Deficiency of Protein C in Congenital Thrombotic Disease

JOHN H. GRIFFIN, BRUCE EVATT, THEODORE S. ZIMMERMAN, ALICE J. KLEISS, and CAROL WIDEMAN, *Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037; Hematology Division, Centers for Disease Control, Atlanta, Georgia 30333*

ABSTRACT A family with a history of recurring thrombosis was studied to determine if a plasma protein deficiency could account for the observed disease. Protein C levels in plasma were determined immunologically using the Laurell rocket technique. The propositus, his father, and his paternal uncle, who are severely affected, had 38–49% of normal levels of protein C antigen, whereas unaffected family members had normal levels. There was no familial deficiency of antithrombin III and plasminogen. Because activated protein C is a potent in vitro anticoagulant enzyme and an in vivo profibrinolytic agent, it is suggested that the recurrent thrombotic disease in this family is due to an inherited deficiency in protein C.

INTRODUCTION

Protein C is a vitamin K-dependent serine protease zymogen (1–2). Both bovine and human activated protein C are potent anticoagulant enzymes that destroy purified bovine Factor Va, Factor VIII:C, and the Factor Xa binding sites and prothrombinase activity of washed bovine platelets (3–10). Purified human activated protein C selectively destroys Factors V and VIII:C in human plasma (6), and it has been suggested that combined Factor V/VIII deficiency disease is due to a deficiency of a plasma inhibitor of activated protein C (11). Endothelial cells contain a cofactor that potently stimulates the activation of protein C by thrombin (12). Infusion of bovine activated protein C into dogs elicits profibrinolytic activity in the blood, presumably by increasing the level of circulating plasminogen activator (13). Thus, the available literature shows that activated protein C is a potent in vitro anticoagulant and in vivo profibrinolytic agent and we hypothesized that an inherited deficiency in protein C would present as a congenital thrombophilia. Until now, inherited thrombophilia has been associated with abnormalities of only three molecules, antithrombin III (14, 15), fibrinogen (16–18), and plasminogen (19, 20). We report here a family with recurring thrombotic disease with an associated protein C deficiency.

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Address reprint requests to Dr. Griffin, La Jolla, Calif.

METHODS

Goat antisera to purified human protein C (6) and prothrombin (a gift from Dr. B. Furie) were prepared by four weekly subcutaneous injections of immunogen in complete Freund's adjuvant. Each antiserum was monospecific by double diffusion and two-dimensional electrophoresis analysis.

Enhancement of Laurell analysis (21) of protein C with ^{125}I-labeled antiprotein C antibodies and autoradiography was necessary for visualizing protein C immunoprecipitin rockets from individuals on coumarin-type anticoagulants. The chloramine T method was used to label antibodies that had been purified by affinity chromatography on protein C-Sepharose. Antiprotein C Laurell plates were prepared by adding ~3 μCi of ^{125}I antibody to 15 ml of molten agarose containing 0.33% whole antiserum. Antiprothrombin Laurell plates contained 2.5% antiserum and were visualized using Coomassie Blue G-250 stain. Each Laurell plate was calibrated with three dilutions of a normal plasma pool (15 normals) and each patient sample was analyzed at two dilutions on at least three plates. Antigen levels were calculated relative to the normal pool level that was defined as 100%. Freezing did not affect the protein C antigen measurement because the same level was determined whether the samples were fresh or frozen.

Methods for performing clotting tests (Table I) have been published (22, 23). Antigenic levels of antithrombin III and plasminogen were measured using Laurell immunoelectrophoresis (21, 23). These activities were determined with chromogenic substrate kits (Quantichrom AT-III kit, Abbott Diagnostics, Diagnostic Products, North Chicago, Ill. and Plasminogen kit, American Dade Div., American Hospital Supply Corp., Miami, Fla.). Reptilase times were determined using Reptilase-R (Abbott Diagnostics).

