

Fibronectin in blood products—an in vitro and in vivo study

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SUMMARY The concentration of fibronectin was assessed in a variety of coagulation factor preparations. Highest concentrations of fibronectin were found in the intermediate purity factor VIII concentrates. Significant amounts were found in cryoprecipitate but high purity factor VIII concentrates contained only small amounts. For practical purposes factor IX concentrates contained no fibronectin. Qualitative estimation of fibronectin showed the presence of an abnormal slow migrating peak on 2 DIEP which was not found in normal plasma. In vivo recovery infused fibronectin was relatively low for all products studied (30–54%). The plasma half life (17–25 h) did not differ significantly depending on whether cryoprecipitate or a factor VIII concentrate was used as a source of fibronectin. No enhancement of plasma fibronectin concentrations was obtained following DDAVP infusion, venous occlusion and exercise. Plasma fibronectin concentrations and 2 DIEP patterns were unaltered following prolonged storage.

Fibronectin is a dimeric $\alpha 2$ -glycoprotein with a molecular weight of 440 000 daltons and a normal plasma concentration of 300 $\mu\text{g/ml}$ in man.¹ Although its functions are not completely understood, it has been shown to possess opsonic activity mediating the phagocytosis of particulate debris by the reticuloendothelial system.² Decreased plasma concentrations have been described in patients after major surgery, trauma, starvation, burns, sepsis and in severely ill patients with evidence of disseminated intravascular coagulation.^{3–8} Reduced plasma concentrations may be of clinical significance since the resulting reticuloendothelial dysfunction leads to impaired circulatory clearance of particulate debris. This may lead to an accumulation of fibrin microaggregates, collagenous debris and immune complexes, all of which can result in increasing damage to the microcirculation and the vital organs.⁹ It has also been possible to show that: (i) patients with low fibronectin concentrations have a reduced resistance to infection;¹⁰ (ii) experimentally the infusion of antifibronectin antibodies reduces the ability to survive shock, trauma and burn injury¹¹ and (iii) a correlation exists between fibronectin concentrations and survival in trauma patients.⁴

These observations would suggest that in certain clinical situations plasma fibronectin replacement

therapy may be of clinical benefit. Indeed, a limited number of patients with sepsis and/or disseminated intravascular coagulation have been treated with cryoprecipitate with apparent efficacy.¹² Since no purified preparation for intravenous use is currently commercially available, we have investigated the fibronectin content, both quantitatively and qualitatively, of a number of coagulation factor preparations. These in vitro studies are accompanied by data on the in vivo recovery and plasma half-life of fibronectin after the infusion of cryoprecipitate and factor VIII concentrates. In addition, we have investigated the response of plasma fibronectin concentration to exercise, 1-deamino-8-D-arginine vasopressin (DDAVP) infusion and venous occlusion and studied the properties of this protein after prolonged storage.

Material and methods

Fibronectin was assayed, both quantitatively and qualitatively, in three intermediate purity factor VIII concentrates (two from commercial sources, one from NHS Elstree), a high purity factor VIII concentrate, a factor IX concentrate (Oxford), a cryoprecipitate and fresh frozen plasma (FFP). After reconstitution according to the manufacturer's instructions all preparations were placed in a water bath at 37°C for 60 min before assay.

Plasma fibronectin was purified, from normal

plasma and factor VIII concentrates, by affinity chromatography on gelatin-Sepharose as described by Engvall and Ruoslahti.¹³ Bound fibronectin was eluted with 4 M urea and the resulting eluate dialysed against 0.15 M NaCl, 0.15 M sodium phosphate, pH 7.4 (PBS).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Weber and Osborn.¹⁴

Fibronectin concentration was determined by Laurell's electroimmunoassay¹⁵ in 1% agarose (containing 2% rabbit antihuman fibronectin) for 18 h at 120 V using a barbital buffer system (pH 8.6, I = 0.02). Antifibronectin antibodies were prepared by immunisation of rabbits with fibronectin purified from human plasma by gelatin-Sepharose chromatography.¹³ After electrophoresis the plates were dried and stained with Coomassie brilliant blue. A protein standard plasma (kindly provided by Behringwerke AG, Marburg—OTFI 02/03), with a fibronectin concentration of 258 µg/ml, served as the primary standard. This was used to determine the concentration of fibronectin in pooled citrated plasma from 45 normal adults (20 men, 25 women). Aliquots of 0.5 ml of pooled plasma, stored at -40°C served as secondary standards.

