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A Shift in Balance Between Profibrinolytic and Antifibrinolytic Factors Causes Enhanced Fibrinolysis in Cirrhosis

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The aim of this study was to assess the cause of enhanced fibrinolysis in cirrhosis by studying the balance between profibrinolytic and antifibrinolytic proteins in 24 patients with mild or severe cirrhosis. Antigen levels of both tissue-type plasminogen activator and plasminogen-activator inhibitor 1 were increased in mild and severe cirrhosis. Activity levels showed a very wide variability, but median activity levels of both proteins were normal. In most patients, the increase in tissue-type plasminogen activator was counterbalanced by the increased levels of plasminogen-activator inhibitor 1, but in a subgroup of patients the change in balance resulted in extremely high tissue-type plasminogen-activator levels. The specific activity of both proteins (activity/antigen quotient) was reduced in either mild or severe cirrhosis. This finding indicates either that more enzyme-inhibitor complexes circulate in the blood of patients with cirrhosis than in normal individuals or that dysfunctional molecules circulate. Plasminogen and α_2-antiplasmin antigen and activity levels were decreased in both mild and severe cirrhosis. The binding of α_2-antiplasmin to fibrin was decreased in severe cirrhosis, making fibrin clots more susceptible to lysis. Clot lysis experiments were performed to see if equal decreases in plasminogen and α_2-antiplasmin levels, as found in cirrhosis, result in a change in the rate of fibrinolysis. It was found that the proportionate decreases led to enhancement of fibrinolysis, indicating that the inhibitor depletion is more important than the proenzyme depletion. The authors conclude that enhanced fibrinolysis frequently found in cirrhosis may be attributed to an increased tissue-type plasminogen-activator activity relative to plasminogen-activator–inhibitor activity and decreased levels of α_2-antiplasmin, resulting in a reduced binding of α_2-antiplasmin to fibrin.

Bleeding is a common complication in patients with cirrhosis, and some bleeding complications can be attributed to changes in hemostasis (1,2). Because the liver plays a critical role in the synthesis of coagulation factors and the clearance of activated coagulation factors, it is clear that a deterioration of liver function results in changes in coagulation. The liver is also involved in the synthesis and clearance of most fibrinolysis proteins, and the impairment of liver function will therefore result in several changes in fibrinolysis (3–10). Changes in fibrinolysis can have a serious impact on the hemostatic balance, as exemplified by congenital disorders of fibrinolysis, such as in patients with a homozygous deficiency of the fibrinolysis inhibitor α_2-antiplasmin, who experience a lifelong hemophilialike bleeding disorder (11,12). The hemophilialike clinical significance of enhanced fibrinolysis in liver disease was indicated by Francis and Feinstein, who found that enhanced fibrinolysis predisposed to soft tissue hemorrhage after trauma and found a trend toward increased intracranial bleeding in patients with severe liver disease (13).

Goodpasture was the first to show that enhanced fibrinolysis was common in cirrhosis (3). Several investigators have since reported enhanced fibrinolysis in liver disease and proposed different mechanisms for their findings (4,5). Some suggest a reduced

Abbreviations used in this paper: α_2-AP, α_2-antiplasmin; ECLT, euglobulin clot-lysis time; FDP, fibrin degradation product; PAI, plasminogen-activator inhibitor; PP, pooled normal plasma; t-PA, tissue-type plasminogen activator.

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inhibition of fibrinolysis, due to decreased levels of plasminogen-activator inhibitors (PAIs) as well as plasmin inhibitors (6–8). Others suggest increased levels of plasminogen activators caused by reduced clearance as a cause for enhanced fibrinolysis (5,9,10). However, there is still no agreement on the role of the different fibrinolysis factors in the enhancement of fibrinolysis. One reason is the recent identification and characterization of several fibrinolytic proteins, another is that most studies focus on only one of the components of the fibrinolytic system. Because multiple changes occur in fibrinolysis in patients with cirrhosis, it may not be sufficient to study individual components; in contrast, these components must be studied in coherence.

We therefore investigated the balance between profibrinolytic and antifibrinolytic factors of the fibrinolytic cascade. First, the balance between tissue-type plasminogen activator (t-PA) and PAI-1 was studied (14). Both t-PA and PAI-1 are synthesized and released mainly by endothelial cells and are cleared by the liver (15–17). Second, we studied the balance between plasminogen and α_2-antiplasmin (α_2-AP). Plasminogen is the proenzyme of plasmin, the key enzyme of fibrinolysis (Figure 1). The most important inhibitor of plasmin, α_2-AP is synthesized by the liver in a very active plasminogen-binding form that is converted in circulation to a less active non–plasminogen-binding form (18). Besides its plasmin-inactivating and plasminogen-binding properties, the plasminogen-binding form of α_2-AP has the ability to crosslink to fibrin, mediated by coagulation factor XIIIA (19). The binding of α_2-AP to fibrin is important because plasmin generated on the fibrin surface is inhibited in situ by the α_2-AP cross-linked to fibrin. This inhibition is more effective than inhibition by circulating α_2-AP (20). α_2-Macroglobulin is a less important inhibitor of plasmin.