RESULTS

Patient's medical history. The propositus is a 22-yr-old Caucasian male with recurrent thrombophlebitis. He was well until July 1977, when he had left-sided pleuritic chest pain associated with transient pulmonary infiltrates and painful swelling in his right leg compatible with thrombophlebitis. The diagnosis of

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TABLE I

Plasma Protein Levels and Coagulation Assays for Plasmas from Family with Recurrent Thrombosis

Assay	Normal range ±2 SD	Propositus*	Father*	Paternal uncle*	Mother	Sister
Protein C antigen	71–154%	37	34	29	106	69
Prothrombin antigen	70–130%	60	72	51	81	69
Antithrombin III antigen	67–113%	77	61	92	113	85
Antithrombin III activity	84–138%	92	68	83	114	80
Plasminogen antigen	70–130%	99	91	87	90	70
Plasminogen activity	1.8–4.5 CTA U/ml	3.0	2.8	2.8	3.6	2.6
Prothrombin time	11.6–13.8 s	13.9	15.5	22.6	12.4	13.9
Activated partial thromboplastin time	36.7–56.7 s	54.3	51.6	65.8	34.2	41.9
Thrombin time	22.1–28.2 s	25.6	24.4	22.6	24.6	23.6
Reptilase time	18–22 s	15.4	18.2	15.2	14.9	14.4
Clottable protein	2.0–4.0 g/liter	2.02	4.05	2.84	2.58	2.14
Fibrinogen (Clauss)	2.0–4.0 g/liter	1.97	3.75	2.70	2.90	2.17
Factor VIII:C activity	50–150%	92	117	104	131	182
Factor V activity	75–150%	86	106	88	120	76

* It is noted that the propositus, his father, and paternal uncle were taking coumarin oral anticoagulant.

pulmonary emboli was made and the patient was anticoagulated with coumarin until June 1978. Antithrombin III levels were found to be normal. In November 1978 the patient was struck by a soccer ball on his right leg, and pain and swelling developed in that leg. Thrombophlebitis was diagnosed by Doppler examination and venograms. The patient has been treated with coumarin since that time without recurrence of disease.

The family history contains numerous episodes of thrombosis. The patient's father, now age 56, developed spontaneous bilateral thrombophlebitis with multiple pulmonary emboli following a minor leg injury at age 24. He had bilateral femoral vein ligations but continued to have recurrent thrombophlebitis in both legs. At age 43, he had a cerebral vascular accident with a mild residual hemiparesis. He had a myocardial infarction at age 45; since then he has been treated with coumarin and has remained asymptomatic. The paternal uncle, now age 60, first had acute thrombophlebitis at age 20 following an injury received while he was playing polo. He has been hospitalized on numerous occasions with recurrent pulmonary emboli. He was last hospitalized for a massive pulmonary embolus in 1977 and has been treated successfully with coumarin ever since. The paternal grandfather died abruptly at age 45. He had sustained a leg injury in a fall from a horse; while he was confined to bed, pulmonary infiltrates developed. These resolved but on the first day out of bed, he collapsed and died after taking several steps. The paternal great grandfather died unexpectedly of a cerebrovascular accident at age 61. The histories of the mother of the propositus and of her family, as well as the history of the only sibling of the propositus, a 24-yr-old sister, were unremarkable.

Laboratory findings and plasma protein analyses. Laboratory studies of plasma from the propositus in the absence of coumarin therapy gave normal results in coagulation screening tests. Platelet count and aggregation studies were normal, and the template bleeding time was normal. The plasma was referred to Scripps Clinic, La Jolla, Calif. for protein C determination and was found to contain 41% of the normal level of plasma protein C antigen where the mean level for 40 normals is 100% (two standard deviations ranging from 71 to 152%). This finding of low protein C led to further studies of the propositus and his family.

Studies of plasma samples from the propositus and his family are summarized in Table I. The notable feature common to affected family members was a low level of protein C antigen. No assay for its activity in plasma is presently available. Prothrombin antigen levels were also decreased. It is noted that in these studies the affected family members were taking variable amounts of coumarin and that this medication has successfully controlled the disease in the father and paternal uncle for the past several years. The therapeutic success of coumarin and the serious nature of the disease made us unwilling to discontinue this medication to obtain plasma samples in the absence of this drug. Although the level of antithrombin III in the plasma of the father is slightly below normal, it was normal in the plasma of the propositus and his uncle and, therefore, the familial disease is not due to a deficiency of this protein.

