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Two case report of protein C deficiency and venous thrombosis.

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Investigation of 5 patients, age 32-39 years, who were admitted to Chulalongkorn Hospital with spontaneous deep vein thrombosis for congenital anticoagulant deficiency, such as protein C using a functional assays, and antithrombin III using an immunological and a functional assays. Subsequent investigations identified two patients to be protein C deficiency.

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ในระยะเวลาตั้งแต่ปี พ.ศ. 2520 เป็นต้นมา มีผู้ป่วยที่เป็นหลอดเลือดดำอุดตันมารับการรักษาที่โรงพยาบาลจุฬาลงกรณ์ จำนวน 504 รายด้วยกัน แต่มีผู้ป่วยที่ต้องอยู่หอผู้ป่วยในของโรงพยาบาลจุฬาลงกรณ์ เพียง 120 ราย อายุระหว่าง 15-75 ปี (เฉลี่ย 48.9) ขณะนั้นยังไม่สามารถทำการทดสอบเกี่ยวกับการหาสาเหตุได้ ตั้งแต่ต้นปี 2532 ทางหน่วยโลหิตวิทยา ภาควิชาอายุรศาสตร์ เริ่มทำการทดสอบเกี่ยวกับสาเหตุการขาด *natural anticoagulant* แต่กำเนิด ได้โดยได้รับทุนวิจัยรัชฎาภิเษกสมโภช จึงเริ่มทำการศึกษาผู้ป่วยที่มาด้วยหลอดเลือดดำอุดตันและมีอายุต่ำกว่า 40 ปี ซึ่งมีเพียง 5 รายพร้อมกับอาสาสมัคร 50 ราย อายุระหว่าง 15-60 ปี พบว่า 2 จาก 5 รายนั้นเกิดจากการขาดโปรตีน ซี แต่กำเนิดเมื่อเทียบกับค่าปกติของอาสาสมัคร

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 Factors Va, VIIIa, Xa binding sites and prothrombinase activity of washed bovine platelets.^(3,9) Purified human activated protein C selectively destroys Factors V and VIII in human plasma,⁽⁵⁾ 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.⁽¹⁰⁾ Endothelial cells contain a cofactor that potentiates the activation of protein C by

thrombin,⁽¹¹⁾ and thrombomodulin as shown in Fig I. Regulation of protein C activation is also dependent on protein S, another vitamin K-dependent plasma protein. 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. About 50% of all patients have thrombotic episodes before the age of 30 years, often occurring without an apparent cause

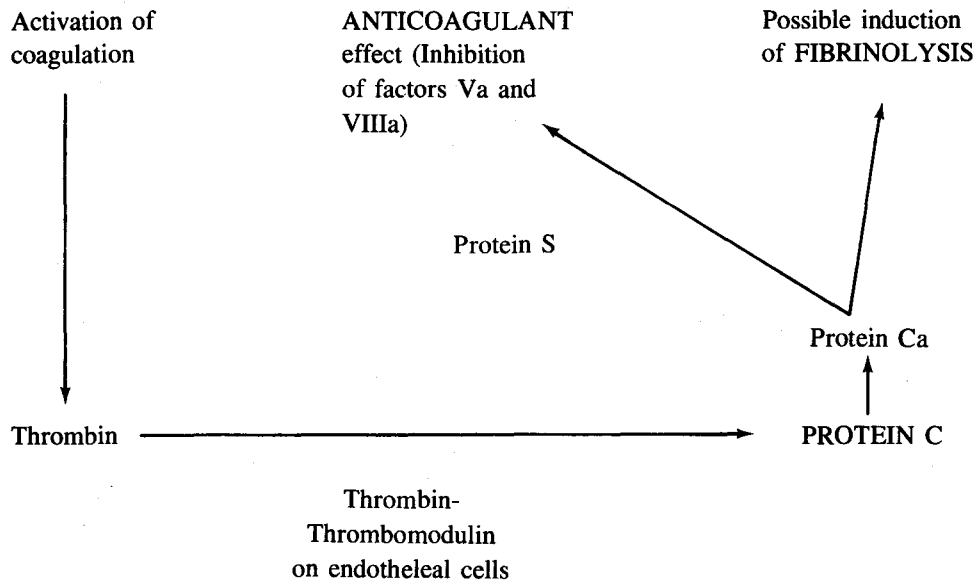


Figure 1. Role of protein C in regulating coagulation.