Two dimensional immunoelectrophoresis (2 DIEP), using 20 µl of sample, was performed on 10 cm² plates run in the first dimension at 300 V (120 min) and in the second dimension, with added 2% rabbit antihuman plasma fibronectin, for 18 h at 120 V.

In vivo recovery and plasma half life estimations were determined by infusing five subjects with severe haemophilia,—that is, factor VIII levels <1 IU/dl, with cryoprecipitate (3 subjects) and an intermediate purity factor VIII concentrate (3 subjects). One subject received both products. At the time of infusion the haemophiliacs had not received any blood products for at least four days. Venous blood samples were taken before infusion and at 0.25, 2, 6, 12, 24, 30 and 42 h post infusion. After addition to a 1/10 volume of 3.8% sodium citrate in

plastic tubes, plasma was separated and stored at -40°C until required.

Plasma volume was calculated from a standard nomogram.¹⁶ The percent in vivo recovery was calculated as follows:

$$\frac{(\text{Fibronectin (post)} - \text{fibronectin (pre)}) \times \text{plasma volume (ml)}}{\text{Fibronectin (conc)} \times \text{vol of concentrate infused (ml)}} \times 100$$

where fibronectin (post) was the highest post infusion level of fibronectin, fibronectin (pre) was the pre infusion level of fibronectin and fibronectin (conc) was the fibronectin concentration of the two infused products.

Total protein concentration was determined using a Technicon Auto Analyser (SMAC).

Storage properties of plasma fibronectin were investigated quantitatively and qualitatively, by serially assaying aliquots of plasma stored at -70°C and -40°C (for up to 3 months) and at 4°C and 25°C (for up to 2 weeks).

To investigate the response of plasma fibronectin concentrations under varying conditions fibronectin was assayed pre and post the following stimuli in three healthy male volunteers:

- (i) Exercise to obtain a pulse rate in excess of 140 beats/min.
- (ii) Venous occlusion achieved by means of sphygmomanometer cuff maintaining a pressure of 110 mm Hg for 10 min.
- (iii) DDAVP—0.3 µg/kg injected intravenously with blood samples obtained at 15 and 30 min post infusion. All subjects experienced facial flushing.

Results

The fibronectin and total protein concentration of the blood products studied are shown in Table 1. The factor VIII concentrates, with the exception of the high purity preparations, contained the highest concentrations of fibronectin ranging from 2100–3600 µg/ml. Cryoprecipitate contained 1500 µg/ml whereas a high purity factor VIII concentrate, as

Table 1 *Fibronectin concentration in blood products, (based on five separate determinations for each product).*

Blood product	Fibronectin (µg/ml)		Total protein (mg/ml)	Fibronectin (%)
	Mean	Range		
Factor VIII concentrate (commercial 1)	3600	2700–3700	35	10
Factor VIII concentrate (commercial 2)	2100	2000–2200	20	10
Factor VIII concentrate (NHS Elstree)	3200	2700–3500	20	16
Cryoprecipitate	1500	1000–1800	57	2.6
Fresh frozen plasma	400	300–500	60	0.7
Factor IX concentrate (Oxford)	<50	<50	12	<0.4
Factor VIII concentrate (high purity)	170	150–200	10	1.7
Pooled normal plasma	300	—	63	0.5

Table 2 *In vivo* recovery and plasma half-life of infused fibronectin in cryoprecipitate and a Factor VIII concentrate (NHS Elstree)

Infused product	<i>In vivo</i> fibronectin recovery (%)	Fibronectin half-life (h)
Cryoprecipitate		
Patient 1	37	22
Patient 2	30	21
Patient 3	54	17
Factor VIII concentrate		
Patient 4	37	22
Patient 3	54	25
Patient 5	44	ND

ND = investigation not performed.

expected, contained significantly lower amounts. In contrast factor IX concentrates contained trace amounts only.