The approach of studying fibrinolytic proteins together may reveal the relevance of the changes in the fibrinolytic balance in patients with liver disease and might therefore result in a better understanding of the mechanism of enhanced fibrinolysis in these patients.

Materials and Methods

Patients

After informed consent was obtained in conformance with the Declaration of Helsinki, 24 outpatients in stable condition with biopsy-proven cirrhosis of different etiologies—alcohol abuse (n = 10), viral hepatitis (n = 5), autoimmune hepatitis (n = 6), and unknown (n = 3)—participated in the study. At the time of blood sampling, none of the patients had bleeding problems. The patients were classified according to Pugh's modification of the Child classification (21). Clinical information was obtained from hospital records. Twelve patients with mild cirrhosis (Child's class A) and 12 patients with severe cirrhosis (Child's class C) were studied. Twelve healthy volunteers served as control group.

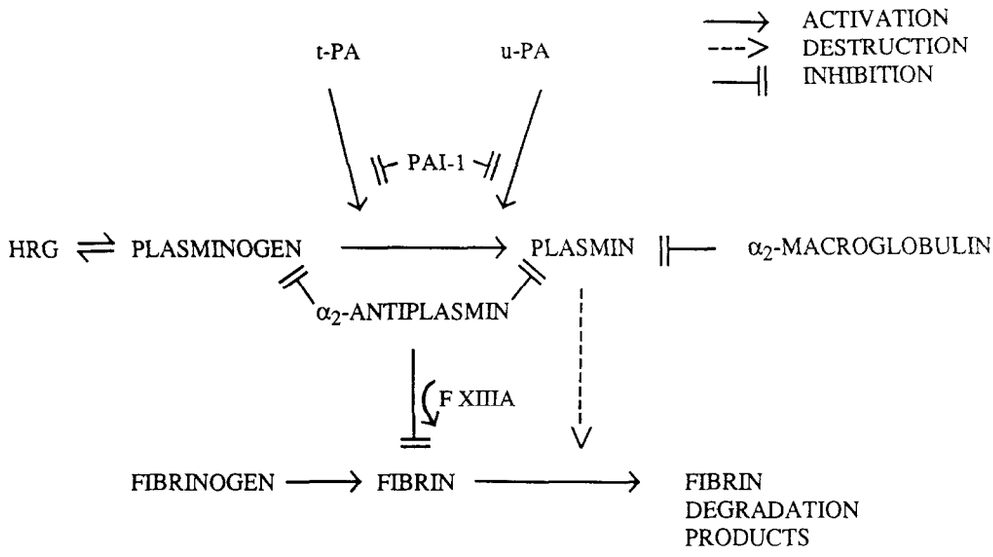

Figure 1. Schematic representation of the fibrinolytic system. During the last step in the coagulation cascade, fibrinogen is converted into fibrin by thrombin. Fibrin has only a temporary function and is degraded to FDPs by plasmin. Plasmin is formed upon activation of plasminogen by plasminogen activators, such as t-PA and urokinase plasminogen activator (u-PA). Plasminogen activator inhibitor 1 controls fibrinolysis by inhibition of t-PA and, to a lesser extent, u-PA. Histidine-rich glycoprotein (HRG) regulates the amount of "free" plasminogen in the circulation by binding to plasminogen and is shown to be in a reversible equilibrium with plasminogen. α_2-Antiplasmin is the most important inhibitor of plasmin in the circulation, by forming plasmin–α_2-AP complexes. As shown here, it also has the ability to bind to plasminogen and to be cross-linked to fibrin, the latter being mediated by coagulation factor XIIIA. α_2-Macroglobulin is also a plasmin inhibitor but is less efficient than α_2-AP.

Plasma

Venous blood, obtained by venapuncture between 10:00 AM and 12:00 noon, was collected in 0.11 mol/L trisodiumcitrate (9:1) and placed immediately on melting ice. Plasma was prepared by centrifugation at 2000*g* for 30 minutes at 4°C and stored at −70°C until use. A laboratory reference value of 100% was obtained from pooled plasma (PP) of 20 healthy volunteers (male-female ratio, 1.0).

