Clinical Topics

Congenital protein C deficiency and thrombotic disease in nine French families

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Abstract

Investigation of 118 patients for protein C deficiency using an immunological and a functional assay, and subsequent investigation of those (nine) found to be deficient, identified 22 patients (14 women, eight men) with protein C deficiency, of whom six were asymptomatic, 15 had histories of venous thromboembolism, and one had a history of arterial thromboembolism. Protein C deficiency was associated in the nine probands with young age at first episode of thromboembolic disease (mean 24·1 (SD 11·9) years), absence of a precipitating condition (five (56%)), and a family history of thromboembolic disease (six (66%)). Investigation of the nine families suggested autosomal dominant transmission of the defect.

Thromboembolic episodes were seen in patients with protein C antigen concentrations below 0·6 U/ml. Mean (SD) protein C antigen concentrations were 0·48 (0·12) U/ml in 18 patients not receiving oral anticoagulant treatment and 0·28 (0·05) U/ml in four receiving such treatment. One patient with severe protein C deficiency (0·16 U/ml) developed skin necrosis soon after starting oral anticoagulant treatment.

Introduction

Recent descriptions of families with thrombotic disease and isolated protein C deficiency[1-6] have confirmed biochemical findings on the anticoagulant properties of protein C recently reviewed by Esmon.[7] Protein C, a vitamin K dependent plasma zymogen synthesised in the liver, is activated by thrombin (fig 1); thrombomodulin, a protein present on the endothelial cell surface, acts as a cofactor in this reaction. Regulation of protein C activation is also dependent on protein S, another vitamin K dependent plasma protein, and on a specific inhibitor. Activated protein C functions as an anticoagulant by inactivating factors Va and VIIIa and, possibly, by stimulating fibrinolysis.

Thrombotic disease is associated with protein C deficiency as the impaired inactivation of factors Va and VIIIa promotes excessive formation of fibrin. We investigated 118 patients with documented venous thromboembolic disease for the presence of isolated protein C deficiency, using both a functional and an immunological assay for protein C.

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Patients and methods

From September 1982 to October 1983 we screened 118 patients for protein C deficiency. Of them, 105 had experienced one or more episodes of deep venous thrombosis or pulmonary embolism, or both. Deep venous thrombosis was confirmed by ultrasonography or phlebography, or both, and pulmonary embolism by perfusion lung scanning or angiography, or both. Twelve patients had only clinical signs of recurrent superficial thrombophlebitis, and the remaining patient had arterial thrombosis and a family history of deep venous thrombosis.

Protein C antigen was assayed by electroimmunoassay, as described by Bertina *et al*,[1] or enzyme linked immunosorbent assay, as described by Soria *et al*,[8] or both. Protein C functional activity was evaluated according to the method described by Bertina *et al*: protein C was extracted from plasma by aluminium hydroxide adsorption and elution; the protein C in the eluate was activated with thrombin; and the amount of activated protein C formed was then estimated from the amidolytic activity towards the peptide substrate S-2366.[9] Protein C activity was only measured in patients not treated with oral anticoagulants. Factor II antigen and factor X antigen were assayed by electroimmunoassay as previously described.[1] Criteria for the identification of protein C deficiency in patients treated and not treated with oral anticoagulants were as previously described.[1]

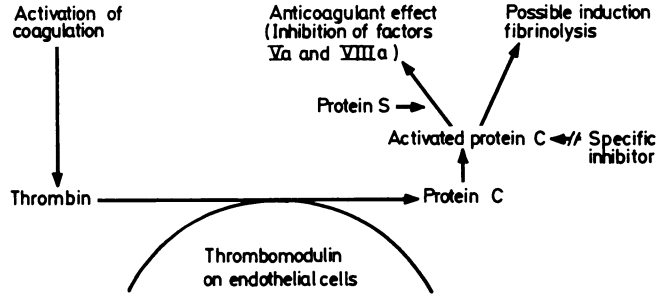

FIG 1—Role of protein C in regulating coagulation.

Other variables of coagulation, such as prothrombin time, partial thromboplastin time, thrombin time, fibrinogen activity, euglobulin clot lysis time, and diluted whole blood lysis time, were measured as described by Caen *et al*.[10] Euglobulin lysis time and diluted whole blood lysis time were measured both before and after 10 minutes' venous occlusion. Plasminogen and antithrombin III activity were assayed with specific amidolytic methods using the substrates S-2251 and S-2238 respectively.