Recent descriptions of families with thrombotic disease and isolated protein C deficiency⁽¹²⁻¹⁷⁾ have confirmed biochemical findings on the anticoagulant properties of protein C recently reviewed by Esmon.⁽¹⁸⁾ The first family with recurrent venous thromboembolism due to protein C deficiency, transmitted as an autosomal dominant trait, was reported in 1981 by Griffin.⁽¹⁴⁾ In heterozygotes, protein C concentration is about 50% of normal. Homozygosity with very low or unmeasurable levels of protein C has been described, particularly in newborns with severe visceral vein thrombosis or purpura fulminans.⁽¹⁹⁾

Congenital protein C deficiency have been

classified into two broad categories: type I with parallel decrease in activity and protein concentration, and type II, with normal protein concentration but reduced activity due to the presence of a dysfunctional protein C.

Functional assays of protein C was chosen for the screening of protein C deficiency because cases of dysfunctional protein C (type II) with normal levels of protein C antigen but low levels of protein C activity would remain undetected if only immunoassays were employed.⁽²⁰⁻²¹⁾ The functional clotting method has the theoretical advantage of exploring indirectly the action of activated protein C on factors Va and VIIIa, the natural protein substrates of the enzyme.

Patients and method

From 1977-1989 we had 120 patient admitted with deep vein thrombosis, from the beginning of 1988 we had 5 patients, age under 40 years whom we screened for protein C and antithrombin III deficiency. The five patients with 3 males and 2 females; none of them had received the contraceptive pills or an anticoagulant, the age was 32-39 years (mean 32.4 years) and they had experienced one to more episodes of deep vein thrombosis or pulmonary or both. Deep vein thrombosis was confirmed by vascular scanning or venography or both and pulmonary embolism by perfusion lung scanning. None of them had a family history of deep vein thrombosis.

Protein C functional activity was evaluated according to the functional clotting method.^(14,20,22,23) Antithrombin III was determined by immunodiffu-

sion using Nor-Partigen immunodiffusion plate, Behring, and assay of antithrombin III with chromogenic substrates.^(24,25) Other variables of coagulation were done such as prothrombin time,⁽²⁶⁻²⁸⁾ activated partial thromboplastin time,⁽²⁹⁻³¹⁾ thrombin time,⁽³²⁾ fibrinogen⁽³³⁾ euglobulin lysis time⁽³⁴⁾, clotting factors II⁽³⁵⁾ VII,⁽³⁶⁾ X⁽³⁷⁻³⁸⁾ and plasminogen was assayed with specific amidolytic method using the substrate S-2251^(39,40)

Results

Table 1 shows the clinical characteristics of the five patients with deep vein thrombosis, age 32-39. all of whom came in with leg edema. Only the first case had recurrent spontaneous deep vein thrombosis and recurrent pulmonary embolism at least 4 times in a 7 month period, the other 4 had only deep vein thrombosis.

Table 1. Clinical characteristics of 5 patients.

No.	Sex	Age (years)	Sites of vein thrombosis	Pulmonary thrombosis	Presenting Symptoms	Precipitating Condition
1	M	36	Inferior vena cava Both common iliac Both femoral Visceral vessels	complete obstruction of right lung	Chest pain, abdominal pain and both leg edema	Spontaneous
2	M	32	Inferior vena cava Both common iliac Both femoral Visceral vessels	-	Both leg edema	Spontaneous
3	M	39	Left femoral	-	Left leg edema	Spontaneous
4	F	36	Left iliofemoral	-	Left leg edema	Post-operative myomectomy
5	F	32	Left femoral	-	Left leg edema	Post partum

Case 1 :

A 36 years old welder who denied any family history of thrombotic disease, smoked 10-20 cigarette a day for 10 years, and always in good health until January