The percentage *in vivo* recovery and plasma fibronectin half-life following infusion of cryoprecipitate and a single intermediate factor VIII con-

centrate (NHS, Elstree) are given in Table 2. Recovery ranged from 30–54% with no significant difference between the two preparations. The plasma half-life varied from 17–25 h. Maximum plasma concentrations of fibronectin in the five subjects studied were found immediately after infusion.

On 2-DIEP plasma samples from 15 normal volunteers gave a single narrow peak (Fig. 1a). This pattern was unchanged following prolonged storage at –40°C. An abnormal 2 DIEP pattern, however, was found in cryoprecipitate and factor VIII concentrates. In addition to the main peak a second slower migrating peak (Fig. 1b) was found. This second peak persisted when fibronectin was separated from the factor VIII concentrates and cryoprecipitate using affinity chromatography. Fibronectin purified from the various blood products studied showed a normal pattern on SDS-PAGE (data not shown).

Fibronectin was found to be, immunologically, a very stable protein. No changes in plasma concent-

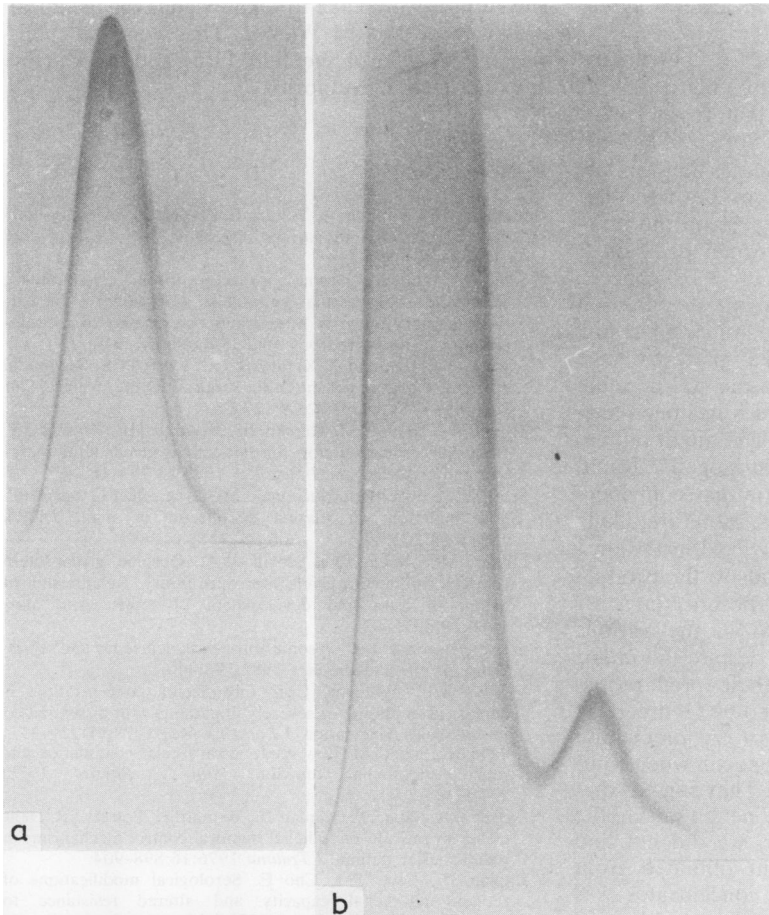


Fig. 1 Two dimensional crossed immunoelectrophoresis incorporating rabbit antihuman antifibronectin antibody (a) normal plasma; (b) a commercial intermediate purity factor VIII concentrate. A similar pattern was obtained with the two other intermediate purity Factor VIII concentrates and cryoprecipitate.

rations or 2 DIEP patterns were observed after storage for 3 months at -70°C and -40°C and 2 wk at 4°C and 25°C .

