Fibrinogen derivatives and platelet activation products in acute and chronic liver disease

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Summary

1. The concentration in plasma of fibrinogen derivatives fibrinopeptide A (FPA) and Bβ1-42 and the platelet release products β-thromboglobulin (βTG) and platelet factor 4 (PF4) have been determined in patients with acute and chronic liver disease.

2. In 21 patients with fulminant hepatic failure on admission in grade III or IV coma the plasma FPA, Bβ1-42, βTG and PF4 levels were significantly increased compared with those in normal control subjects. On heparinization before haemoperfusion the FPA levels returned to the normal range and during resin and charcoal haemoperfusion there were no significant changes in the coagulation or platelet factors, except for a small increase in FPA with charcoal haemoperfusion.

3. In ten patients with compensated chronic liver disease there was a significant increase in Bβ1-42 and βTG levels but not FPA and PF4 as compared with normal controls.

4. Interpretation of the results is complicated by the possible reduced clearance of these proteins as a result of renal failure in some of the patients with fulminant hepatic failure and also by the damaged liver itself. However, these results have confirmed that disseminated intravascular coagulation can occur in both acute and chronic liver disease.

Key words: fibrinogen, fibrinopeptide, haemoperfusion, liver disease, platelets.

Abbreviations: FPA, fibrinopeptide A; PF4, platelet factor 4; βTG, β-thromboglobulin.

Introduction

The liver plays a central role in haemostasis not only because it is the site of synthesis of a majority of the clotting factors, but also because it clears many factors or their derivatives from the circulation. Bleeding is a major complication of both acute and chronic liver disease and many studies have shown gross abnormalities in blood clotting times, including prolongation of the prothrombin time, as well as low levels of many of the individual clotting factors [1-3]. The main natural inhibitors of the coagulation proteinases, e.g. antithrombin III [4] and α2-antiplasmin [5], are also found in reduced concentrations in liver disease and, furthermore, abnormal proteins may be produced, which leads to impaired function [6]. Increased consumption of the clotting factors is also found, probably as a result of the release of thromboplastic material from the damaged liver. A similar mechanism may be responsible for the reduced platelet count and impaired platelet aggregation [7] in liver disease. In acute liver failure, where there is massive hepatic necrosis, these changes are so marked that disseminated intravascular coagulation (DIC) has been reported with thrombocytopenia, clotting factors <30% of normal and the presence of fibrinogen degradation products and fibrin strands in the circulation [8].

Recent developments have led to specific and sensitive assays of activation products in vivo of coagulation and fibrinolytic systems and also of platelet α-granule proteins that are released during the platelet release reaction in vivo. Radioimmuno-
assays of fibrinopeptide A (FPA), cleaved by the action of thrombin on fibrinogen [9], and of fibrinogen fragment Bβ1-42 [10], cleaved by plasmin, can be used to detect any imbalance that may occur in the coagulation and fibrinolytic systems. The platelet α-granule proteins [11, 12] β-thromboglobulin (βTG) and platelet factor 4 (PF4) are sensitive markers of platelet activation. The advantage of determining these activation products is that they are normally cleared very rapidly from the circulation and thus reflect ongoing dynamic events rather than those which have occurred at an earlier stage. Hence in this study we have assayed plasma levels of the fibrinogen derivatives and platelet release products in patients with fulminant hepatic failure on admission and during their treatment by extracorporeal haemoperfusion, as well as in patients with compensated chronic liver disease.

Patients
Twenty-nine patients with fulminant hepatic failure were studied (20 as a result of paracetamol overdose, seven after viral hepatitis, one after halothane anaesthesia and one after antituberculous drugs), of whom 21 were seen on admission in either grade III or IV encephalopathy, and five of these had renal failure as defined by a plasma creatinine > 300 μmol/l. A further ten patients had chronic liver disease (three alcoholic cirrhosis, four primary biliary cirrhosis, one primary hepatocellular carcinoma, one cryptogenic cirrhosis, one Wilson’s disease). In every instance the diagnosis was proven histologically and all this group were well compensated biochemically. The results of some of the routine tests of liver function and haematology are given in Table 1.