1988 when he had the first episode of spontaneous chest pain and the diagnosis of pulmonary thrombosis of the right lung, thrombosis of left femoral vein, both common iliac veins and inferior vena cava was made by vascular

scanning using 99 mTc-MAA alpha DTPA. His hemoglobin was 10.4 gm% Wbc $9.8 \times 10^9/L$, N 87%, L 13 %, Platelet $205 \times 10^9/L$, aPTT 37.8/39.6 seconds, PT 11.6/12.3 seconds, TT 12.4/12.2 seconds fibrinogen 2.37 gm/L, factors II, V, VIII and X were within normal limits. Antithrombin III was 0.13 gm/L by immunodiffusion and was 1110% assayed with chromogenic substrates. The plasminogen was 132% assayed with specific amidolytic method using the substrate S-2251 and the protein C functional activity was only 4.6% and other detail of coagulation tests are shown in the table 2. He was

then treated with a continuous infusion heparin of 30,000-35,000 units daily for 3 weeks, and maintained on coumarin until April, 1988 when he stopped taking the medicine and developed severe continuous abdominal pain and upper thighs edema. Repeated vascular scanning showed about the same results as the previous study. He was immediately heparinized on continuous infusion with little improvement of his abdominal pain; 3 days after admission he was underwent abdominal exploratory laparotomy because of guarding, tenderness, mucous bloody stool and a diagnosis of acute peritonitis.

Table 2. Laboratory Results in 5 Cases.

Tests	Normal Range $\pm 2SD$ (n=50)	Case 1	Case 2	Case 3	Case 4	Case 5
Protein C activity %	161.4+43.50 (149.83-172.97)	4.6	16.8	143.5	132.0	165.3
Antithrombin III Immunological (gm/L)	0.24 \pm 0.06 (0.232-0.248)	0.31	0.29	0.36	0.25	0.24
Functional %	115.69 \pm 19.32 (113.01-118.37)	110	126	137	129	132
Plasminogen activity %	126.50+36.22 (122.2-130.8)	132	140	110	128	132
Activated partial thromboplastin time (sec.)	34-45	53	49	37	42	37
Prothrombin time (sec.)	11.5-13.0	12.5	11.8	12.4	11.5	12.0
Thrombin time (sec.)	10.5-13.4	12.0	12.5	11.6	12.0	11.8
Fibrinogen (gm/l)	1.5-4.0	2.5	2.2	2.8	2.7	2.7
Factor II activity %	90-120	110	125	119	125	134
Factor V activity %	90-120	132	116	126	138	126
Factor VII activity %	90-120	137	126	118	112	106
Factor X activity %	90-120	130	116	120	121	130
Hematocrit %	35-45	40	36	38	35	36
White cell count $\times 10^9/L$	5-10	6.5	7.8	5.9	7.9	5.7
Platelet count $\times 10^9/L$	150-400	210	268	310	320	268

() = 95% confidence interval)

The surgeon found marked dilatation of the subcutaneous preperitoneal vein, serosanguinous intraperitoneal fluid of 200 ml, dilated mesenteric vein and tributaries, swelling and congestion of small bowel; all secondary to inferior vena cava, and mesenteric venous thrombosis. The surgeon did not do anything further because of the patient's condition. He was given heparin continuously postoperatively for 4 weeks with good response in the sense that there was much less guarding, tenderness and reduction of the abdominal pain and leg edema. He was discharged on coumarin anticoagulation therapy. He was again hospitalized for a massive pulmonary embolus in July 1988 when he suddenly developed severe chest pain, shortness of breath, hemoptysis and cyanosis after he again stopping he coumarin for 2 weeks. His blood pressure was 70/0 mmHg. at the time of admission, respiration rate was 46 per/minute. He was immediately heparinized 40,000 units/24 hours continuous infusion with little improvement. In spite of other supportive treatment his general condition deteriorated and he expired shortly after admission.

The case described here demonstrated that case No 1 had a marked decrease in functional assay for protein C in the plasma and is associated with a history of recurrent and massive thromboses. No plasma protein C antigen is available so that study of protein C was limited to functional assay. John H Griffin⁽⁴¹⁾ reported that protein C levels between 38-49% are sufficiently low to result in thrombotic disease. It is an impression that long-term treatment with oral anticoagulant drug is beneficial for the prevention of recurrence venous thrombosis. Although this is not proven it is supported by the facts that this patient had recurrences of the disease shortly after the withdrawal of the coumarin and remained asymptomatic during long-term treatment.