Results

Of the 118 patients studied (117 with venous thrombosis, one with arterial thrombosis), nine (7·6%) were found to have isolated protein C deficiency. Further studies of their families permitted the identification of a further 13 patients with isolated protein C deficiency (fig 2).

Table I shows the clinical characteristics of the 22 patients with isolated protein C deficiency. Six patients were asymptomatic. Thirteen had presented with deep vein thrombosis (distal in three, proximal in 10, and complicated by pulmonary embolism in four). Two had presented with only

TABLE I—*Clinical manifestations of thrombotic disease in 22 patients with isolated protein C deficiency*

	Case No	Sex	Deep vein thrombosis	Pulmonary embolism	Recurrent superficial thrombophlebitis	Arterial thrombosis	Age at onset (years)	Precipitating condition
Family 1	1	F*	++	–	++	–	28	Delivery
	2	F	+	–	++	–	32	Caesarean section
	3	F	+	–	+	–	4	Tonsillectomy
	4	F	–	–	–	–		
Family 2	5	M*	+	–	–	–	18	Spontaneous
	6	M	–	–	–	–		
	7	M	–	–	–	–		
	8	M	–	–	–	–		
Family 3	9	M*	++	–	–	–	10	Appendicectomy
Family 4	10	F*	+	+	–	–	17	Spontaneous
Family 5	11	F*	–	–	–	+	52	Spontaneous
	12	F	–	–	+	–	37	Pregnancy
	13	F	–	–	–	–		
Family 6	14	M*	++	–	–	–	16	Trauma
	15	M	–	–	–	–		
	16	F	–	–	+	–	37	Pregnancy interruption
Family 7	17	F*	++	–	++	–	24	Spontaneous
	18	M	+	++	–	–	32	Spontaneous
Family 8	19	F*	++	+	–	–	25	Spontaneous
	20	F	+	+	+	–	33	Delivery
Family 9	21	F*	+	–	–	–	26	Delivery
	22	F	+	+	–	–	28	Delivery

*Propositus
++, more than one episode.

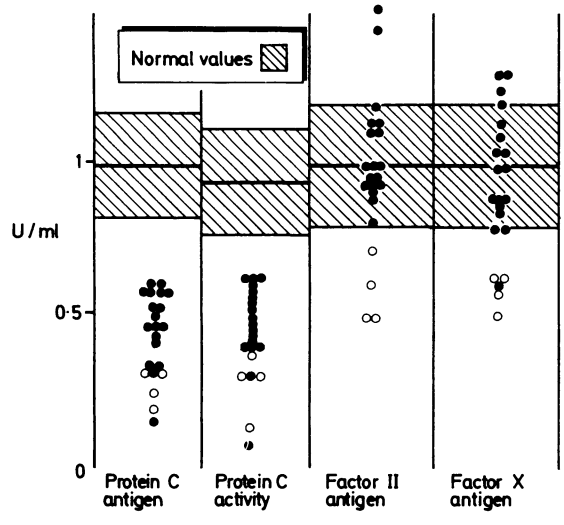

FIG 2—Laboratory findings in 22 patients with isolated protein C deficiency, four of whom (○) received anticoagulant treatment and 18 of whom (●) did not.

isolated superficial thrombophlebitis. Interruption of vena cava was performed in one case. The mean (SD) age at the onset of thromboembolic disease was 24·5 (9·7) years (range 4-37). One woman (case 11) had a history of arterial thrombosis without either deep vein thrombosis or superficial thrombophlebitis; she developed a humeral arterial thrombosis, which was treated by thrombectomy followed by venous graft after a second occlusion; cardiac investigations showed ectasia of the left coronary artery and ascending aortic dilatation. Her sister (case 12) developed recurrent superficial thrombophlebitis during pregnancy; her niece (case 13) was asymptomatic.

Table II compares the clinical characteristics of the nine probands and 13 affected members of their families with protein C deficiency with those of the other subjects investigated for venous thromboembolic diseases.

