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SYSTEMIC EFFECTS OF TISSUE PLASMINOGEN ACTIVATOR–ASSOCIATED FIBRINOLYSIS AND ITS RELATION TO THROMBIN GENERATION IN ORTHOTOPIC LIVER TRANSPLANTATION[1]

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Orthotopic liver transplantation is frequently associated with hyperfibrinolysis, the origin and clinical relevance of which is largely unknown. In 20 orthotopic liver transplantations, we studied the occurrence and systemic effects of hyperfibrinolysis. Severe fibrinolysis was defined to be present when the euglobulin-clot lysis time and the whole-blood-clot lysis time, as measured by thrombelastography, were shorter than 60 and 90 min, respectively, at some time during the operation. Based on these criteria, 7 patients had minimal fibrinolysis (group I), and 13 patients had severe fibrinolysis (group II). In group II a gradual increase of tissue-type plasminogen activator (t-PA) activity was seen during the anhepatic stage, followed by an "explosive" increase immediately after graft reperfusion (P=0.0004, compared with group I), and a reduction of plasminogen activator inhibitor (PAI) activity. Plasma degradation products of fibrinogen and fibrin increased parallel to t-PA activity, and levels were significantly higher at 45 min after graft reperfusion in group II compared with group I (P<0.04). Thrombin-antithrombin III complexes showed an identical steady increase in both groups, indicating that increased t-PA activity was not related to thrombin formation. A combination of increased endothelial release and reduced hepatic clearance may have caused the increased t-PA activity. The t-PA–associated destruction of fibrinogen and fibrin after graft reperfusion is consistent with the clinical signs of severe oozing often seen in this period. These observations may have important clinical implications for the treatment of bleeding in patients undergoing orthotopic liver transplantation.

Orthotopic liver transplantation has become an accepted and clinically useful treatment for patients with a variety of irreversible liver disease (*1*). The gradual improvements of the surgical technique, anesthesiologic management, and immunosuppressive therapy have contributed to an increase in the success rate and long-term survival (*1, 2*). The surgical operation however is an extensive procedure, which may be frequently associated with serious bleeding, requiring massive blood transfusions (*3*). Maintenance of surgical hemostasis may be seriously complicated by disturbances in the hemostatic system. Previous studies have suggested an important role of hyperfibrinolysis in the origin of bleeding complications during orthotopic liver transplantation (*4, 5*).

Recently, studies involving only a few subjects have shown that the hyperfibrinolysis during orthotopic liver transplantation may be related to increased plasma levels of tissue-type plasminogen activator (t-PA)* (*6, 7*), a key enzyme of the fibrinolytic system (*8*). Under normal physiologic conditions, t-PA activity in the circulation is low, due to its rapid inactivation by formation of complexes with PA-inhibitors (PAI), but t-PA activity may increase several fold after specific stimuli (*8*). Both t-PA and its major inhibitor (PAI-1) are produced and secreted by endothelial cells, whereas hepatocytes and blood platelets are additional sources of PAI-1 (*8, 9*). Elimination of t-PA from the blood is mainly regulated by the liver with a t½ of 3–5 minutes (*10*). Increased levels of t-PA during orthotopic liver transplantation probably result from a combination of increased endothelial release and decreased hepatic clearance during the anhepatic stage (*6*). The mechanisms underlying the increased release of t-PA and the role of PAI in the regulation of t-PA activity during liver transplantation however are still unknown.

Some investigators have suggested that hyperfibrinolysis in orthotopic liver transplantation may be secondary to disseminated intravascular coagulation (DIC) (*7, 11, 12*). Differentiation between secondary and primary fibrinolytic activity however has been difficult, mainly due to lack of appropriate laboratory tests. Lack of specific parameters has also hampered the assessment of the role of increased fibrinolytic activity in the actual breakdown of coagulation factors and the development of a bleeding diathesis. Especially under strongly hyperfibrinolytic conditions, fibrinogenolysis may also occur (*13*). Although increased serum levels of fibrin(ogen) degradation

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* Abbreviations: AU, arbitrary units; DIC, disseminated intravascular coagulation; ELT, euglobin clot lysis time; FbDP, fibrin degradation products; FgDP, fibrinogen degradation products; PAI, plasminogen activator inhibitor; TAT, thrombin-antithrombin III; TEG, thrombelastography; t-PA, tissue-type plasminogen activator; WBLT, whole blood clot lysis time.

products have been found during liver transplantation (*4, 11*), whether these were the result of fibrin breakdown or plasmin destruction of fibrinogen could not be determined.

