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SUCCESSFUL TREATMENT OF HOMOZYGOUS PROTEIN C DEFICIENCY BY HEPATIC TRANSPLANTATION

James F. Casella[1], **Jessica H. Lewis**[2,3], **Francis A. Bontempo**[2,3], **Basil J. Zitelli**[4], **Howard Markel**[1], and **Thomas E. Starzl**[5]

[1] Department of Pediatrics, Division of Hematology, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

[2] Central Blood Bank of Pittsburgh

[3] Department of Medicine, University of Pittsburgh

[4] Department of Pediatrics, University of Pittsburgh

[5] Department of Surgery, University of Pittsburgh

Summary

A child with homozygous protein C deficiency was treated at age 20 months by orthotopic hepatic transplantation. Postoperatively there was complete reconstitution of protein C activity and resolution of the thrombotic condition.

Introduction

Protein C is a vitamin-K-dependent anticoagulant glycoprotein which, once activated by thrombin, inhibits activated factors V and VIII and stimulates fibrinolysis.[1] Current evidence suggests that protein C deficiency is inherited as an autosomal codominant trait.[2–4] Patients heterozygous for protein C deficiency usually have about 50% of normal protein C activity. An increased incidence of serious thrombosis has been reported amongst the heterozygous relatives of several kindreds.[2,5–8] Patients with homozygous protein C deficiency typically present shortly after birth with purpura fulminans, retinal haemorrhage, and evidence of central nervous system or renal thrombosis.[9–17] Protein C activity levels have been undetectable in all reported cases, and the disease is invariably fatal if left untreated.

Although fresh frozen plasma (FFP), coumarin anticoagulants, and factor IX concentrates have been used successfully in the treatment of homozygous protein C deficiency, all existing treatments have substantial drawbacks. The biological half-life of injected protein C (<8 h) necessitates unacceptably frequent administration of plasma products, and poor access to veins is often another limiting factor. The thrombotic tendency can be controlled with coumarin anticoagulants, but only in doses that severely restrict normal childhood activities and carry a risk of fatal haemorrhage.

Several lines of evidence suggest that the liver is the major site of protein C synthesis. First, other vitamin-K-dependent factors (II, VII, IX, and X) are synthesised in the liver; second, protein C levels are reduced in liver disease;[18] third, synthesis of soluble protein C by a human hepatoma line has been demonstrated in vitro;[19] and, fourth, cDNA inserts in a gt11 library prepared from human liver mRNA have been shown to code for protein C.[20] These

Correspondence should be addressed to J. F. C., Room 201, Brady Building, Johns Hopkins Hospital, Baltimore, MD 21205, USA.

observations led us to attempt orthotopic hepatic transplantation as primary therapy for a patient with protein C deficiency.

Case-report and Methods

The patient, born at full term, was the child of first cousins. Details of his clinical course and of protein C levels in the pedigree have been presented elsewhere.[17] Retinal and vitreous haemorrhages (ultimately resulting in complete blindness[7]) were noted shortly after birth. Purpuric skin lesions were first observed at 24 h of age. Protein C antigen was present at 17% of normal levels, but protein C activity was undetectable. Therapy with FFP was instituted at 48 h of age, and was continued until $8\frac{1}{2}$ months of age. Two attempts at withdrawal of FFP were associated with renal failure, microangiopathic haemolytic anaemia, and fibrinolysis. At age $8\frac{1}{2}$ months, warfarin ('Coumadin') therapy was successfully instituted during phased withdrawal of FFP. At age 15 months an intracranial haematoma developed after a fall from bed. Warfarin was discontinued and treatment with FFP was reinstituted for six weeks. Warfarin was then resumed, and the haematoma resolved without neurological sequelae.

At age 20 months, the patient was transferred to the Children's Hospital of Pittsburgh for orthotopic hepatic transplantation. Before surgery, he had been receiving warfarin 0·5 mg/kg per day—3·0 mg in the morning and 2·5 mg at night. The last dose (2·5 mg) was administered 6 h before induction of anesthesia. At the time of induction the prothrombin and partial thromboplastin times were 25·6 s (control 11·8) and 57·2 s (control 25), respectively. 5 mg vitamin K_1 was given intramuscularly, followed by an intravenous infusion of 10 ml/kg FFP. The protein C antigen and activity levels immediately after the infusion were 29% and 24%, respectively. Maximum reduction of the prothrombin time was achieved $4\frac{1}{2}$ h after induction, at which time the prothrombin time was 14·8 s with a partial thromboplastin time of 31·6 s. Revascularisation of the graft was complicated by hepatic artery and portal vein thrombosis. Heparin, 3 mg/kg as a single intravenous bolus, was administered immediately before thrombectomy of both vessels; however, thrombectomy of the portal vein failed, necessitating placement of a vein graft from the confluence of the splenic and superior mesenteric arteries to the hilum of the liver. A Roux-en-Y choledochojejunostomy was performed.