Methods

Plasminogen was measured using streptokinase for activation of plasminogen and HD-Nva-CHA-Lys-pNA as a substrate, both obtained from Behringwerke (Marburg, Germany) (22). Tissue-type plasminogen activator activity and PAI-1 of plasma were measured by spectrophotometric assays according to Verheijen et al. (23,24). The coefficients of variation of these assays are 9% and 10%, respectively. Antigen levels of t-PA were determined using an enzyme-linked immunosorbent assay (Biopool, Umea, Sweden) (coefficient of variation, 8%) (25). Levels of PAI-1 antigen were measured using a TintElize TM PAI-1 assay (cat no. 210220) from Biopool with a coefficient of variation of 5%. Specific activity of t-PA and PAI-1 of the individual patients was calculated as the ratio of activity to antigen of t-PA and PAI-1, respectively. To determine the euglobulin clot-lysis time (ECLT), standard euglobulin fractions of plasma were prepared at pH 5.9 with a plasma dilution of 1:10 (26). Precipitates were redissolved in Tris/Tween buffer (0.1 mol/L Tris/HCl; 0.1% Tween 80 (vol/vol), pH 7.5), and 0.2 mL of the euglobulin fractions were clotted by addition of 0.1 mL of calcium-thrombin solution (25 mmol/L $CaCl_2$ and 10 NIH/L thrombin). The lysis time of the clot was recorded. The disappearance of air bubbles was regarded as the endpoint. Activity of α_2-AP was measured using Behrichrom α_2-AP, using HD-Nva-CHA-Lys-pNA as a substrate (Behringwerke) (27). Levels of α_2-AP antigen were measured by Rocket immunoelectrophoresis according to Laurell, using a 1.5% anti-α_2-AP antiserum (Nordic Immunology, Tilburg, The Netherlands) (28). The ratio of plasminogen-binding to non–plasminogen-binding α_2-AP was determined by modified crossed immunoelectrophoresis (29). In this method we added monoclonal antibodies directed against the COOH-terminal part, the plasminogen-binding site, of α_2-AP (30) in the first-dimension gel and polyclonal antiserum (Nordic Immunology) against α_2-AP in the second dimension. The two obtained immunoprecipitation peaks represent the plasminogen-binding and the non–plasminogen-binding forms of α_2-AP, respectively, and the ratio was calculated as the ratio of the surfaces of the immunoprecipitation peaks determined with a computerized program using a Hipad digitalizer (Geveke Electronics, Amsterdam, The Netherlands). The coefficient of variation of this assay is 2.5%. Binding of α_2-AP to fibrin was studied by clotting 180 μL plasma with 120 μL of a mixture of thrombin (4.17 NIH/mL) and $CaCl_2$ (10.4 μmol/L) in 0.11 mol/L NaCl, which was left standing at 37°C for 1 hour. In the serum supernatant and in a plasma sample incubated with 120 μL of 0.15 mol/L NaCl, α_2-AP activity was assayed as described above. The difference represented the amount of α_2-AP bound to fibrin and was expressed relative to the patient's own plasma level (relative value) and in absolute amounts (absolute value). Factor XIIIA was determined according to the method of Laurell using an anti–factor XIIIA antiserum from Behringwerke (28). α_2-Macroglobulin was assayed according to Laurell using a 2% rabbit anti-human α_2-macroglobulin antiserum (Nordic Immunology) (28).

Fibrin-degradation products (FDPs) were measured in plasma using an enzyme immunoassay (obtained from Organon Teknika, Boxtel, The Netherlands) using a monoclonal antibody against fragment E as catching antibody and a monoclonal antibody against the D-dimer as tagging antibody (31). Albumin levels were determined using the Bromocrysolgreen principle on a chemical analyzer (SMAC; Technicon Instruments Corp., Tarrytown, NY).

In Vitro Studies

Clot lysis experiments were performed to determine the effect of decreasing levels of plasminogen and α_2-AP on the rate of fibrinolysis. Pooled normal plasma was depleted in plasminogen and plasminogen-binding α_2-AP by affinity chromatography on Lysine Sepharose (32), followed by affinity chromatography on Kringle I-III Sepharose (prepared at Gaubius Institute). To this plasma, different amounts of both purified Glu-plasminogen and plasminogen-binding α_2-AP were added to concentrations of 12.5%, 25%, 50%, 100%, and 150%. The concentrations of plasminogen and plasminogen-binding α_2-AP in the PP (100% value) were 1.5 μmol/L and 0.7 μmol/L, respectively. Plasminogen was prepared from human Cohn III fraction (32), and purified plasminogen-binding α_2-AP was obtained from Biopool. Ninety microliters of these plasma samples was clotted by addition of a mixture of 0.3 NIH thrombin and 4.5 IU single-chain melanoma t-PA (33) in 7.5 μL of a 0.2 mol/L $CaCl_2$ solution and 37.5 μL phosphate-buffered saline. These plasma samples were incubated at 37°C, and FDPs were measured after 10 minutes and every 5 minutes thereafter.

Statistical Analysis

Statistical analysis was performed using the Wilcoxon rank-sum test and the Spearman rank-correlation test. A P value of < 0.05 was considered significant. Unless otherwise stated, the values are expressed as median and range.

Results

Balance Between Tissue-Type Plasminogen Activator and Plasminogen-Activator Inhibitor 1 in Cirrhosis

We measured the total amount of t-PA and PAI-1 in the circulation, using suitable antigen assays. As shown in Table 1, both t-PA and PAI-1 antigen levels are strongly increased in both mild and severe cirrhosis. In Figure 2A, a relationship between