Case 2 :

A 32 years old mentally retarded was first admitted at Chulalongkorn Hospital in February 1987 because of spontaneous bilateral leg edema for many months prior to admission. The diagnosis of thrombosis of the inferior vena cava, both common iliac and both femoral veins was made by venography. He was treated with continuous heparin infusion 20,000-30,000 units per day with very good response, then he was discharged on coumarin. He was lost to follow up and did not take coumarin for 5-6 months. He was admitted for the second time in August 1988 with the same symptoms and signs and venogram. His hemoglobin was 12.2 gm%, wbc $7.4 \times 10^9/L$, N 74%, L 26%, platelet $301 \times 10^9/L$, aPTT 36.4/37.6 seconds, PT 12.1/12.8 seconds, TT 14.2/13.6 seconds, fibrinogen 2.68 gm/L, factors II, V, VII and X

were within normal limits, Antithrombin III was 0.29 gm/L by immunodiffusion and was 126% assayed with chromogenic substrates, the plasminogen was 140% assayed with specific amidolytic method using the substrate S-2251 and the protein C functional activity was 16.8% and other detail of coagulation tests are shown in the table 2. He was treated with a continuous fusion of heparin 30,000 units daily for 3 weeks and anticoagulated with coumarin with good results. Because of his mental retardation and no one to take care him at home, he could not take coumarin regularly, However, he is still alive at the time of reporting and came to the hospital from time to time when ever the leg edema became worse.

Discussion

Antithrombotic treatment with vitamin K antagonist^(13,15,43) is effective for the prevention of thrombosis. However, during the initial phase of oral anticoagulant treatment, protein C deficient patients are particularly susceptible to coumarin-induced hemorrhagic skin necrosis; and long-term treatment with coumarins itself carries a risk of bleeding complication. Recently, it has been demonstrated that stanozolol, an anabolic steroid can raise plasma protein C concentrations 1.5 folds in healthy volunteers.⁽⁴²⁾ Since most patients with hereditary protein C deficiency are heterozygotes⁽⁴³⁾ having protein C concentrations around 0.5 U/ml, it was hypothesized that oral administration of stanozolol may raise protein C concentration to normal level, thus correct the phenotypic defect in these patients.

Oral administration of stanozolol to type I protein C deficient patients will raise plasma protein C approximately 1.6 folds to subnormal or normal levels. This is in accordance with previous observations in individual patients treated with stanozolol or danazol, another anabolic steroid.⁽⁴⁴⁻⁴⁶⁾ Even after one week of treatment with stanozolol (5 mg bid), the increase in plasma protein C is already significant. Prolonged treatment for a period of 3 months will keep the protein C level within the normal range, as has been demonstrated in an Italian type I protein C deficient patient.⁽⁴⁴⁾ Shortly after withdrawal of stanozolol, the plasma concentration of protein C returns to pre-treatment level. During the treatment period the ratio between protein C activity and protein C antigen does not change; indicating that the biological activity of protein C as assessed in our functional assay was not influenced by stanozolol the mechanism of action of stanozolol remains obscure. Whether stanozolol increases protein C synthesis in the liver or its release from the liver, or whether it reduces the catabolic rate of protein C, is not known. However, it has been suggested that hepatocytes contain steroid receptors capable of reacting with anabolic

steriod⁽⁴⁵⁾, in this way increasing the synthesis rate of protein C.

The effect of stanozolol is not restricted to protein C alone, for stanozolol has also a substantial effect on other plasma proteins involved in fibrin formation and degradation. In healthy individuals stanozolol changes fibrinolytic parameters in such a way that the net effect seems to favour fibrinolysis.⁽⁴⁶⁾ This effect is thought to be mediated by an increase both in tPA activity which is mainly caused by a decrease in tPA inhibitor activity and in the concentration of free plasminogen, on the other hand in healthy individuals stanozolol will increase the

plasma levels of the procoagulant factors II, IX and X while it has no effect on factor VII and VIII and decreases the fibrinogen concentration.^(42,46) A.M. Broekmans found that stanozolol can increase not only antithrombin III and protein C but also heparin cofactor II, a plasma inhibitor of thrombin.⁽⁴⁷⁾ Stanozolol has no effect on plasma proteins, the protein cofactor of activated protein C.⁽⁴⁸⁾

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