Postoperatively, the patient was maintained on aspirin 40 mg/kg once daily, dipyridamole 1 mg/kg orally twice daily, tapering doses of prednisone, and cyclosporin adjusted to maintain serum levels of approximately 500 ng/ml as measured by radioimmunoassay. Protein C levels improved steadily after reaching a nadir 24 h postoperatively (figure). In the second week after transplantation an episode of presumed rejection characterised by lymphocytic infiltration of the hepatic parenchyma and portal areas and mild increases in serum transaminases was treated with OKT3 monoclonal antibody. The liver enzymes fell, but four weeks after transplantation the patient became feverish and irritable, with atypical lymphocytosis and recurrent modest rises in transaminases. A cerebrospinal fluid mononuclear cell pleocytosis reached a maximum of 247/μl six weeks after transplantation; this remained unexplained and the patient improved spontaneously. He has been treated subsequently for two episodes of pneumococcal septicaemia without meningitis, the second within three weeks of administration of pneumococcal vaccine ('Pnu-immune'). He remains symptom-free with regard to his initial coagulation disorder. Protein C levels 7 months after hepatic transplantation are normal.

Laboratory Methods

Coagulation factors were assayed by published methods.[21–23] Protein C functional assay was done by the method of Bertina[4] and of Comp et al[24] (reagents from American Diagnostica Inc, Greenwich, CT). A venom derivative, 'Protac C', converts human protein C to the active protease (aPC) which is then measured on a chromogenic substrate, 'Spectrozyme-aPC'. Protein C immunological assay was done by the rocket technique in prepared plates from Helena Laboratories (Beaumont, TX). For both kinetic and immunological assays, the normal ranges were 65–145% of laboratory normal standard. Protein S immunological assay was likewise done with the rocket technique, antibody being purchased from American Diagnostica. The normal range was 50–150% of laboratory normal standard. The C4 bound protein was precipitated with polyethylene glycol at 3·75%. Crossed immunoelectrophoresis[25] was used if the result with rocket assay was low.

Results

The accompanying figure shows post-transplantation levels of factor X and functional and antigenic protein C, and the table gives results of additional coagulation assays. The time-course and pattern of protein C recovery did not differ noticeably from that of other vitamin-K-dependent procoagulant factors. There were no thrombotic complications in the postoperative period.

Discussion

The successful reconstitution of protein C activity in this patient establishes hepatic transplantation as a useful treatment for an otherwise catastrophic illness. The results also unequivocally confirm that the liver is a major site of protein C synthesis. Preoperatively, there was cause for concern not only about the ability of the transplanted liver to synthesise protein C but also about the pattern of reconstitution. If protein C had been synthesised more slowly than the procoagulant proteins, for example, then an imbalance between procoagulant and anticoagulant activities might have led to postoperative thromboses. A similar imbalance may account for the episodes of skin necrosis that occur in heterozygotes for protein C deficiency who arc receiving warfarin.[26,27] The explanation, presumably, is that protein C concentrations fall quickly after initiation of warfarin, because of the short half-life of the protein in vivo, whereas most of the procoagulant proteins have much longer half-lives. Fortunately, it seems that protein C is elaborated on a time-course similar to that of other vitamin-K-dependent factors.

The occurrence of intraoperative thrombosis in this patient suggests that perioperative management of his protein C abnormality could have been improved upon, although thrombosis of the hepatic arteries and portal veins has also been seen in liver-transplant patients without protein C deficiency. Perhaps continued anticoagulation during the operative procedure will turn out to be the best course despite the added risks. Alternatively, higher doses of FFP before and during the operation might provide enough protein C to prevent thromboses.