Twelve of the fulminant hepatic failure patients were also studied during treatment by haemoperfusion, in six by using albumin coated resin columns (Immuno AG, Vienna, Austria) and six by using polymer coated charcoal columns (Haemocol 100, Smith and Nephew Pharmaceuticals, Romford, Essex, U.K.). For resin haemoperfusion, heparin (mucous, Leo Labs Ltd, Princes Risborough, Bucks., U.K.) was administered initially as an intravenous bolus (7500 i.u.), followed by continuous infusion (≈ 2500 i.u./h) into the extracorporeal circuit to maintain a whole body activated clotting time (Haemachron International Technidyne Corp., NJ, U.S.A.) of 250-300 s [13]. With charcoal haemoperfusion a bolus (7500 or 5000 i.u.) of heparin was given with an infusion (≈ 1000 i.u./h) as well as infusion of eprostenol (Wellcome Research Laboratories, Beckenham, Kent, U.K.) in glycine buffer, pH 10.5, to achieve a blood concentration of 5 ng/ml for platelet protection [14]. Twenty-two normal hospital and laboratory staff served as controls with a mean age of 33 years (range 18-56, 13 females, nine males).

Methods
Blood samples were collected into tubes on ice either by clean venepuncture or else from the arteriovenous shunt before haemoperfusion. During haemoperfusion the blood samples were collected from the inlet line to the columns, after the entry of the heparin infusion. For the fibrinopeptide assays nine parts of blood were collected into 1 part of heparin (1000 i.u./ml) and Trasylol (1000 units/ml) and platelet poor plasma was obtained by centrifugation at 4°C. FPA was determined by radioimmunoassay on plasma from which fibrinogen had been removed by bentonite, as already described in detail [15]. Fibrinogen fragment Bβ1-42 was determined by radioimmunoassay on ethanol precipitated plasma [15, 16], essentially as described by Nossel et al. [10], except an unsolubilized second antibody (donkey anti-rabbit, Sac Cel; Wellcome Reagents, Dartford, Kent, U.K.) was used to separate free from bound antigen [17]. Blood (2.5 ml) for radioimmunoassay of the platelet release proteins

<table>
<thead>
<tr>
<th>Table 1. Results of biochemical and haematological tests in patients studied</th>
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<td>Values are given as means with ranges in parentheses.</td>
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<tr>
<th></th>
<th>n</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Prothrombin time (s)</th>
<th>Platelet count (x 10^9/l)</th>
<th>AST (i.u/l)</th>
<th>Bilirubin (μmol/l)</th>
<th>Creatinine (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulminant hepatic failure (on admission)</td>
<td>21</td>
<td>35</td>
<td>M</td>
<td>31-195</td>
<td>89</td>
<td>178</td>
<td>2406</td>
<td>–</td>
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<td></td>
<td></td>
<td>(16-58)</td>
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<td>(67-937)</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>10</td>
<td>51</td>
<td>M</td>
<td>14-27</td>
<td>18</td>
<td>187</td>
<td>98</td>
<td>73</td>
</tr>
<tr>
<td></td>
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<td>(16-73)</td>
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<td>(53-89)</td>
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<td>Normal range</td>
<td>22</td>
<td>(12-14)</td>
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was collected into a cold solution consisting of 100 μl of theophylline (5.4 mg/ml), 100 μl of EDTA (100 mg/ml) and 100 μl of PGE₁ (1 μg/ml) kept on melting ice. Plasma was recovered after centrifugation at 1500 g for 30 min and assayed for βTG as described by Ireland et al. [15]. PF4 was determined by minor modification of the Abbott Laboratories radioimmunoassay (Basingstoke, Hants, U.K.).

Statistics

The data was analysed by using the Wilcoxon Rank Sum Test and Test for Pair Differences and also with linear regression as appropriate.

Results

Fulminant hepatic failure

The results for the different factors measured are given in Table 2. In the 21 patients studied on admission the plasma levels of both FPA and Bβ1-42 were slightly but significantly increased, as compared with the normal control values, with a greater proportionate increase in Bβ1-42 (Fig. 1).