In the present study, we examined the origin and clinical relevance of hyperfibrinolysis in orthotopic liver transplantation. Measurement of t-PA and PAI activity in combination with the separate quantitation of plasma degradation products of fibrinogen and fibrin enabled us to study the origin of hyperfibrinolysis and its role in the development of a systemic lytic state. Thrombin-antithrombin III (TAT) complexes were measured to study the role of clotting activation and thrombin formation in the origin of hyperfibrinolysis.

MATERIALS AND METHODS

Patients. Twenty-three adult patients who underwent their first liver transplantation at Presbyterian University Hospital between June 1 and 27, 1988 were observed prospectively. In an otherwise consecutive series, 3 patients, of whom more than 2 blood samples were missing due to technical errors, were omitted. The remaining 20 patients were categorized by pathological diagnosis as described previously (*14*). Five different diagnostic groups could be distinguished, as shown in Table 1. Orthotopic liver transplantation was performed by a standard technique, using a venovenous bypass in all patients (*15*). The surgical procedure can be divided into 3 stages. During the preanhepatic stage (stage I), the host liver is isolated. The anhepatic stage (stage II) begins with the clamping of the vessels of the native liver and ends with the completion of the vascular anastomosis of the graft liver. The postanhepatic stage (stage III) lasts from graft reperfusion to the end of surgery. Intraoperative blood loss was compensated by the transfusion of modified whole blood (from which platelets have been removed) or packed RBC and fresh frozen plasma in an approximate ratio of 1:1. In case of massive blood loss, a rapid infusion system was used, by which a mixture of packed RBC, fresh-frozen plasma, and Plasmalyte A was infused in a ratio of 1 U:1 U:250 ml. Platelets and cryoprecipitate were usually not given before stage III. All patients gave their informed consent for blood sampling during the operation, as part of the intraoperative patient care.

Intraoperative blood samples for hemostasis monitoring were collected from an arterial line. Blood (9 ml) was collected in 1 ml 0.13 mol/L trisodium citrate and immediately centrifuged at 2800×g for 10 min. Plasma was either directly used for testing or frozen at −70°C. Whole blood (0.36 ml) was used for thrombelastographic monitoring within 2 min after sampling. Blood samples were taken according to the following schedule: immediately after induction of anesthesia (BASE); 30 min before removing the liver (II-30); 5 min in the anhepatic stage (II+5); 5 min before graft reperfusion (III-5); 5 min after graft reperfusion (III+5); 45 min after reperfusion (III+45); 150 min after reperfusion (III+150), and at the end of the operation (END).

Assays. Standard hemostasis tests were performed using previously described methods (*16, 17*). Thrombelastographic monitoring of whole blood coagulation and fibrinolysis was performed using a Thromb Elastograph-D (Haemoscope Corporation, Morton Grove, IL). The whole blood clot lysis time (WBLT) was defined as the time between the maximum amplitude and the registration of complete lysis on the thrombelastographic recording (normal >150 min) (*18*).

Levels of t-PA activity (normal range, 0–1 IU/ml) and PAI activity were measured using chromogenic substrate methods (Coasets t-PA and PAI, Kabi Vitrum Hematology, Stockholm, Sweden). For the measurement of t-PA activity, 100 μl of plasma was acidified (pH 4.0–4.1) with 100 μl of acetate buffer and 20 μl of 20% acetic acid, both supplied in the assay kit. T-PA activity was determined by measuring the amidolytic activity of plasmin onto the chromogenic substrate S-2251, after incubation in the presence of plasminogen and human fibrin(ogen) fragments (*19, 20*). The fibrinolytic activity of t-PA was expressed in International Units assessed by calibration against the international standard of t-PA from human melanoma cells (lot 83/517, National Institute for Biological Standards and Control, London, UK). PAI activity was measured by adding 40 IU/ml t-PA to an equal volume of plasma. After incubation for 10 min at room temperature, samples were diluted with sterile water (1:80), and residual t-PA activity was determined as described above. PAI activity was expressed in arbitrary units (AU), defined as the amount that inhibits 1 IU of t-PA in 10 min (*21*) (normal, 0–40 AU/ml).