Severe homozygous protein C deficiency joins the growing list of metabolic disorders including alpha-1-antitrypsin deficiency,[28] Wilson's disease,[29] hereditary tyrosinaemia,[30] type 1 and 4 glycogen storage[31,32] disease, Crigler-Najjar syndrome,[33] porphyria,[34] familial hypercholesterolaemia,[35] factor VIII[36,37] deficiency, and primary hyperoxaluria[38] that are correctable by hepatic transplantation. Yet, protein C deficiency is distinguished from all but the last three in that hepatic failure is not a primary feature of the disease. In fact, to our knowledge, protein C is the first coagulation defect in which liver transplantation has been done to correct the specific disorder and not end-stage liver disease related to previous blood

product therapy. Removal of the need for continuous warfarin therapy or frequent infusions of plasma products has resulted in a sustained improvement in the quality of life for this child, despite the need for continuing immunosuppression.

Further observation of this and other patients will be necessary before liver transplantation can be declared the treatment of choice for protein C deficiency. Future difficulties with this approach may be encountered. For example, protein C, like many other proteins, may exhibit polymorphisms.[4,39,40] Therefore, protein C synthesised by donor livers may differ from that formerly synthesised by the recipient's liver. As a consequence, the patient's immune system might respond by forming antibodies against the foreign protein C. In the future, other treatment methods will need to be pursued—in view of the inherent risks of liver transplantation and the fact that early morbidity such as blindness cannot conceivably be prevented by transplantation. Ultimately, a genetic approach is likely to be most successful, either through carrier detection in high-risk populations or through gene therapy.

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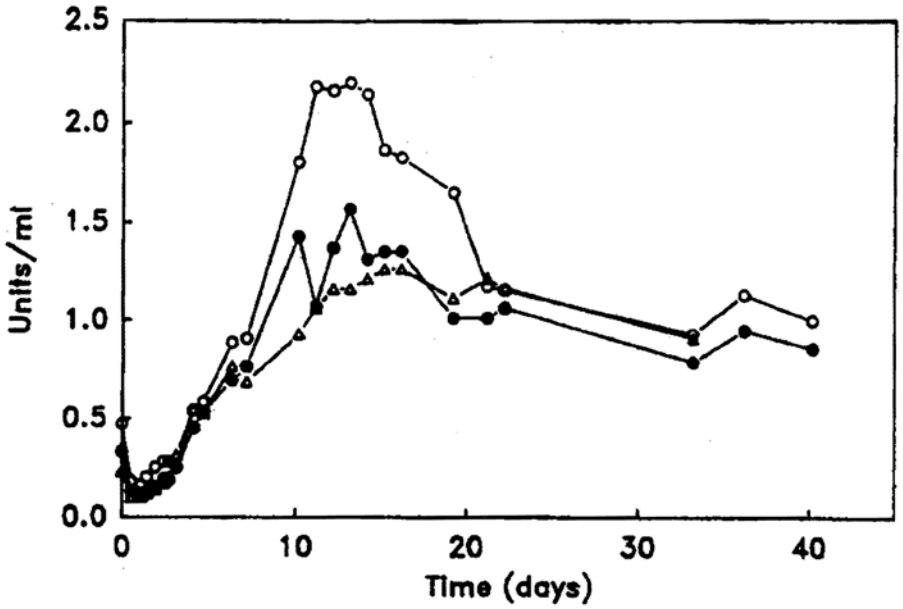

Figure. Preoperative and postoperative protein C levels in relation to factor X

Levels of protein C activity (○) and antigen (●) are shown in relation to factor X levels (△) before (time 0) and after hepatic transplantation.

Table

LEVELS OF ADDITIONAL COAGULATION FACTORS PREOPERATIVELY AND POSTOPERATIVELY★

	Factor								
Time	**II**	**V**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**Protein S**
Day I (preop)	0·29	0·76	0·33	2·70	0·39	0·23	0·69	0·96	
Day 1 (postop)	0·09	0·13	0·07	0·65	0·02	0·10	0·03	0·36	
Day 2	0·13	0·27	0·08	1·20	0·31	0·12	0·07	0·15	<0·12
Day 3	0·31	0·36	0·29	1·30	0·61	0·20	0·18	0·13	0·64
Day 4	0·37	0·46	0·32	1·60	0·78	0·31	0·27	0·13	
Day 5	0·56	0·74	0·42	2·25	1·10	0·54	0·42	0·32	1·37
Day 6	0·54	1·00	0·58	2·55	1·10	0·64	0·55	0·47	1·66
Day 8	0·54	0·80	0·64	2·75	1·05	0·68	0·72	0·69	1·37
Day 10	1·60	1·50	0·60	3·70	1·50	0·90	0·96	1·05	

★All assays are functional, with normal values of 50–150%, expressed as units/ml.