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

JT REILLY, BA MCVERRY, MJ MACKIE

From the University Department of Haematology, Royal Liverpool Hospital, Liverpool

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 α2-glycoprotein with a molecular weight of 440 000 daltons and a normal plasma concentration of 300 μg/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
Table 1: Fibronectin concentration in blood products (based on five separate determinations for each product).

<table>
<thead>
<tr>
<th>Blood product</th>
<th>Fibronectin (μg/ml)</th>
<th>Total protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII concentrate</td>
<td>300-3000</td>
<td>10-30</td>
</tr>
<tr>
<td>Fresh frozen plasma</td>
<td>200-3000</td>
<td>10-30</td>
</tr>
<tr>
<td>Cryoprecipitated Factor VIII concentrate (commercial)</td>
<td>1500-2000</td>
<td>10-30</td>
</tr>
<tr>
<td>Pooled normal plasma (high purity)</td>
<td>100-1500</td>
<td>10-30</td>
</tr>
<tr>
<td>Pooled normal plasma (low purity)</td>
<td>&lt;50</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Results

The fibronectin and total protein concentration of the blood products studied are shown in Table 1. The Factor VIII concentrate, cryoprecipitated Factor VIII concentrate (commercial), and pooled normal plasma (high purity) all contained over three times the fibronectin concentration of pooled normal plasma (low purity). In vivo recovery and plasma half-life estimations were determined by infusing five subjects with 1 IU/dl or 10 IU/dl against 0-15 with chromatography. One subject received both products at one time. The two haemophilia were not received at the same time. The factor VIII concentrate was used as the standard.

In vivo recovery and plasma half-life estimations were determined by infusing five subjects with 1 IU/dl with chromatography. One subject received both products at one time. The two haemophilia were not received at the same time. The factor VIII concentrate was used as the standard.

(iii) A 0.1 μg/ml fibronectin, 0.03 μg/ml Factor VIII concentrate, and 0.01 mg/ml total protein were stored in citrate at 3°C (for up to 2 weeks), then incubated at 37°C (for 4°C for up to 3 months) and at 4°C for up to 3 months.

(ii) Factor VIII concentrate was stored in citrate at 3°C (for up to 2 weeks), then incubated at 37°C (for 4°C for up to 3 months) and at 4°C for up to 3 months.

(i) Venous occlusion achieved by means of sphygmomanometer cuff maintaining a pressure of 110 mm Hg for 10 min.

(ii) Exercise to obtain a pulse rate in excess of 140 beats/min.

(iii) Venous occlusion achieved by means of sphygmomanometer cuff maintaining a pressure of 110 mm Hg for 10 min.
Fibronectin in blood products—an in vitro and in vivo study

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

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

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 concentrate (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.

The authors would like to acknowledge the technical assistance of Mrs M Wilmot. This work was supported by the Carol Shepherd Fund and the Mersey Regional Health Authority.

References

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


Requests for reprints to: Dr JT Reilly, University Department of Haematology, Royal Liverpool Hospital, Liverpool, England.