Two different sandwich-type enzyme-linked immunosorbent assays (Fibrinostika, Organon Teknika, Turnhout, Belgium) were used for the quantitation of plasma levels of fibrinogen degradation products (FgDP; normal <0.5 μg/ml) and fibrin degradation products (FbDP; normal <0.5 μg/ml). In both ELISAs, a monoclonal antibody, which reacts exclusively with FgDP and FbDP and not with intact fibrinogen or fibrin, is used as catching antibody. The FgDP ELISA contains a monoclonal tagging antibody that is specific for covalently bound fibrinopeptide A. Since fibrinopeptide A is split off during the activation of fibrinogen by thrombin, this ELISA tags only FgDPs that result from the plasmin-mediated destruction of fibrinogen (*22*). The FbDP ELISA gets its specificity for FbDP by using a monoclonal antibody that is elicited with D-dimer as immunogen (*23*).

TAT complexes (normal range, 1.0–4.1 μg/L) were measured by an ELISA (Behringwerke, Marburg, FRG), based on rabbit antibodies to human thrombin and antithrombin III, respectively (*24*). Samples with TAT levels exceeding the highest standard contained in the assay kit (60 μg/L), were diluted (1:2 or 1:4) in normal pooled plasma, which had been shown to have a TAT concentration of 1.2 μg/ml.

Statistical analysis. Statistical analysis was performed using the NPAR1WAY computer program of the Statistical Analysis System (SAS Institute Inc., Cary, NC). The significance of differences within and between groups were tested using the Wilcoxon rank-sum test and two-sample test, respectively. Values for $P<0.05$ were considered to be significant.

TABLE 1. Diagnosis and characteristics in 20 patients undergoing orthothopic liver transplantation

Diagnosis	No.	F	M	Age range
Postnecrotic cirrhosis	9	3	6	27–54
Primary biliary cirrhosis	5	5	–	27–60
Sclerosing cholangitis	3	1	2	29–41
Carcinoma	1	–	1	63
Miscellaneous[a]	2	1	1	22, 37
Total	20	10	10	22–63

[a] Two patients with Wilson's disease.

RESULTS

In all but 1 patient, slightly to severely increased fibrinolytic activity, as measured by shortening of the euglobulin clot lysis time (ELT; normal >120 min) or WBLT (normal >150 min), was found in at least 1 blood sample during the operation. Signs of hyperfibrinolysis were most frequent at the end of the anhepatic stage and early after graft reperfusion of the donor liver. Fibrinolysis was defined as minimal if the ELT was longer than 60 min or when the WBLT was longer than 90 min in all blood samples. Severe fibrinolysis was defined as being present when the ELT and WBLT were shorter than 60 and 90 min, respectively, in at least one of the intraoperative blood samples. According to these criteria, the patients were divided into 2 groups. Group I was formed by 7 patients with minimal fibrinolysis. Group II consisted of 13 patients with severe fibrinolysis. Comparison of the preoperative hemostasis parameters showed no significant differences between the 2 groups (Table 2). Both groups included patients with different diagnoses,

TABLE 2. Comparison of preoperative hemostasis profile in patients with minimal (group I) and severe (group II) intraoperative fibrinolysis

Variables	Reference values	Median (range)	
		Group I	Group II
Coagulation:			
PT (sec)	10.8–13.0	11.3 (10.4–21.2)	12.2 (9.7–21.2)
aPTT (sec)	26–34	36.0 (28.9–51.6)	42.9 (29.1–127)
ThT (sec)	13–18	17.1 (15.3–32.9)	22.0 (14.3–47.7)
Fibrinogen (mg/dl)	150–450	285 (159–460)	140 (85–350)
Factor II (%)[a]	50–150	66 (32–130)	38 (15–135)
Factor VIII (%)[a]	50–150	185 (130–300)	120 (82–280)
TAT complex (μg/ml)	1.0–4.1	8.0 (2.0–16.0)	4.3 (1.6–60.0)
Platelets (10^9/L)	150–450	81 (56–510)	118 (39–336)
Fibrinolysis:			
ELT (min)	>120	>120 (105–>120)	60 (15–>120)
WBLT (min)	>150	>150[b]	>150[b]
t-PA act. (IU/ml)	0–1.0	2.4 (0–6.0)	1.4 (0–8.0)
PAI act. (IU/ml)	0–40.0	18.5 (14.0–36.0)	18.8 (3.0–37.5)
FgDP (μg/ml)	<0.5	0.28 (0.20–3.0)	0.50 (0.20–2.6)
FbDP (μg/ml)	<0.5	0.30 (0.26–4.5)	0.84 (0.22–6.0)

[a] Percentage of pooled normal plasma.
[b] For all patients.

without an accumulation of any diagnostic group in either of the 2 groups.