In contrast to plasminogen activator and FVIII:AG levels (results not shown), no increase in plasma fibronectin concentrations was demonstrated following venous occlusion, exercise or the intravenous administration of DDAVP.

Discussion

Our study demonstrated that fibronectin is a significant component of cryoprecipitate accounting for 2.6% of the total protein present. However, it would appear that factor VIII concentrates are a richer source of fibronectin (10–16% of the total protein) although, as might be expected, the more highly purified factor VIII preparation contained significantly less fibronectin than cryoprecipitate. Factor IX concentrates contain negligible amounts of fibronectin and should not be used for replacement therapy. These results are in general agreement with previous reports.^{17 18}

Infusion studies demonstrated a percentage recovery of infused fibronectin of only 30–54%. This is significantly lower than that reported for other proteins such as FVIII:C (>80%).¹⁹ Sherman and Lee²⁰ have shown in animal studies that this low recovery might be due in part to intravascular/extravascular redistribution. However, in the present study the finding of an abnormal peak on 2 DIEP of cryoprecipitate and factor VIII concentrates suggests that a fraction of the infused fibronectin may be either denatured or circulate in a complexed form. Similar 2 DIEP patterns have recently been described in plasma from some patients with acute leukaemia and it has been suggested that the slower migrating peak might represent fibronectin complexed with fibrinogen.²¹ Rapid clearance of this altered fibronectin may contribute to the low recovery observed. It is, therefore, likely that the purification processes involved in the production of the blood products leads to the production of small quantities of altered fibronectin.

The recovery of fibronectin from the various blood products studied is similar despite the major methodological differences in their preparation. This data differs from that reported by Gomperts *et al*,¹⁷ who claim that the infusion of cryoprecipitate results in a better recovery of fibronectin when compared to factor VIII concentrates. They suggest that this is due to denaturation of fibronectin during the preparation procedure. However, we did not find any differences when fibronectin obtained from cryoprecipitate and factor VIII concentrates was assessed by 2 DIEP and SDS-PAGE.

Thus our results would suggest that factor VIII concentrates, with the exception of high purity preparations, are the most efficient available material for replacement therapy. Any such potential benefit must be weighed against the relative hepatitis risks²² and the recent possible association between concentrate use, immunological abnormalities and the acquired immunodeficiency syndrome (AIDS).²³

It would obviously be of considerable value with regard to replacement therapy if the fibronectin plasma content could be enhanced. Two procedures investigated, DDAVP infusion and exercise, have been shown to increase the plasma concentration of certain proteins, such as FVIII:AG and plasminogen activator, found in endothelial cells. From a practical point of view the infusion of DDAVP to blood donors has been used to increase the yield of FVIII:C.²⁴ We were, however, unable to show that these procedures had any significant effect on plasma fibronectin concentrations.

