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 thrombellastography, 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. 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 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 t1/2 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 fibrinolytic conditions, fibrinogenolysis may also occur (13).

* Abbreviations: AU, arbitrary units; DIC, disseminated intravascular coagulation; ELT, euglobin clot lysis time; Fdp, 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 prehepatic 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 posthepatic 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 packed RBC and fresh frozen plasma in an approximate ratio of 1:1. 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 (FDP; normal <0.5 µg/ml). In both ELISA, a monoclonal antibody, which reacts exclusively with FGDP and FDP 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 FGDP that result from the plasmin-mediated destruction of fibrinogen (22). The FDP ELISA gets its specificity for FDP 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 NPARiWay 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.

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.
significantly were found during the postanhepatic stage. and levels were still
when compared with group I
levels were still moderately increased (3.4 and
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mechanism, and at none of the time points were TAT levels
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creased sharply. resulting in a more than doubling at 5 min
reperfusion. compared with the values at 5 min before
May intraoperative levels of t-PA activity and PAI activity
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 in all the stages 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, P0₂, 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 α₁-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 Palairet et al. (7) have
reported a similar increase of t-PA during the anhepatic stage
in a limited number of patients undergoing OLT. However,
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 pO₂ 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 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

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**FIGURE 1.** Intraoperative levels of t-PA activity and PAI activity (mean ±SEM) in patients with minimal (O—O, 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. 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).
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 activity, 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 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 increase in 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, 31). 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.

REFERENCES

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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

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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 (CPl) and human complement, XozaMyme-H65 (anti-T cell ricin A chain immunotoxin), or soybean agglutinin (SBA) alone or in combination with sheep erythrocytes (SBA) 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<sub>AE</sub> 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<sub>AE</sub> (99.8%), CPl+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<sub>AE</sub> 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 CPl+C', XozaMyme-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%.

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) + 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; CPl, Campath-1; E<sub>AE</sub>, 2-aminoethylisothiouronium-treated erythrocytes; HSA, human serum albumin; SBA, soybean agglutinin; TCD, T cell depletion.