Mean intraoperative levels of t-PA activity and PAI activity of group I and II are depicted in Figure 1. There were no significant changes in t-PA and PAI activity during the preanhepatic stage in both groups. In group I t-PA levels remained below 12 IU/ml during the rest of the operation in all patients. In group II t-PA activity increased after clamping of the vessels of the native liver, and levels were significantly higher at the end of the anhepatic stage (III-5), compared with group I (P=0.002). During reperfusion of the graft, t-PA levels increased sharply, resulting in a more than doubling at 5 min after reperfusion, compared with the values at 5 min before reperfusion (P<0.007). At this time t-PA activity in group II (65.1±8.5 IU/ml, mean ±SEM) was about 30 times higher than the preoperative value and more than 10 times higher than t-PA activity in group I (P=0.0004). Later in the postanhepatic stage, a rapid disappearance of t-PA activity was seen, and levels fell into the normal range (0–1 IU/ml) at the end of the operation in all but 2 patients. In these 2 patients t-PA levels were still moderately increased (3.4 and 10 IU/ml). In group II free PAI activity showed a pattern inverse that of t-PA activity, and only minimal PAI activity (1.1±0.7 AU/ml) was left at the peak of fibrinolytic activity, but levels increased during the later postanhepatic period in both groups.

Mean plasma levels of FbDP and FgDP in groups I and II are shown in Figure 2. Although an increase of FbDP and FgDP was seen in both groups, levels in group II were significantly higher in the postanhepatic period at 45 min after graft reperfusion when compared with group I (P<0.04). In group II the highest FgDP level (18.4±7.9 μg/ml) coincided with the peak in t-PA activity (II+5), whereas the maximum in FbDP (32.5±11.2 μg/ml) occurred somewhat later in the postanhepatic stage (III+45).

Mean levels of TAT complexes in group I and II are shown in Figure 3. An identical increasing pattern was seen in both groups, and at none of the time points were TAT levels significantly different between the 2 groups. Highest TAT levels were found during the postanhepatic stage, and levels were still above the normal upper limit (>4.1 μg/L) at the end of the operation in all patients.

Comparison of changes in arterial pH, pO_2, and blood pressure in group I and II are shown in Table 3. There was no evidence for a relationship between intraoperative changes in hemodynamics and the increase in t-PA activity. Periods of shock, as determined by a drop in blood pressure and pH, were found among both patients with high and low t-PA activities.

The intraoperative use of blood products is shown in Table 4. There were no differences in the use of cryoprecipitate and platelets between the 2 groups. However, intraoperative blood loss, as reflected by the total use of modified whole blood or packed RBC and fresh-frozen plasma, was significantly higher in group II than in group I (P<0.02).

DISCUSSION

In earlier studies we have found that increased fibrinolytic activity, as measured by the ELT or thrombelastography (TEG), occurs in about 80% of the patients undergoing orthotopic liver transplantation (*18, 25*). Fibrinolytic activity may increase during the anhepatic stage and is most often severe early after graft recirculation. A simultaneous decrease of plasminogen and α_2-antiplasmin, the main inhibitor of plasmin, has been found during this period, which supports the view of an active fibrinolytic process (*5*). However, the use of ELT and TEG in these studies did not allow an exact characterization of the fibrinolytic defect, and the origin and clinical relevance of the increased fibrinolytic activity have remained largely unclear.

In this study we found an extreme increase of t-PA activity, and concomitant decrease of PAI activity during the anhepatic and early postanhepatic period in patients with severe fibrinolysis, as measured by the ELT and TEG. Reduction in PAI activity can be explained by the formation of complexes with t-PA. After saturation of free PAI, a further increase of t-PA will result in the increase of free t-PA activity in the circulation (*9, 21*). Recently, Dzik et al. (*6*) and Palareti et al. (*7*) have reported a similar increase of t-PA during the anhepatic stage in a limited number of patients undergoing OLT. However,

