotting fibrinogen by functional assay with thrombin. The functional assay is carried out on diluted plasma with an
excess of thrombin and is unlikely to be interfered with by low levels of fibrin degradation products. The reduced value (95 mg per dl) seems likely to be due to slow polymerization. When the clot weight method is employed, time of clotting is not critical since the plasma is incubated with thrombin for 30 minutes and the clot is then washed and weighed. Inhibitors of clotting would be expected to interfere with the clot weight assay since the plasma is not diluted, and a poor clot would be formed. On the other hand, a slowly polymerizing fibrinogen could clot completely because of the longer incubation period.

Lipinski et al. have reported a study of 22 patients with advanced liver disease who had a predominance of lower molecular weight (LMW) fraction of fibrinogen on 3.5 percent SDS polyacrylamide gel electrophoresis. These fractions, though completely clottable, gave substantially longer thrombin times. These authors and others demonstrated that normal fibrinogen was made up of higher molecular weight (HMW) fibrinogen and a smaller fraction of LMW fibrinogen which has a partially degraded A α chain and is believed to be a derivative of HMW (“native”) fibrinogen.

In the patients with cirrhosis studied by Lipinski, most, and in two cases all, of their fibrinogen migrated in the region of LMW bands occasionally seen in normal plasma. Whether or not these altered fibrinogen molecules result from a production defect in the damaged liver cell as postulated by von Felton, Verhaeghe, and by Morse or by partial degradation as postulated by Lipinski et al., Mosesson et al. and Sherman et al. remains to be established.

Lane et al. have confirmed the occurrence of acquired dysfibrinogenemia in liver disease and suggest that it is more common than the congenital form. They demonstrated that purified fibrin monomers from the plasmas of patients with both acute and chronic liver disease exhibited delayed polymerization rates. However, Lane et al. found that electrophoresis of isolated fibrins on SDS polyacrylamide gel did not show any abnormalities. Thus, in some patients with liver disease the abnormality must be a relatively minor change in terms of the overall structure and charge of the fibrinogen molecule. Sherman and Mosesson have shown that early products of fibrinogen digestion by plasmin have both electrophoretic mobility changes and slow polymerization rates. Mosesson has objected to the theoretical concept of production of abnormal fibrinogen by sick liver cells and suggests fibrinolysis could account for the observed findings. Von Felten has indicated that lack of fibrinolysis in his patient was sufficiently demonstrated.

A patient with metastatic carcinoma in the liver had the same electrophoretically abnormal fibrinogen without other lytic fragments being demonstrated during the last ten days before her demise. These findings suggest plasmin digestion was not necessarily involved, and that another mechanism, such as derepression of the genes for fetal fibrinogen, may have occurred as a result of malignancy in the liver.

Palascak and Martinez demonstrated delayed polymerization of purified fibrin monomer from plasmas of five patients with liver disease. The patients had plasma thrombin times that were prolonged at least 40 percent longer than normal. None of the patients had evidence of DIC or fibrinolysis. There were no abnormalities by electrophoresis. Palascak and Martinez also showed that desialylation of fibrinogen by treatment with vibrio cholerae neuraminidase releases 90 percent of its sialic acid without evidence of proteolysis. The desialylated fibrinogen shows more rapid thrombin and reptilase times than normal fibrinogen. Asialofibrin monomer aggregation was increased al-
though fibrinopeptide release was normal. Infusion of the desialylated fibrinogen into rabbits showed only a modest shortening of the disappearance time. Marshall et al.\textsuperscript{15} raised the possibility that desialylated glycoproteins might accumulate in the circulation in patients with compromised hepatocellular function, since he could demonstrate reduced binding of these compounds to liver cell membranes. Alternatively, Marshall pointed out accumulation of desialylated glycoproteins in the circulation could result from defective protein synthesis by the diseased liver. It seems possible that alterations in the amount or configuration of sialic acid in fibrinogen, a post translational event, could effect a delay of fibrin formation.

Thus, while there is controversy over the existence of electrophoretically separable fibrin fragments in liver disease in the absence of evidence for active fibrinolysis, and there is argument over the mechanisms involved, there is no disagreement that abnormal polymerization occurs when purified fibrin monomers from patients with liver disease are tested. Whether the abnormality is caused by a subtle alteration in peptide structure owing to derepression of fetal genes, a post translational change in carbohydrate moiety within the damaged liver cell, or early degradation after the fibrinogen leaves the cell, still remains to be established.

Summary

The functional relationship of the fibrinogen molecule to its structure, as it is now elucidated, appears more complex than had been conceived earlier. It is gradually becoming understood through amino acid sequence studies of normal and abnormal fibrinogens and the degradation products of their fibrins.

Acquired dysfibrinogenemia associated with liver disease results from slow polymerization of abnormal fibrin monomers which may result from at least three proposed mechanisms associated with damaged liver cells: (1) abnormal peptide structure as might be produced by genetic changes in liver cells affected by malignancies (derepression of genes for fetal fibrinogen), (2) post translational changes in carbohydrate composition affecting rate of fibrin monomer aggregation, or (3) fibrinolysis of varying degrees associated with liver disease and producing clottable fibrinogen fragments which may or may not have altered electrophoretic mobility.

References