Table 1. Tissue-Type Plasminogen Activator and Its Inhibitor in Cirrhosis

	Control (n = 12)	Child's class A (n = 12)	Child's class C (n = 12)
t-PA antigen (*ng/mL*)			
Median (range)	3.8 (2.0–8.0)	11.7 (3.1–38.1)[a]	27.1 (13.5–129)[b]
Mean ± SD	4.2 ± 1.9	13.6 ± 9.6	36.8 ± 30.6
t-PA activity (*mIU/mL*)			
Median (range)	127 (1–590)	28 (0–1770)[c]	29 (1–6220)[c]
Mean ± SD	196 ± 211	222 ± 499	899 ± 1728
t-PA specific activity (*mIU/ng*)			
Median (range)	41 (0–295)	2 (0–183)[d]	12 (0–84)[c]
Mean ± SD	74 ± 99	29 ± 55	23 ± 28
PAI antigen (*ng/mL*)			
Median (range)	7.0 (2.3–18.5)	18.5 (5.0–48.8)[e]	21.6 (13.3–108)[a]
Mean ± SD	8.2 ± 4.8	18.6 ± 13.7	35.8 ± 33.6
PAI activity (*IU/mL*)			
Median (range)	2.5 (0.9–7.8)	4.6 (0–22.0)[c]	4.2 (0–67.8)[c]
Mean ± SD	2.8 ± 2.1	5.5 ± 6.2	9.8 ± 18.9
PAI specific activity (*IU/ng*)			
Median (range)	0.31 (0.16–0.96)	0.24 (0–0.45)[c]	0.18 (0–0.65)[d]
Mean ± SD	0.35 ± 0.21	0.21 ± 0.15	0.20 ± 0.18

NOTE. Results of t-PA antigen levels and activity and PAI-1 activity and antigen levels of plasma in patients with Child's class A and Child's class C cirrhosis compared with a reference group. The specific activities of both t-PA and PAI-1 were calculated as the activity-to-antigen ratio of the individual patients.
[a]$P < 0.002$, [b]$P < 0.0001$, [c]NS, [d]$P < 0.05$, [e]$P < 0.01$, all vs. control.

individual levels of t-PA antigen and PAI-1 antigen in blood, which is well-known in normal individuals and maintained in patients with cirrhosis, can be seen. The t-PA and PAI-1 antigen levels of the total group of patients correlated significantly ($r = 0.71$; $P < 0.001$). In the total amounts, reflected in the antigen concentrations, the balance of t-PA/PAI-1 seemed to change in patients with cirrhosis. This finding is illustrated in Figure 2*B* using the ratio of t-PA antigen to PAI-1 antigen (in mol/mol), showing a trend toward increased levels of t-PA compared with PAI-1.

A main question is how increases in the counteracting components t-PA and PAI-1 balance out in final availability of t-PA. In this respect, evaluation of the median values of the groups showed no devations.

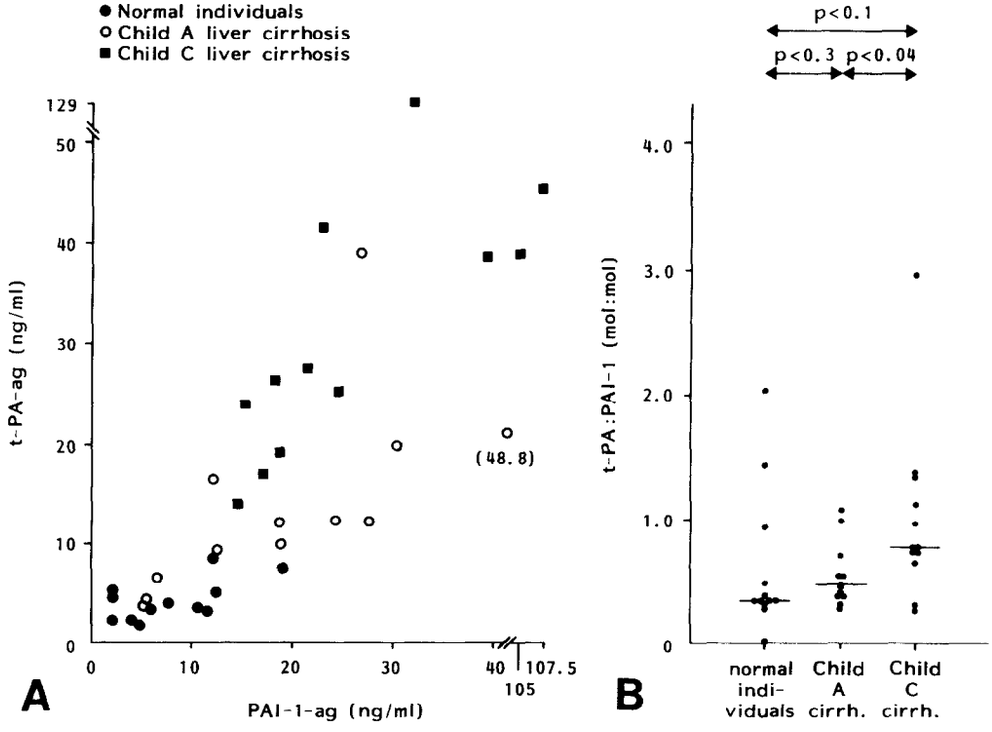

Figure 2. Antigen levels of t-PA and PAI-1 in cirrhosis.

A. Antigen levels of t-PA and PAI-1 in healthy individuals and patients with mild (Child's class A) and severe (Child's class C) cirrhosis. The correlation coefficients of the normal individuals and those with mild and severe cirrhosis are 0.48 ($P < 0.05$), 0.83 ($P < 0.001$), and 0.83 ($P < 0.001$), respectively.