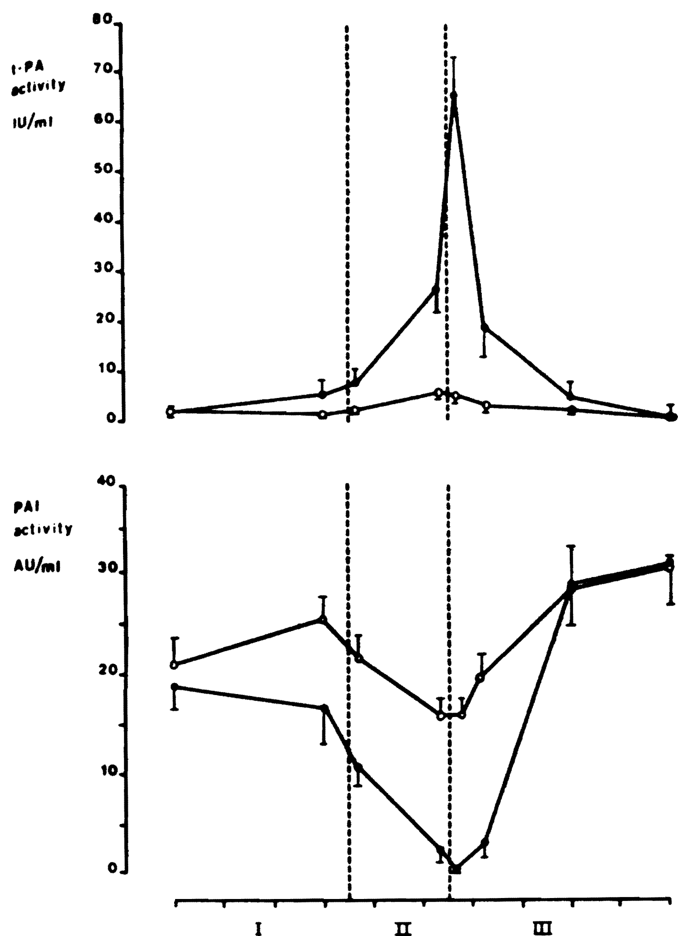

FIGURE 1. Intraoperative levels of t-PA activity and PAI activity (mean ±SEM) in patients with minimal (○—○, group I, n=7) and severe fibrinolysis (●——●, group II, n=13). Each tic on the abscissa indicates 1 hr. The area between the dotted lines represents the anhepatic stage (stage II).

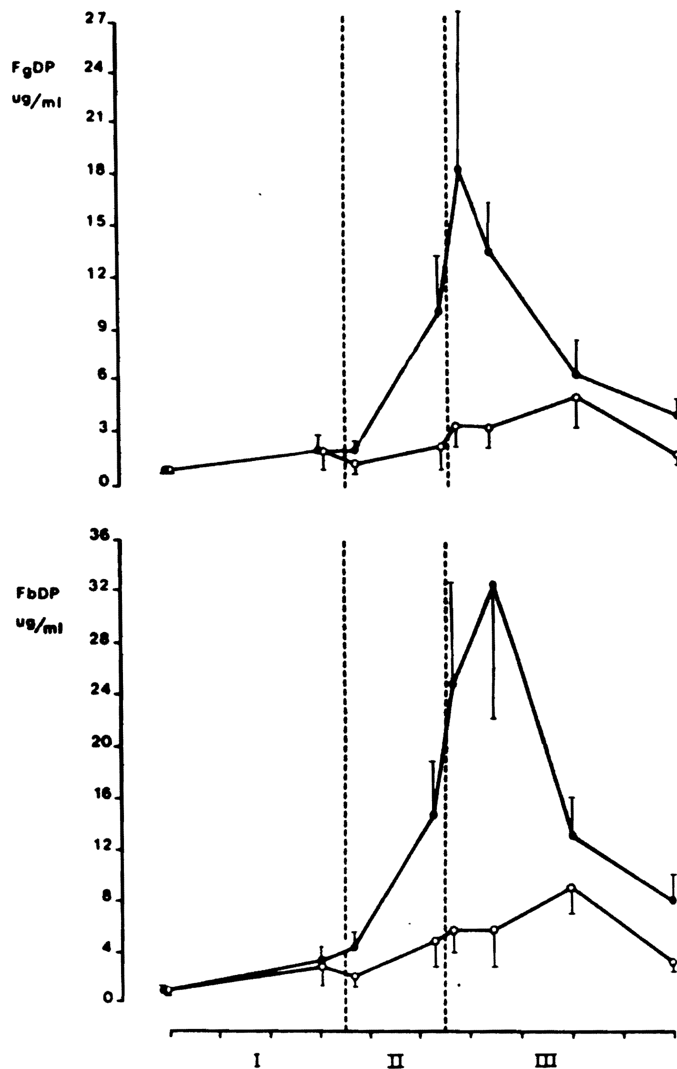

FIGURE 2. Intraoperative levels (mean ±SEM) of fibrinogen degradation products (FgDP) and fibrin degradation products (FbDP) in orthotopic liver transplantation. ○——○, group I: patients with minimal fibrinolysis (n=7); ●——●: Group II: patients with severe fibrinolysis (n=13). Each tic on the abscissa indicates 1 hr. The area between the dotted lines represents the anhepatic stage (stage II).

these studies did not show the explosive t-PA increase, occurring directly after graft reperfusion, as seen in our patients. Since we observed a rapid normalization of t-PA activity after its maximum, this peak could have been easily missed in the other studies if no blood samples were taken within 10 min after reperfusion.