The platelet release proteins βTG and PF4 were also significantly increased in these patients and in this case there was no significant proportionate change compared with the controls (Fig. 2), as indicated by the plasma βTG/PF4 ratio (mean

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<tr>
<th></th>
<th>n</th>
<th>FPA (pmol/ml)</th>
<th>Bβ1-42 (pmol/ml)</th>
<th>βTG (pmol/ml)</th>
<th>PF4 (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulminant hepatic failure</td>
<td>21</td>
<td>*2.64 (0.39-8.44)</td>
<td>**10.24 (1.53-36.0)</td>
<td>**4.09 (0.86-10.0)</td>
<td>**3.51 (1.16-7.19)</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>10</td>
<td>1.01 (0.36-2.47)</td>
<td>**4.87 (1.07-11.7)</td>
<td>**2.31 (1.58-4.17)</td>
<td>0.16 (0.08-0.35)</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>22</td>
<td>1.05 (0.54-2.34)</td>
<td>1.57 (0.65-4.00)</td>
<td>0.69 (0.43-1.57)</td>
<td>0.23 (0.08-0.65)</td>
</tr>
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*P < 0.05, **P < 0.01 vs control.

FIG. 1. (a) Plasma fibrinopeptide A levels in patients with fulminant hepatic failure (FHF), control subjects and chronic liver disease (CLD). (b) Plasma Bβ1-42 antigen levels in patients with fulminant hepatic failure (FHF), control subjects and chronic liver disease (CLD). *P < 0.05, **P < 0.01 vs control.
There was a significant linear correlation between plasma FPA and βTG \((r = 0.72, P < 0.01)\), Bβ1-42 and PF4 \((r = 0.80, P < 0.001)\), and βTG and PF4 \((r = 0.63, P < 0.01)\). The only correlation found between these parameters and the routine biochemical and haematological measurements was between FPA and the plasma creatinine \((r = 0.65, P < 0.01)\).

**Effect of haemoperfusion**

The initial samples in these studies were taken after the bolus administration of heparin (5000 or 7500 i.u.) to the patients, and the plasma FPA levels (mean 0.96 pmol/ml, range 0.37-3.53 pmol/ml) in this group were significantly lower \((P < 0.02)\) after heparin administration than in the patients with fulminant hepatic failure on admission (mean 2.64 pmol/ml, range 0.39-8.44 pmol/ml) and were similar to the normal values. During resin haemoperfusion there was no significant change over 4 h in levels of FPA, Bβ1-42 or βTG (Fig. 3) (PF4 was not measured in the haemoperfusion studies). The mean initial platelet count was 118 x 10⁹/l (range 74-210) and after 4 h was 111 x 10⁹/l (range 50-213 x 10⁹/l). With charcoal haemoperfusion there was a gradual increase in FPA over 4 h (Fig. 4) and the mean level at the end of haemoperfusion was different from the initial value \((P = 0.05)\). Plasma Bβ1-42 rose for the first 2 h and then fell, but there was considerable variation in these values. The plasma

**FIG. 2.** (a) Plasma β-thromboglobulin levels in patients with fulminant hepatic failure (FHF), control subjects and chronic liver disease (CLD). (b) Plasma platelet factor 4 levels in patients with fulminant hepatic failure (FHF), control subjects and chronic liver disease (CLD). **P < 0.01** vs control.

**FIG. 3.** Plasma fibrinopeptide A (○), Bβ1-42 antigen (■) and β-thromboglobulin (▲) levels in patients with fulminant hepatic failure during resin haemoperfusion; means ± SE, \(n = 6\).
Fibrinopeptides in liver disease

Perfusion (h)

FIG. 4. Plasma fibrinopeptide A (○), Bβ1-42 antigen (●) and β-thromboglobulin (▲) levels in patients with fulminant hepatic failure during charcoal haemoperfusion; means ± SE, n = 7. *P = 0.05 vs initial value.

levels of βTG remained constant during haemoperfusion as did the platelet count, which was $163 \times 10^9/\text{l}$ (range 99–253 × 10^9/l) initially, and $174 \times 10^9/\text{l}$ (range 97–268 × 10^9/l) after 4 h.

Chronic liver disease

In the ten patients with chronic liver disease there was a significant increase in Bβ1-42 levels as compared with the normal controls (Fig. 1), but not in FPA levels. The βTG level was also significantly increased in these patients, as was the βTG/PF4 ratio (mean 32.45, range 5.94–189, $P < 0.001$) (Fig. 2), but no similar increase was found in PF4 levels. No significant correlations were found between the FPA, Bβ1-42, βTG and PF4 in these patients, though there was a significant correlation between PF4 and the blood platelet count ($r = 0.82$, $P < 0.01$) but not with the other routine biochemical and haematological measurements.