Of the 22 patients with isolated protein C deficiency, three were treated with heparin and four with oral anticoagulants. A decreased concentration of protein C antigen associated with normal levels of factor II and factor X antigen was observed in the 18 patients without anticoagulant treatment (table III). In the four patients treated with oral anticoagulants, protein C antigen concentration and the ratios of protein C antigen to factor II antigen and protein C antigen to factor X antigen were all below the lower limit of normal (fig 2) criteria developed by Bertina *et al*. Table III compares data on these 22 patients with those obtained on the patients with venous thromboembolism but without isolated protein C deficiency. All patients with protein C deficiency had normal antithrombin III and plasminogen activities; also, the fibrinolytic activities before and after 10 minutes' venous occlusion were normal (details in Conard *et al*, The fibrinolytic system in patients with congenital protein C deficiency, submitted for publication). Of the 22 patients with isolated protein C deficiency, 21 were found to be heterozygous for the defect. Only one patient (case 14) showed a low protein C antigen concentration and protein C activity, with the concentrations of the other vitamin K dependent factors within the normal range. Both his parents (cases 15 and 16)—who were cousins—had isolated protein C deficiency. This patient (case 14) might have been either a homozygote with a mild defect or a heterozygote with an extremely low protein C activity.

Protein C activity could be determined in only 33 of 64 patients who did not receive oral anticoagulant treatment, so decreased functional activity (molecular variant) could not be excluded in 31 patients with thrombotic disease who had normal protein C antigen concentrations.

Discussion

Clinical manifestations of venous thromboembolism in 15 patients out of 22 with isolated protein C deficiency confirmed the previously described association between thromboembolic disease

TABLE II—*Clinical characteristics of 118 patients investigated for venous thromboembolic disease. (Values are numbers (%) of patients except where indicated)*

	Probands with protein C deficiency (n=9)	Patients without protein C deficiency	
		Deep vein thrombosis (n=97)	Superficial thrombophlebitis (n=12)
No of men	3	39	7
Mean (SD) age, in years	29·8 (13·2)	37·8 (12·2)	44·2 (15·6)
Mean (SD) age at first episode	24·1 (11·9)	30·8 (11·2)	39·0 (14·1)
Precipitating factor: present	4 (44)	74 (76)	4 (33)
absent	5 (56)	23 (24)	8 (67)
Pulmonary embolism	1 (10)	49 (50·5)	
Interruption of vena cava	1 (10)	22 (22·7)	
Recurrences	5 (56)	57 (58·8)	8 (66)
Familial history of thromboembolic disease	6 (66)	31 (32)	3 (25)

TABLE III—*Mean (SD) protein C antigen concentration and factor II antigen and factor X antigen in patients investigated for venous thrombosis*

	Patients with protein C deficiency	Patients without protein C deficiency	
		Deep vein thrombosis	Superficial thrombophlebitis
		No oral anticoagulant treatment	
	(n=18)	(n=54)	(n=10)
Protein C antigen (U/ml)	0·48 (12)	1·25 (0·33)	0·99 (0·10)
Factor II antigen (U/ml)	1·05 (0·16)	1·24 (0·34)	0·97 (0·04)
Factor X antigen (U/ml)	1·01 (0·20)	1·13 (0·31)	0·99 (0·09)
		Oral anticoagulant treatment	
	(n=4)	(n=43)	(n=2)
Protein C antigen (U/ml)	0·28 (0·05)	0·55 (0·16)	0·47 (0·05)
Factor II antigen (U/ml)	0·58 (0·11)	0·57 (0·18)	0·59 (0·24)
Factor X antigen (U/ml)	0·56 (0·09)	0·48 (0·18)	0·49 (0·12)

and isolated protein C deficiency.[1-6] Deep vein thromboses were the most common clinical manifestations (13 cases), with recurrences in 10 patients (of deep vein thrombosis in seven, of superficial thrombophlebitis in three). Superficial thrombophlebitis was encountered less often in our series of patients (seven out of 22 (30%)) than in the patients studied by Broekmans *et al* (18 out of 30 (60%)).[11]

Arterial pathology was reported in some patients with protein C deficiency.[3 5] Griffin *et al* described a patient aged 24 at onset of deep vein thrombosis who suffered myocardial infarction and a transient ischaemic attack at 44. One patient in our series (case 11), with a family history of superficial thrombophlebitis (her sister) and protein C deficiency (sister and niece), had presented with arterial thrombosis without venous manifestations. Aortic endothelium lesions, seen in this patient, may have been the origin of thrombi: this raises the question of whether the arterial thrombosis was associated with protein C deficiency or with the endothelial lesions.