B. The ratios between t-PA and PAI-1 antigen levels of all healthy individuals (N) and patients with mild (Child's class A) and severe (Child's class C) cirrhosis. The *horizontal lines* indicate the median.

There is a normal median t-PA activity in both patient groups, suggesting that the increased t-PA concentration is controlled by the increased PAI-1 level. These findings are supported by the fact that the specific activity of t-PA, calculated as the quotient (t-PA activity:t-PA antigen) of the individual patients, is reduced in mild cirrhosis ($P < 0.05$), whereas the same trend is seen in severe cirrhosis ($P = 0.06$). Specific activity of PAI-1 is also reduced in severe cirrhosis, as shown in Table 1. However, the evaluation of median values does not appropriately reflect the situation and does not account for the very wide variability of t-PA activity levels found in both mild and severe cirrhosis. In Figure 2, the individual levels of t-PA activity are plotted against the individual PAI activity levels of both healthy volunteers and patients with severe cirrhosis. As can be seen, t-PA activity levels correlated negatively with PAI activity levels in both controls ($r = -0.75$; $P < 0.01$) and patients ($r = -0.68$; $P < 0.02$). When a curve is plotted through the individual levels of t-PA activity and PAI activity of the controls and the group of patients with severe cirrhosis, a shift in the balance of these parameters can be seen (Figure 3). This shift accounts for a much wider variation in t-PA activity values and especially for extremely high t-PA activity in a subgroup of patients. In patients with severe cirrhosis, low levels of PAI activity (<2 IU/mL) are associated with extremely high levels of t-PA activity. In the other patients (PAI activity levels >2 IU/mL), t-PA activity is not increased. The net result of these changes in t-PA and PAI-1 on overall fibrinolytic activity was also measured by the ECLT. The ECLT was >180 minutes in all healthy individuals. In mild cirrhosis, the ECLT was >180 minutes in 7 patients and between 107 and 172 minutes in the other 5 patients. In severe cirrhosis, the ECLT was >180 minutes in 6 patients and between 18 and 130 minutes in the other 6 patients. The 4 patients with the highest t-PA activity all had shortened ECLTs (18,27,51, and 73 minutes).

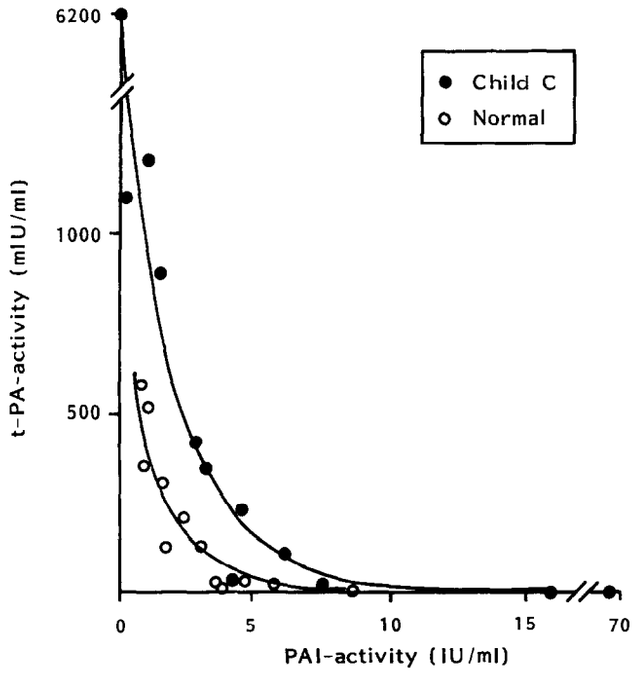

Figure 3. Tissue-type plasminogen activator and its inhibitor in cirrhosis. Levels of t-PA activity and PAI-1 activity of individual patients with severe cirrhosis and normal subjects. A shift in balance between these two fibrinolysis parameters is shown in severe cirrhosis.

Balance Between Plasminogen and α_2-Antiplasmin

The amount of plasminogen was decreased in both mild (66; 42%–95%; $P < 0.005$) and severe (41; 13%–67%; $P < 0.0001$) cirrhosis compared with the control group (88; 71%–126%). In mild and severe cirrhosis, both α_2-AP activity and antigen levels were decreased (Figure 4). The ratio between the two molecular forms (plasminogen-binding and non–plasminogen-binding forms) was measured using modified crossed immunoelectrophoresis (Figure 5). The ratio was increased in mild cirrhosis (2.8; 2.1–3.7; $P < 0.001$) and in severe cirrhosis (4.0; 1.7–15.4; $P < 0.005$) (control, 2.2; 1.8–2.7).

The relative amount of α_2-AP bound to fibrin is normal in mild but increased in severe cirrhosis (Figure 4). Levels of coagulation factor XIIIA, which mediates the binding of α_2-AP to fibrin, are decreased in mild (76; 52%–119%; $P < 0.025$) and severe (76; 41%–116%; $P < 0.005$) cirrhosis compared with the control group (94; 81%–122%). Although the relative amount of α_2-AP bound to fibrin is increased in severe cirrhosis, the absolute amount is decreased because of the strong reduction of total α_2-AP level. Levels of α_2-macroglobulin, also capable of inhibiting plasmin, were increased in mild cirrhosis (132%; 98%–257%; $P < 0.005$) and normal in severe cirrhosis (105%; 82%–178%) (control, 89%; 59%–127%) (data not shown).