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References

- 1 Stathakis NE, Fountas A, Tsianos E. Plasma fibronectin in normal subjects and in various disease states. *J Clin Pathol* 1981;**34**:504–8.
- 2 Saba TM, Jaffe EA. Plasma fibronectin (opsonic glycoprotein). Its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticulo-endothelial function. *Am J Med* 1980;**68**:577–94.
- 3 Aronsen KF, Ekelund G, Kindmark CO, Laurell CB. Sequential changes of plasma proteins after surgical trauma. *Scand J Clin Lab Invest (Suppl)* 1972;**29**:127–36.
- 4 Scovill WA, Saba TM, Kaplan JE, Bernard HR, Powers SR. Disturbances in circulatory opsonic activity in man after operative and blunt trauma. *J Surg Res* 1977;**22**:709–16.
- 5 Scott RL, Sohmer PR, Macdonald MG. The effect of starvation and repletion on plasma fibronectin in man. *JAMA* 1982;**248**:2025–7.
- 6 Lanser ME, Saba TM, Scovill WA. Opsonic glycoprotein (plasma fibronectin) levels after burn injury: Relationship to extent of burn and development of sepsis. *Ann Surg* 1980;**192**:776–82.
- 7 Lanser ME, Saba TM. Opsonic fibronectin deficiency and sepsis: Cause or effect? *Ann Surg* 1982;**195**:340–5.
- 8 Mosher DF, Williams EM. Fibronectin concentration is decreased in plasma of severely ill patients with disseminated intravascular coagulation. *J Lab Clin Med* 1978;**91**:729–35.
- 9 Kaplan JE, Saba TM. Low grade intravascular coagulation and reticulo-endothelial function. *Ann J Physiol* 1978;**234**:H323–29.
- 10 Scovill WA, Saba TM, Kaplan JE, Bernard H, Powers SR. Deficiency in reticulo-endothelial humoral control mechanisms in patients after trauma. *J Trauma* 1976;**16**:898–904.
- 11 Kaplan JE, Saba TM, Cho E. Serological modifications of reticulo-endothelial capacity and altered resistance to traumatic shock. *Circ Shock* 1976;**2**:203–11.

- ¹² Saba TM, Blumenstock FA, Scovill WA, Bernard H. Cryoprecipitate reversal of opsonic $\times 2$ surface binding glycoprotein deficiency in septic surgical and trauma patients. *Science* 1978;**201**:622.
- ¹³ Engvall E, Rouslahti E. Binding of soluble form of fibroblast surface protein fibronectin to collagen. *Int J Cancer* 1977;**20**:1-5.
- ¹⁴ Weber K, Osborn M. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* 1969;**244**:4406-12.
- ¹⁵ Laurell CB. Electro-immunoassay. *Scand J Clin Lab Invest* 1972;**29** (suppl 124): 21-37.
- ¹⁶ Dagher FJ, Lyons JH, Finlayson DC. Blood volumes measurement: a critical study prediction of normal values: controlled measurement of sequential changes: choice of bedside method. *Ad Surg* 1965;**1**:69-109.
- ¹⁷ Gomperts ED, Izadi P, Berg D. Fibronectin in commercial coagulation factor concentrates and response of fibronectin plasma levels to infusions. *Thromb Haemost* (Abstract) 1981;**46**:55.
- ¹⁸ Broughton BJ, Simpson A. Plasma fibronectin in acute leukaemia. *Br J Haematol* 1982;**51**:487-91.
- ¹⁹ Allain JP, Verroust F, Soulier JP. In vitro and in vivo characterisation of Factor VIII preparations. *Vox Sang* 1980;**38**:68-80.
- ²⁰ Sherman LA, Lee J. Fibronectin: blood turnover in normal animals and during intravascular coagulation. *Blood* 1982;**60**:558-63.
- ²¹ Klingemann HG, Kosukavak M, Hofeler H, Havemann K. Fibronectin and Factor VIII—Related antigen in acute leukaemia. *Hoppe-Seyler's Z Physiol Chem* 1983;**364**:269-77.
- ²² Levine PH, McVerry BA, Attock B, Dormandy KM. Health of the intensively treated haemophiliac, with special reference to abnormal liver chemistries and splenomegaly. *Blood* 1977;**50**:1-9.
- ²³ Ragni MV, Lewis JH, Spero JA, Bontemp FA. Acquired immunodeficiency like syndrome in two haemophiliacs. *Lancet* 1983;**i**:213-4.
- ²⁴ Nilsson IM, Walter H, Mikaelsson M, Vilhardt H. Factor VIII concentrate prepared from DDAVP stimulated blood donor plasma. *Scand J Haematol* 1979;**22**:42-6.

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