Relatively young age at the first episode of thrombosis, absence of precipitating factors, and familial history of deep vein thrombosis characterised the nine probands with protein C deficiency in a large population of patients with thromboembolic disease. Protein C deficiency was detected in eight (7·6%) of the patients with a first episode before 40 years and in five (7%) of the patients with recurrent thromboembolic episodes. Protein C deficiency seems to be more common than antithrombin III deficiency, which has been detected in 4·4% of patients with recurrent venous thrombosis.[12] Pabinger-Fasching *et al* observed a higher incidence (2-3%) of antithrombin III deficiency compared with protein C deficiency (1%). The lower average age of our patients may explain this discrepancy: Broekmans *et al* observed an incidence of 8·1% in patients younger than 40, compared with 1·2% in patients over 40.

As previously described, a protein C antigen concentration lower than 0·6 U/ml is associated with a history of deep vein thrombosis. Not all our patients with protein C activity lower than 0·6 U/ml, however, had experienced thromboembolic episodes. In addition, we identified one who might have been homozygous for a mild deficiency. Homozygotes with a severe deficiency (plasma protein C antigen activity less than 0·05 U/ml) have been reported: all were newborn infants with diffuse thromboses[13] or purpura fulminans.[14]

Broekmans *et al* proposed that protein C deficiency is transmitted as an autosomal dominant disorder.[2] A recessive autosomal transmission has, however, been suggested by Seligsohn *et al*.[13] Autosomal dominant transmission was confirmed in seven out of the nine families assessed: transmission from mother to daughter was found in five families, from father to daughter in one, from father to son in one. One patient (case 11) with severe protein C deficiency whose parents were both heterozygous might have been a heterozygote with an extremely low protein C activity or a homozygote for a mild defect.

Choice and duration of the antithrombotic treatment in protein C deficiency continue to be discussed: heparin has been observed to be effective in the acute phase of deep vein thrombosis. Introduction of oral anticoagulation may be associated with skin necrosis in patients with protein C deficiency, as recently described[15 16]: only one of our patients (case 11, with severe protein C deficiency) showed recurrent skin necrosis at the introduction of oral anticoagulation.[16] Thirteen others were treated on several occasions without any complications. If protein C deficiency is suspected the introduction of oral anticoagulant treatment must be progressive and associated with intensive heparin anticoagulation to prevent thrombosis of the microcirculation occurring with low protein C activities.

Long term treatment with an oral anticoagulant drug seems to be beneficial in preventing recurrences of venous thromboembolism in patients with protein C deficiency: of our patients who did not receive anticoagulant treatment, seven had recurrent deep vein thrombosis and five recurrent superficial thrombophlebitis. Fifteen of the 22 patients with protein C deficiency, however, did not receive oral anticoagulant treatment. Longer follow up is necessary to make a definite statement on the prevention of recurrence by long term treatment with oral anticoagulant.

Protein C deficiency seems to be one of the most commonly observed inherited abnormalities of coagulation predisposing to thrombosis (roughly 7% incidence in our series), others being quantitative or qualitative alterations in antithrombin III, plasminogen, and fibrinogen activities. Thus evaluation of protein C activity is of interest in patients with thromboembolic disease, particularly in young patients without precipitating conditions of the thromboembolic episode or with a familial history of thromboembolism, or with both.