In Vitro Clot-Lysis Studies

As stated above, we found that plasminogen and α_2-AP levels are decreased about equally in cirrhosis. Because plasminogen is the proenzyme of plasmin and α_2-AP its inhibitor, these changes would theoretically counteract each other. By performing clot-lysis studies we tried to assess the influence of this equal decrease on the rate of fibrinolysis. After depleting PP of plasminogen and α_2-AP, we added back proportionate measured amounts of both pro-

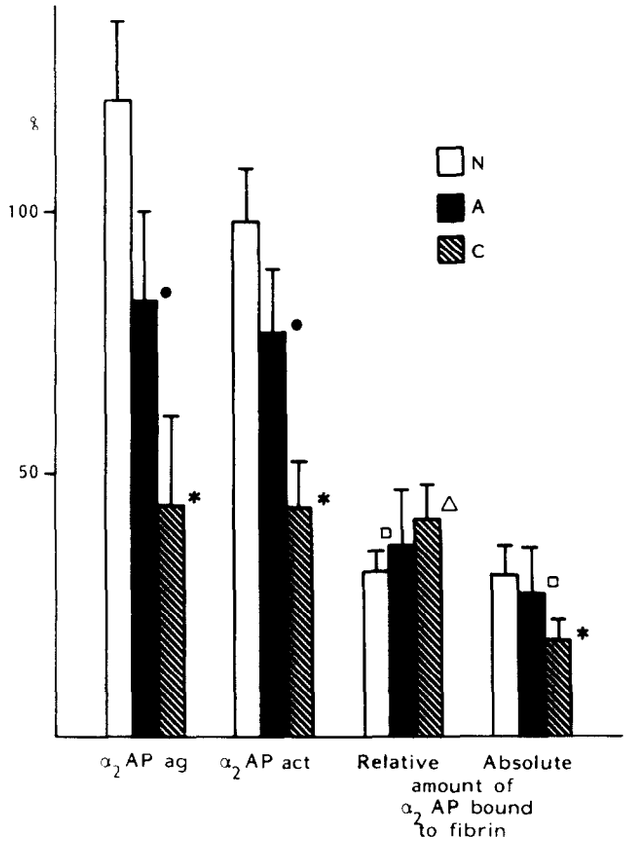

Figure 4. α_2-Antiplasmin in cirrhosis. α_2-Antiplasmin activity and antigen levels and the relative and absolute amount of α_2-AP bound to fibrin in normal subjects (control; n = 12) and those with Child's class A (n = 12) and Child's class C (n = 12) cirrhosis. *Bars* indicate mean values; *lines* indicate SD. *$P < 0.005$, •$P < 0.001$, △$P < 0.01$, ⌜NS, all vs. the control group.

teins. These plasma samples were clotted by adding thrombin and lysed by addition of t-PA. Lysis was recorded by measuring FDPs in the fluid phase of the clot. Figure 6 shows that equal decreases of plasminogen and α_2-AP levels result in a shortening of the lysis time. For instance, it takes 100 minutes to achieve complete lysis of the clot if both concentrations are 100%, but it only takes 35 minutes if both concentrations are 25% compared with PP. The clot-lysis experiments suggest that the equal decreases in plasminogen and α_2-AP levels found in cirrhosis result in enhancement of fibrinolysis.

Discussion

Earlier studies on fibrinolysis in cirrhosis suggested that reduced inhibition of fibrinolysis or increased levels of plasminogen activators could be responsible for enhanced fibrinolysis (3–10). However, all these studies focused mainly on one of the components of the fibrinolytic system only. In our study of patients with different degrees of cirrhosis, we considered various components together, notably the profibrinolytic and antifibrinolytic factors at two levels of the fibrinolytic cascade.

The increase of both t-PA antigen and PAI-1 antigen levels in our patients is in accordance with the findings of most other studies (34–40). However, Hersh et al. report decreased t-PA inhibition in patients with liver disease and suggest that the enhancement of fibrinolysis was related to a decreased t-PA-inhibitor antigen level (39). We could not support these data by our study, nor could others (37–39). Tran-Thang et al. suggest that the increased PAI-1