Because coumarin therapy is known to decrease the antigenic and functional levels of the vitamin K-

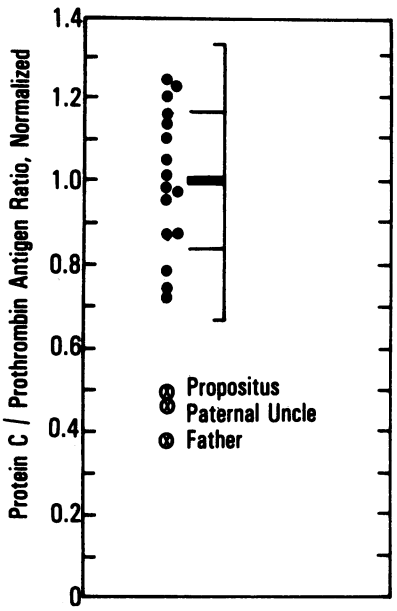

FIGURE 1 Determination of protein C antigen levels in plasmas from three affected members of a family with recurrent thrombotic disease. The protein C to prothrombin antigen ratio for 16 patients (controls) and three family members undergoing coumarin anticoagulant therapy was determined (mean 1.25±0.21 SD). The normalized values obtained by dividing by 1.25 are shown (solid circles). Brackets represent normalized mean (1.0) and one or two SD.

dependent plasma proteins, Factor IX (24), Factor X (25), and prothrombin (26), it would be expected to alter protein C levels. Hence, we sought a strategy that would allow an assessment of normal protein C levels in the presence of coumarin therapy. Protein C and prothrombin antigen levels were studied in plasma samples from 16 adult patients undergoing coumarin therapy at a time when they were not hospitalized and in stable condition. Their prothrombin time values ranged from 1.5 to 2.5 of normal. Protein C antigen levels ranged from 41 to 120% and prothrombin antigen levels from 35 to 78%. The mean protein C value was 68% and the mean prothrombin value was 54%, in agreement with a previous report (26). For each patient the ratio of protein C antigen to prothrombin antigen was calculated and found to range from 0.90 to 1.55 with a mean value of 1.25 (mean±SD = 1.25±0.21). The ratios were normalized by dividing them by 1.25 for convenience of presentation (Fig. 1). The normalized protein C/prothrombin antigen ratios for 16 patients undergoing coumarin therapy are seen in Fig. 1. The observed values range from 0.72 to 1.24 (mean 1.0±0.168 SD). Thus, there is a characteristic ratio between protein C antigen and prothrombin antigen levels, and this ratio exhibits a reasonable distribution in spite of great variability among patients in their response to anticoagulant therapy (Fig. 1). The normalized protein C/prothrombin antigen ratios were calculated for the propositus and affected family members on coumarin therapy, and the values are seen in Fig. 1. The father, the uncle, and the propositus have values that are 38, 45, and 49%, respectively, of normal values (1.0±0.168). This implies that the level of plasma protein C antigen in these patients in the absence of coumarin is significantly reduced and ranges from 38 to 49%. In the case of the propositus in the absence of coumarin therapy, the observed level of protein C antigen was 41±5% (six determinations). According to the analysis presented in Fig. 1, his protein C level in the absence of coumarin is inferred to be 49% of normal, thus supporting the validity of this approach.

DISCUSSION

The results described here demonstrate that a marked decrease in plasma protein C antigen is associated with a history of recurrent thrombosis in three affected members of a family with a history of recurrent thrombosis. No functional assay for protein C in plasma is currently available so that studies of protein C were limited to immunologic assays. The 22-yr-old propositus in the absence of coumarin therapy has 41% of normal plasma protein C levels. Because anticoagulant therapy is useful for recurrent thrombosis and because this decreases antigenic levels of vitamin K-dependent plasma proteins (21–23), we used the ratio of protein C to prothrombin antigen as an index based on which the protein C level could be estimated. Based on this index, the propositus has 49% protein C. The father and the paternal uncle have 38 and 45% of normal protein C levels, respectively. Thus, it appears that one-third to one-half of normal levels of protein C may compromise normal regulation of thrombosis and predispose to thrombotic disease.

Antithrombin III appears to be a major regulatory protein limiting the activity of procoagulant plasma enzymes and activated protein C may represent a major regulatory protein limiting the activity of activated procoagulant cofactors, Factors Va and VIIIa. In this respect, the anticoagulant properties of activated protein C and antithrombin III are complementary. Inherited abnormalities in antithrombin III, fibrinogen, or in plasminogen are associated with recurrent thrombotic disease (14–20). In the case of antithrombin III, a plasma level of 50% of normal is sufficient to predispose the patient to disease. Similarly, it appears that protein C levels between 38 and 49% in the family reported here are sufficiently low to result in thrombotic disease. At present we do not know whether the protein C molecules present in reduced levels in the propositus are functionally normal or abnormal. Assuming they are at least partially active, it is speculated

that a total deficiency in protein C or antithrombin III might not be found because this may be a severe threat to life.

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