The intraoperative course of t-PA activity suggests that there are 2 different mechanisms responsible for the increase of t-PA during orthotopic liver transplantation. The initial rise of t-PA during the anhepatic stage has been explained by a combination of increased release of t-PA and reduced hepatic clearance (*6*). This view is supported by the lack of fibrinolytic activation during auxiliary, heterotopic liver transplantation in which the native liver is not removed (*26, 27*). Dzik et al. (*6*) found that increase of t-PA during orthotopic liver transplantation may be associated with signs of shock. They suggested a mechanism of increased t-PA release due to hypotension and acidosis. We could not find any differences in blood pressure, arterial pH, or pO_2 in patients with high or low t-PA levels. Probably mechanisms other than changes in the arterial circulation may also attribute to an increased release of t-PA. Patients with liver disease are known to be prone to activation of their fibrinolytic system upon specific stimuli such as physical stress and exercise (*28, 29*). The surgical stress, with the manipulations and extensive trauma to the vascular bed and abdominal circulation therefore might have contributed to an increased release of t-PA.

The rapid increase during graft reperfusion suggests a second mechanism that is associated with the restoration of blood flow through the donor liver. In studies with pigs, we recently demonstrated that fibrinolytic activity in the hepatic venous outflow immediately after graft reperfusion is significantly higher than the fibrinolytic activity in the systemic circulation (Porte RY, Blauw E, Knot EAR, 1988, unpublished data). These data, in combination with the "explosive" t-PA increase after reperfusion in this study, strongly suggest an increased release of t-PA from the reperfused donor liver. Several factors, including vasoreactive agents, venous occlusion, anoxia, and thrombin, have been found to stimulate t-PA release from endothelial cells in in vitro and in vivo studies (*8, 30, 31*). It can be theorized that, during graft reperfusion and the subsequent restoration of normal portal blood flow, one or more of

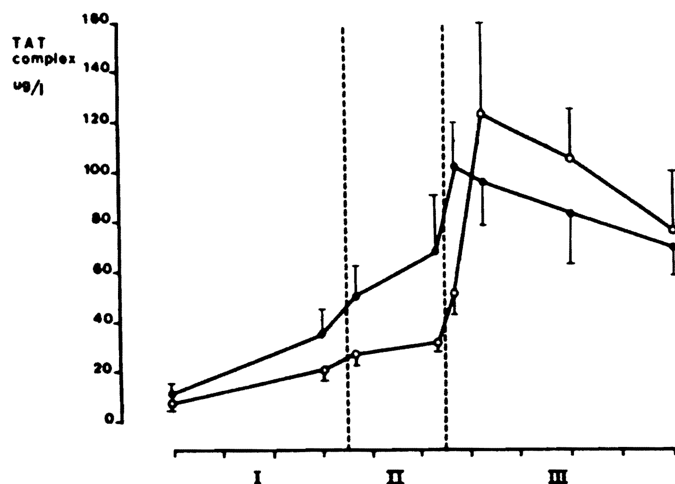

FIGURE 3. Intraoperative levels (mean ±SEM) of thrombin-antithrombin III (TAT) complexes in orthotopic liver transplantation. O——O, group I: patients with minimal fibrinolysis (n=7); ●——●, group II: patients with severe fibrinolysis (n=13). Each tic on the abscissa indicates 1 hr. The area between the dotted lines represents the anhepatic stage (stage II).

TABLE 3. Comparison of intraoperative hemodynamic changes in patients with low (group I) and high (group II) t-PA activity

Hemodynamics	Group I (n=7)	Group II (n=13)
At the end of stage II:		
Arterial pH[a]	7.37 (7.33–7.45)	7.40 (7.34–7.46)
Arterial pO_2 (mm Hg)	218 (113–311)	279 (122–397)
No. patients with a hypotensive period	1/7 (14%)	3/13 (23%)
Early in stage III:		
Arterial pH	7.31 (7.22–7.42)	7.32 (7.15–7.42)
Arterial pO_2 (mm Hg)	272 (150–486)	275 (114–531)
No. patients with a hypotensive period	1/7 (14%)	5/13 (38%)

[a] The pH and pO_2 are expressed in median (range).