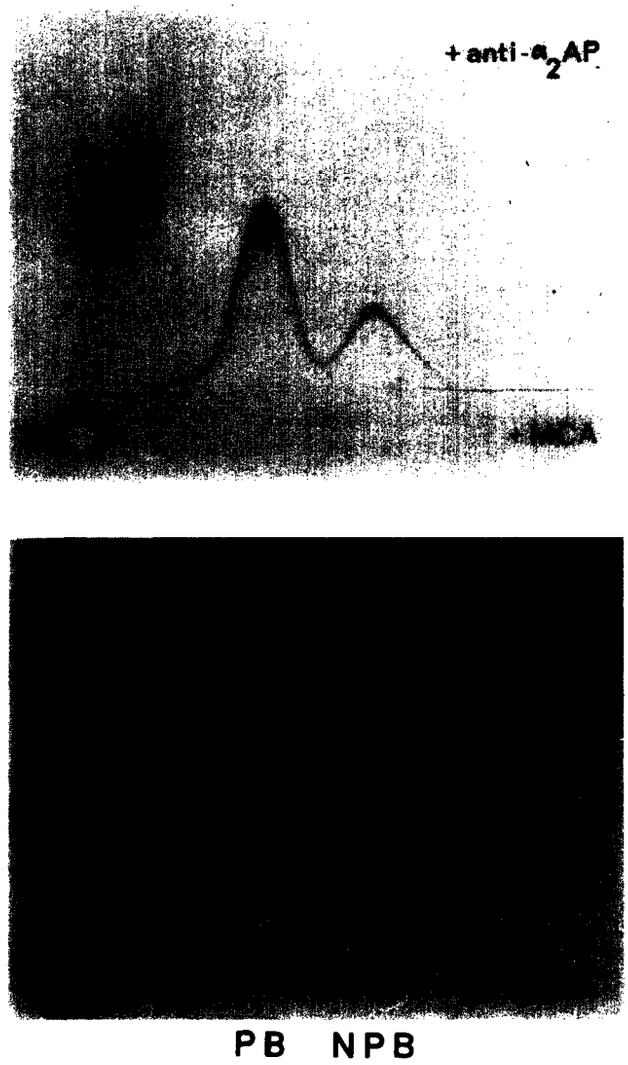

Figure 5. The two molecular forms of α_2-AP. Modified crossed immunoelectrophoresis to assay the plasminogen-binding (PB) and the non–plasminogen-binding (NPB) forms of α_2-AP in plasma of a healthy volunteer, with a PB-NPB ratio of 2.3 (*A*) and of a patient with severe cirrhosis, with a PB-NPB ratio of 4.0 (*B*). Monoclonal antibodies (MCA) were added to the first-dimension gel. In the second-dimension gel, polyclonal antibodies against α_2-AP were added. The ratio of PB-NPB forms of α_2-AP was calculated as the ratio of the surfaces of the immunoprecipitation peaks.

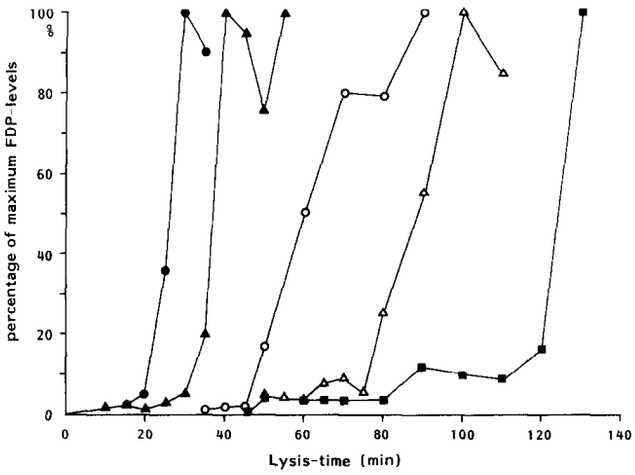

Figure 6. In vitro plasma clot-lysis experiments. Plasma samples containing various but equal concentrations of plasminogen-binding α_2-AP and plasminogen were clotted and incubated at 37°C. The lysis of the clots was recorded by measuring FDPs in the fluid phase of the clot. The FDP levels, expressed as percentages of the maximum FDP level (obtained after complete lysis of the clot), are given on the *vertical axis*. Levels of FDPs were measured every 5 minutes up to 140 minutes of incubation. Plasminogen and α_2-AP concentrations are given as a percentage of PP (100% represents 1.5 μmol/L and 0.7 μmol/L, respectively): ●, 12.5%; ▲, 25%; ○, 50%; △, 100%; ■, 150%.

antigen level was related to its acute-phase reactant property and that the PAI-1 increase may serve as an early indicator of liver defects (39). However, we found that both t-PA and PAI-1 antigen levels increased concomitantly with the severity of the disease. This increase can be caused by decreased clearance by the liver. Normally, t-PA and PAI-1 are cleared rapidly by the liver, with half-lives of 4 and 10 minutes, respectively (17). Our results support the conclusions of earlier studies that a detoriation of liver function will lead to a decreased clearance and increase of both proteins in the circulation (35,38). However, this does not explain the findings of our study that the balance between t-PA and PAI-1 antigen changes slightly in favor of t-PA, as shown in Figure 2*B*. The fact that PAI-1 is synthesized partly by the liver and partly by endothelial cells, whereas t-PA is synthesized only by endothelial cells, may account for this change in t-PA and PAI-1 balance. Metabolism studies have not yet been performed to study the clearance of t-PA and PAI-1 and their complexes in cirrhosis and remain necessary to confirm the conclusions of our study and earlier ones. By calculating the specific activity of t-PA and PAI-1, we provided evidence for a shift in the composition of the circulating amounts of t-PA and PAI-1. The specific activities of both t-PA and PAI-1 were decreased, indicating larger amounts of enzyme-inhibitor complexes in the patients than in the healthy individuals. Although we did not directly show t-PA bound to inhibitors by measuring the complexes, our results are supported by recent findings of Leiper et al., who showed an increase of t-PA–PAI-1 complexes in cirrhosis (41). The possibility cannot be excluded that the decreased specific activity of t-PA or PAI-1 is caused by the circulation of dysfunctional molecules. It is known that in most patients with cirrhosis, abnormal coagulation factors (i.e., dysfunctional fibrinogen molecules) are synthesized by the diseased liver (42). The antigen assays used in this study for t-PA and PAI-1 are recording free and complexed molecules equally effective (43). Therefore, changes in the balance between t-PA and PAI-1 antigen levels in cirrhosis cannot be attributed to the circulation of t-PA–PAI-1 complexes.