Discussion

On first consideration it might seem that these results with elevated plasma concentrations of the activation products of coagulation and fibrinolysis (FPA and Bβ1-42) and platelet release products (βTG and PF4) would support the view that acute and chronic liver disease induces disseminated intravascular coagulation. In several other clinical situations such findings have been taken to be good evidence for an imbalance in haemostasis. However, the plasma concentration of a protein or protein fragment depends on its rate of production, volume of distribution and rate of elimination from the circulation, and it is likely that in the present patients one or more of these factors is altered. Thus, in renal failure, investigations both in patients [18] and in experimental animals [19, 20] have suggested that the plasma concentration of Bβ1-42, βTG and to a lesser extent FPA may be influenced by decreased elimination through the kidneys, as well as an increased rate of production. Renal failure is an important complication of acute liver failure and was present in five of the 21 patients studied on admission, and it is possible, therefore, that in these cases the elevation of the coagulation and platelet products was caused in part by the renal impairment. Some support for this is provided by the significant correlation found between plasma FPA and the serum creatinine levels. PF4 levels in plasma are not influenced by renal failure, but the possibility should be considered that the liver may be a site of catabolism of this protein and thus the reduced hepatic function could lead to accumulation. Impaired clearance of denatured protein due to defective reticuloendothelial cell function has been found in fulminant hepatic failure [21].

In spite of the above reservations the results indicate increased activity of both thrombin and plasmin in the patients. FPA levels in plasma are not so affected by renal failure as are Bβ1-42 and βTG and an increased plasma level is likely to be caused at least in part by increased thrombin action on fibrinogen. Support for this is provided by the haemoperfusion studies. Administration of heparin at the initiation of haemoperfusion resulted in a significant fall in mean FPA levels to the normal control levels and, because heparin can rapidly accelerate thrombin inhibition, such a decrease in FPA levels is usually taken to be good evidence for increased thrombin activation in plasma. Continuous heparin administration during haemoperfusion using the resin column suppressed thrombin activity and FPA generation, which is reasonable as, during haemodialysis of patients with renal failure, less heparin was used without fibrinopeptide release [22, 23]. However, a small increase in FPA levels was observed during charcoal haemoperfusion, where less heparin was administered. Patients with fulminant hepatic failure have reduced antithrombin III levels and thus may require more heparin to obtain an adequate response. Also the PGI2 infusion may have affected the whole blood activated clotting time in the extracorporeal circuit [24], so that the value did not reflect the level of anticoagulation in the patient. There was no evidence of platelet activation during resin haemoperfusion
due to the albumin-coating and the effect of PGI₂ during charcoal haemoperfusion.

We were unable to demonstrate an elevated FPA in chronic liver disease, a result that appears to be at variance with that of the study by Coccheri et al. [25], but this may be because our patients were stable and compensated biochemically. However, in these patients ß1-42 and ßTG levels were significantly elevated. It is unlikely that these increases were caused by impaired renal clearance as the serum creatinine levels were normal in these patients and thus increased plasmin and platelet releasing activities are indicated. It is perhaps surprising to find the slightly increased ß1-42 levels in the patients without an apparent increase in FPA levels, as to date there is not firm evidence to suggest that plasmin acts directly on fibrinogen rather than fibrin. There are two possible explanations for this result. Firstly a greater molar rise of ß1-42 than FPA has been observed in human disseminated intravascular coagulation [10] and thus small amounts of FPA released may not be as readily detected as the secondary rise in ß1-42 resulting from plasmin action. Secondly, the liver is the major organ of catabolism of the fibrinolytic system activator, tissue plasminogen activator, and increased plasma levels of tissue plasminogen activator due to reduced clearance in liver disease [26] may increase the level of ß1-42 in plasma without the need for a prior increase in fibrin formation. Our results are consistent with those of Stein & Harker [27], who proposed that in patients with cirrhosis there is a complex process with platelet consumption on incompletely endothelialized surfaces in the liver, leading to local fibrin deposition and secondary activation of the fibrinolytic system.

In summary, interpretation of increased plasma levels of fibrinogen and platelet activation products in liver disease is complicated because the clearance of these products may be impaired. Nevertheless, our results have shown that during both acute and chronic liver disease, there is platelet activation and increased fibrinolysis, which is likely to be the result of low-grade intravascular coagulation.

Acknowledgments
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References