TABLE 4. Intraoperative blood use in patients with low (group I) and high (group II) t-PA activity

		Group I (n=7)	Group II (n=13)
RBC[a] (U)	Mean	5	19
	Median (range)	4 (1–8)	10 (3–101)
FFP (U)	Mean	1	11
	Median (range)	0 (0–8)	4 (0–68)
RBC + FFP (U)	Mean	6	30
	Median (range)	4 (2–16)	10 (3–169)
Cryoprecipitate (U)	Mean	0	3
	Median (range)	0[b]	0 (0–12)
Platelets (U)	Mean	3	5
	Median (range)	0 (0–10)	8 (0–28)

[a] RBC includes packed red blood cells and modified whole blood.
[b] For all patients.

these factors, or leakage from ischemic damaged endothelial cells, contribute to an increase of t-PA. The normalization of t-PA activity during the late postanhepatic stage can be explained by the restoration of the normal hepatic clearance of t-PA after the implantation of a viable donor liver. Reduction of t-PA activity might have been enhanced by an increase of PAI toward the end of the operation, which is generally seen after major surgery and which is consistent with the behavior of PAI as an acute phase reactant (*32*).

Some investigators have suggested that hyperfibrinolysis in orthotopic liver transplantation may be secondary to thrombin formation during DIC (*7, 11*). For several reasons we do not believe that DIC is the main cause of t-PA increase during orthotopic liver transplantation.

First of all, in large series of patients, we previously found no evidence for a combined decrease of coagulation factors and inhibitors, as occurs during DIC (*24, 33*). Repeatedly negative findings regarding thromboembolic processes in histopathologic and clinical examination support the view that DIC does not play a clinically important role in OLT (*34, 35*).

Second, this study provided evidence for a thrombin-independent increase in t-PA during liver transplantation. Although TAT complexes, which are formed early during clotting activation by complex formation between thrombin and antithrombin III (*24*), increased steadily from the beginning of the operation and were still elevated at the end of the operation, t-PA increase was clearly limited to the anhepatic and early postanhepatic stage. Additionally, there was no difference in TAT increase in patients with high or low t-PA levels, indicating that the t-PA increase was independent of thrombin formation. The rise in TAT levels in our patients was most probably a sign of local clotting activation at the wound surface. Further studies, preferably with patients undergoing other major abdominal surgical procedures as control group, however, are necessary to establish the role of increased TAT levels in orthotopic liver transplantation.

Third, secondary fibrinolysis during DIC is probably a more local process that does not result in detectable increased fibrinolytic activity in the systemic circulation. In an analysis of 346 patients with DIC, Spero et al. (*36*) found evidence of systemic fibrinolytic activation, as demonstrated by shortened ELT or recalcified clot lysis time, in only 10% of the patients. Francis and Seyfert (*37*) recently demonstrated that although t-PA antigen is elevated in patients with DIC, detectable free t-PA activity is less frequently present than in hospitalized controls. These investigators concluded that PAI levels are also increased in DIC, leading to masking of the increased endothelial secretion of t-PA. This view is supported by experiments with cultured endothelial cells in which thrombin was shown to stimulate the release of both t-PA and PAI (*30*), resulting in no detectable net free t-PA activity despite increased levels of t-PA antigen. We observed a *40–50*-fold systemic increase of t-PA activity and a concomitant saturation of PAI, resulting in a decrease of free PAI activity during the period of hyperfibrinolysis. Another process than DIC therefore seems responsible for the increased t-PA activity in orthotopic liver transplantation. Why some patients develop a high increase of t-PA activity, whereas others do not, remains unclear. We could not find any difference in diagnosis, preoperative hemostasis profile, or intraoperative hemodynamics in patients with high or low intraoperative t-PA activity levels.