For the first time, both t-PA and PAI-1 activities in a well-defined population of patients with cirrhosis were studied. Although a wide range of activity levels of both t-PA and PAI-1 was found, the median values of both are not significantly different in mild and severe cirrhosis compared with controls. The results of the few earlier studies on t-PA activity levels are contradictory in this respect. Boks et al. (34) did not find an increase of t-PA activity, whereas Hersh et al. (40) found increased t-PA activity in a subgroup of patients with cirrhosis. In the only study that reported PAI activity levels in liver disease, Kruithof et al. (37) found increased PAI activity in seven unclassified patients with hepatic insufficiency, which might have been due to two patients with extremely high levels of PAI activity. We classified the patients according to severity of disease and found no relationship between the severity of disease and t-PA or PAI-1 activity levels. Therefore, we suggest that patients should be evaluated as individuals rather than as groups. In individual patients, this increased t-PA activity relative to PAI-1 activity can result in extremely high t-PA activity, as is shown in Figure 3. This finding is in accordance with the shortening of the ECLT. Only these patients may be at risk for a bleeding tendency due to increased fibrinolysis.

The balance between profibrinolytic and antifibrinolytic factors was also studied at the plasminogen and α_2-AP level. Antigen and activity levels of α_2-AP were decreased in mild and severe cirrhosis. This finding confirms those of earlier studies by Arnman et al. (44) and Aoki et al. (45), who suggested that the decrease in α_2-AP resulted in enhanced fibrinolysis. They did not study this decrease in relation to plasminogen, which is also decreased in cirrhosis (46–48). The decrease in plasminogen theoretically counteracts the decrease in α_2-AP. To evaluate the importance of equally decreased plasminogen and α_2-AP levels for clot lysis, we performed in vitro clot-lysis studies. We depleted PP from plasminogen and α_2-AP and added back proportionate amounts of both proteins. The

samples were clotted, then clot lysis was recorded. Equal decreases in plasminogen and α_2-AP resulted in an increased rate of fibrinolysis. Therefore, we suggest that the net effect of decreased levels of both plasminogen and α_2-AP is an enhancement of fibrinolysis due to a dominating role of α_2-AP. As shown in Figure 6, the pattern of the lysis of the various fibrin clots is similar and the difference in lysis time is dependent on the lag time. This is in accordance with other clot-lysis tests, such as the ECLT, in which the lag time also determines the length of the lysis time.

We also studied the binding of α_2-AP to plasminogen and fibrin. The proportion of α_2-AP in the circulation that is bound to plasminogen is increased as determined by an increase in the ratio of the active plasminogen-binding form and the less active non–plasminogen-binding form of α_2-AP. This might be caused by a decrease in the normally occurring conversion of the plasminogen-binding form into the non–plasminogen-binding form. The mechanism of the conversion is still unknown, but our findings may indicate that the liver, or a factor derived from the liver, is involved in this process.

Because of the low levels of total α_2-AP, the (absolute) amount of α_2-AP binding to fibrin is decreased in severe cirrhosis. The relative amount of α_2-AP binding to fibrin, however, is increased in severe cirrhosis. This finding might be attributed to the relative increase in plasminogen-binding α_2-AP compared with non–plasminogen-binding α_2-AP, as mentioned above, because only the plasminogen-binding form of α_2-AP binds to fibrin (19). The reduced inhibition of plasmin, both in circulation due to a decrease in total α_2-AP and at the fibrin surface due to a reduced cross-linking of α_2-AP to fibrin, may make fibrin more susceptible to lysis and lead to enhanced fibrinolysis in cirrhosis. α_2-Macroglobulin does not seem to play a role in enhancing fibrinolysis in patients with cirrhosis.

Conclusions

Our study showed that several changes occur at the two levels of the fibrinolytic mechanism studied. The changes include increased t-PA and PAI-1 antigen levels, abnormal correlation between t-PA and PAI-1 blood levels, reduced plasminogen and α_2-AP levels, a changed balance between plasminogen-binding α_2-AP and non–plasminogen-binding α_2-AP, and a reduced binding of α_2-AP to fibrin. We suggest that enhanced fibrinolysis frequently seen in patients with cirrhosis is caused by increased t-PA activity relative to PAI-1 activity and decreased levels of α_2-AP, resulting in a reduced binding of α_2-AP to fibrin.

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