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References

1 Bertina RM, Broekmans AW, Van Der Linden IK, Mertens K. Protein C deficiency in a Dutch family with thrombotic disease. *Thromb Haemost* 1982;**48**:1-5.
2 Broekmans AW, Veltkamp JJ, Bertina RM. Congenital protein C deficiency and venous thromboembolism. A study of three Dutch families. *N Engl J Med* 1983;**309**:340-4.
3 Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Widemann C. Deficiency of protein C in congenital thrombotic disease. *J Clin Invest* 1981;**68**:1370-3.
4 Horellou MH, Conard J, Bertina RM, Samama M. Thromboses veineuses associées à un déficit constitutionnel en protéine C: trois observations. *Presse Médicale* 1983;**12**:2259.
5 Marlar RA, Endres-Brooks J. Recurrent thromboembolic disease due to heterozygous protein C deficiency. *Thromb Haemost* 1983;**50**:331.
6 Pabinger-Fasching I, Bertina RM, Lechner K, Niessner H, Korninger CH. Protein C deficiency in two Austrian families. *Thromb Haemost* 1983;**50**:810-3.
7 Esmon CT. Protein C: biochemistry, physiology and clinical implications. *Blood* 1983;**62**:1155-8.
8 Soria J, Soria C, Samama M, Nicolas C, Kisiel W. Severe deficiency in protein C in a congenital thrombotic disease. Description of an immunoenzymological assay for protein C determination. *Thromb Haemost* (in press).
9 Bertina RM, Broekmans AW, Krommenkoek Van Es C, Van Wijingaarden A. The use of a functional and immunological assay for assay for plasma protein C in the study of heterogeneity of congenital protein C deficiency. *Thromb Haemost* 1984;**51**:1-5.
10 Caen J, Larrieu MJ, Samama M. L'hémostase. In: *Méthode d'exploration et diagnostic pratique*. Paris: Expansion Scientifique, 1975.
11 Broekmans AW, Van Der Linden IK, Veltkamp JJ, Bertina RM. Prevalence of isolated protein C deficiency in patients with venous thromboembolic disease and in the population. *Thromb Haemost* 1983;**50**:350.
12 Conard J, Veuillet-Duval A, Horellou MH, Samama M. Etude de la coagulation et de la fibrinolyse dans 131 cas de thromboses veineuses récidivantes. *Nouv Rev Fr Hematol* 1982;**4**:205-9.
13 Seligsohn U, Berger A, Abend M, *et al*. Homozygous protein C manifested by massive venous thrombosis in new-born. *N Engl J Med* 1984;**310**:559-62.
14 Branson H, Katz J, Marble R, Griffin J. Inherited protein C deficiency and coumarin-responsive chronic relapsing purpura fulminans in a new-born infant. *Lancet* 1983;**11**:1165-7.
15 Broekmans AW, Bertina RM, Loeliger EA, Hofmann V, Klingeman HG. Protein C and the development of skin necrosis during anticoagulant therapy. *Thromb Haemost* 1983;**49**:251.
16 Samama M, Horellou MH, Soria J, Conard J, Nicolas G. Successful progressive anticoagulation in a severe protein C deficiency and previous skin necrosis at the initiation of oral anticoagulant treatment. *Thromb Haemost* 1984;**51**:132-3.

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What treatment is advised for a patient with temporal arteritis and an active peptic ulcer?

Lurking behind this question is the fear that giving corticosteroids to a patient with a peptic ulcer will cause that ulcer to perforate or bleed or, at least, will prevent it healing. Though widespread, this fear is not soundly based. The evidence that corticosteroids induce peptic ulcer is conflicting and unconvincing,[1] and there is no better evidence that steroids worsen the course of the disease. I would recommend that the patient should be given prednisolone in the normal way and also that cimetidine or ranitidine are given in full dosage for four weeks, when endoscopy is advisable to discover if the ulcer has healed. If it has the dose of the H_2-blocking drug may be reduced to 400 mg cimetidine or 150 mg ranitidine at bedtime. To be on the safe side, this dose should be continued until the corticosteroid drug has been tailed off. If the ulcer has not healed at four weeks or if symptoms persist after the first few days of H_2-blocking treatment I would recommend adding a locally active drug such as sucralfate or dicitratopotassium bismuthate (De-Nol).—K W HEATON, reader in medicine, Bristol.

1 Conn HO, Blitzer BL. Non-association of adrenocorticosteroid therapy and peptic ulcer. *N Engl J Med* 1976;**294**:473-9.

Are asbestos fire blankets effective and are they hazardous when coming into contact with fires?

Asbestos fibre blankets are effective, but nowadays "fire blankets" (made from non-asbestos textiles) may be used. When asbestos fire blankets are pulled out of a container and shaken some asbestos fibres are released into the atmosphere but disperse quite soon. There is, therefore, a theoretical risk from the asbestos, but it must be infinitesimal compared with that from the fire.—W R LEE, professor of occupational health, Manchester.