Irrespective of its origin, the extremely high t-PA levels during the early postanhepatic stage may be clinically important. Especially in this period, formation of fibrin and stable hemostatic clots is necessary to prevent or stop bleeding from the vascular anastomosis and the extensive wound surface. Although the systemic increase of t-PA activity had a transient character, the clinical effect may extend over a considerably

longer period, as t-PA may bind to fibrin and become incorporated in newly formed hemostatic clots, resulting in early lysis and delayed bleeding from fresh wounds (*38*). The role of t-PA in the development of a systemic lytic state was demonstrated in this study by the increase of plasma levels of both FbDP and FgDP in patients with high t-PA levels. This confirms earlier studies, which suggested a plasmin-mediated destruction of coagulation factors during orthotopic liver transplantation (*5, 33*). FgDPs are known to have an anticoagulant effect by inhibiting the polymerization of fibrin monomers into cross-linked fibrin (*39*). The occurrence of FgDPs in the circulation therefore may well be attributed to a further deterioration of the hemostatic function in patients undergoing orthotopic liver transplantation, as has been suggested before by Blecher et al. (*40*). The peak of FbDPs in the early postanhepatic stage is consistent with the clinical picture of delayed oozing and increased blood loss in this period (*18, 25*). We indeed found an significantly higher blood loss in patients with severe fibrinolysis (group II). However, these data should be interpreted with some reserve, as blood loss is influenced by multiple factors and larger series of patients are necessary to confirm this observation.

Since the primary increase in t-PA activity was associated with an active proteolytic destruction of fibrinogen, and fibrin and a high blood loss, the use of antifibrinolytic drugs seems justified in patients with life-threatening hemorrhages where active fibrinolysis is likely. To identify these patients, it is advisable to include at least 1 test method for the assessment of fibrinolytic activity in the intraoperative hemostasis monitoring in orthotopic liver transplantation.

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T CELL DEPLETION OF HUMAN BONE MARROW

COMPARISON OF CAMPATH-1 PLUS COMPLEMENT, ANTI–T CELL RICIN A CHAIN IMMUNOTOXIN, AND SOYBEAN AGGLUTININ ALONE OR IN COMBINATION WITH SHEEP ERYTHROCYTES OR IMMUNOMAGNETIC BEADS[1]

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The aim of this study was to compare the extent of in vitro T cell depletion and recovery of hematopoietic progenitor cells achieved with five methods of T cell depletion. Bone marrow samples from the same source were treated with monoclonal antibody Campath-1 (CP1) and human complement, XomaZyme-H65 (anti–T cell ricin A chain immunotoxin), or soybean agglutinin (SBA) alone or in combination with sheep erythrocytes (E_{AET}) or a cocktail of immunomagnetic beads (B) directly coated with anti-CD2, anti-CD3, or anti-CD8 monoclonal antibodies. Residual T cells were enumerated by limiting dilution analysis, E_{AET} rosetting, and proliferative responses to phytohemagglutinin. The results of this study demonstrated the following reductions in BM T cells as detected by limiting dilution analysis (mean % control): SBA+B (99.9%), SBA+E_{AET} (99.8%), CP1+C′ (99.4%), anti–T cell ricin A chain immunotoxin (99.0%), and SBA alone (94.2%). Neither PHA response nor enumeration of residual E_{AET} rosettes provided discriminating differences in the degree of T cell depletion by treatment method when T cell reductions exceeded 99.0% by LDA. These results demonstrate the ability of CP1+C′, XomaZyme-H65, and SBA plus sheep erythrocyte or magnetic bead depletion to achieve a greater than 99% reduction of BM T cells and the importance of limiting dilution analysis in defining differences in T cell numbers when depletion exceeded 99%.

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Pretransplantation depletion of donor T cells from rodent (*1, 2*) or human bone marrow grafts has produced significant reduction in the incidence and severity of acute graft-versus-host disease (*3–8*). A number of methods for in vitro T cell depletion (TCD)* have been evaluated, including the use of monoclonal antibody(s) (MoAb) + complement (*4–7*), soybean agglutination (SBA) plus sheep erythrocyte depletion (*8, 9*), elutriation (*10*), anti–T cell ricin (*11*) and ricin A chain immunotoxins (*12*), and immunomagnetic beads coated with anti–T cell MoAb (*13*). Methods for enumerating the extent of TCD have included E-rosette analysis, proliferative responses to phytohemagglutinin, and immunofluorescence analysis with anti–T cell MoAbs. However, quantitating residual BM T cells by E-rosette analysis, PHA response, and immunofluorescence with anti–T cell MoAb has not provided a consistent correlation between the number of residual T cells transplanted and the subsequent development or severity of GVHD (*14–16*).

* Abbreviations: B, beads; BFU-E, burst-forming unit-erythroid; CD, cluster designation; CP1, Campath-1; E_{AET}, 2-amino-ethyliosothiouronium-treated erythrocytes; HSA, human serum albumin; SBA, soybean agglutinin; TCD, T cell depletion.