Fibrinolysis During Liver Transplantation in Humans: Role of Tissue-Type Plasminogen Activator

By Walter H. Dzik, Charles F. Arkin, Roger L. Jenkins, and David C. Stump

Human liver transplantation is frequently associated with a coagulopathy and bleeding diathesis developing during the anhepatic phase of surgery. The hemostatic defect has been attributed in part to accelerated fibrinolysis. In this study we evaluated changes in specific blood fibrinolytic parameters occurring in eight adult patients undergoing first-time orthotopic liver transplantation. Five of the eight patients experienced moderate to severe systemic fibrinolysis as reflected by α2-antiplasmin consumption and fibrinogen degradation with the concomitant appearance of fibrinogen degradation products. In association with these changes, an increase in tissue-type plasminogen activator (t-PA) activity and t-PA antigen levels was also observed. Fibrinolysis was most pronounced during the anhepatic phase of surgery and decreased after revascularization of the grafted liver. Three additional patients who underwent the same procedure manifested much less evidence of systemic fibrinolytic activation and had minimal elevation of t-PA antigen levels or activity. Urokinase-type plasminogen activator levels, although elevated in three patients, were disassociated from increased t-PA levels and concomitant systemic fibrinolysis. The operative course of those patients developing t-PA-associated fibrinolysis was characterized by shock, acidosis, generalized bleeding, and a need for substantially greater blood product support during surgery. These findings suggest that the observed fibrinolytic defect is related to increased circulating plasma levels of t-PA, presumably resulting from a combination of increased intravascular release and decreased hepatic clearance of t-PA. These observations may have implications for intraoperative therapy for the transplant-related coagulopathy and its associated bleeding.

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Orthotopic liver transplantation is performed with increasing frequency as a treatment for patients with a wide variety of serious liver diseases. The surgical procedure is technically demanding and can be associated with massive hemorrhage. Several factors contribute to the increased frequency of bleeding that occurs during liver transplantation. Excessive surgical blood loss may occur during removal of the recipient liver in patients with previous hepatobiliary surgery. Increased bleeding may also occur during the vascular anastomoses of the donor and recipient portal vein, inferior vena cava, and hepatic artery. In addition, bleeding from multiple collateral vessels may occur in patients with preexisting portal hypertension. Moreover, patients come to surgery with varying degrees of coagulopathy associated with end-stage liver disease. Finally, orthotopic liver transplantation has been associated with an additional coagulopathy developing during the anhepatic phase of surgery that is due, at least in part, to pathological fibrinolysis. In some patients this coagulopathy results in life-threatening bleeding; in other patients the effects are mild and less clinically relevant. However, the exact mechanism of the underlying fibrinolytic defect during the anhepatic phase of liver transplantation is unknown.

Tissue-type plasminogen activator (t-PA) is a potent activator of the fibrinolytic system in humans. Under normal physiological conditions it is present in the circulation at low levels (5 to 10 ng/mL). The activation of plasminogen by t-PA is markedly stimulated in the presence of fibrin as a result of their specific molecular interaction. The activator can be rapidly released into blood in response to a variety of stimuli such as exercise, venous stasis, vasoactive agents, anoxia, and acidosis. Physiological opposition to this profibrinolytic response normally occurs through the presence of a specific inhibitor and through rapid clearance of t-PA by the liver with a plasma half-life in humans on the order of three to five minutes. Because many of the stimuli for t-PA release might be expected to occur during liver transplantation and because the effects of t-PA might be further enhanced by interruption of its clearance during the anhepatic phase of surgery, we investigated its role in the development of systemic fibrinolysis during orthotopic liver transplantation.

MATERIALS AND METHODS

Sampling procedure. Before surgery patients provided informed consent to intraoperative blood sampling. Samples were obtained from nine consecutive first-time adult liver transplant patients, one of whom was excluded from study because of early intraoperative death resulting from bleeding from friable collateral vessels. The surgical procedure used has been described in detail elsewhere. Briefly, the vessels supplying the recipient liver are isolated and the recipient common bile duct ligated. Clamps are placed on the portal vein, the inferior vena cava above and below the liver, and the hepatic artery, after which the recipient liver is removed. The period of time from clamping the recipient vessels to completing the anastomoses of the graft vessels is referred to as the anhepatic phase of surgery. During this phase venous blood draining from the femoral and portal veins flows through a nonanticoagulated extracorporeal circuit bypassing the liver and returns to the heart through
an axillary vein. After subsequent anastomoses of the donor and recipient hepatobiliary vessels, the extracorporeal bypass circuit is removed.

The protocol for blood transfusion support during surgery was the same for each patient. Fresh-frozen plasma was transfused at an approximate ratio to packed RBCs of 1:2. Additional fresh-frozen plasma was transfused if the prothrombin time was prolonged to greater than 20 seconds (normal, 11.5 to 13.5 seconds). Platelet concentrates were transfused to maintain a platelet count of approximately 100,000/µL. Cryoprecipitate was transfused if the fibrinogen level fell significantly below 100 mg/dL (Table 1).

Samples for fibrinolytic analysis were collected from arterial blood in 0.01 mol/L sodium citrate anticoagulant and immediately centrifugated at 1,500 g for ten minutes. Cell-free plasma was immediately frozen at −70°C until testing.

Arterial blood samples for the measurement of t-PA activity were also collected in citrate and were acidified within seconds of collection by the addition of 200 µL glacial acetic acid (pH 3.9) to 400 µL whole blood. After centrifugation at 1,500 g for ten minutes, one part of acidified plasma was mixed with an equal part of a neutralizing concentration of goat anti-t-PA antibody (background sample). A second aliquot of acidified plasma was mixed with an equal part of a similar concentration of nonspecific goat immunoglobulin (test sample).

**Assay methods.** Fibrinogen was measured by a clotting rate assay.16 α2-Antiplasmin activity was determined by measurement of the residual activity of exogenously added plasmin.15 Functional plasminogen was assayed by the method of Friberger and Knog.18 Levels of fibrinogen/plasminogen degradation products (FDP) were measured by hemagglutination inhibition of fibrinogen-tanned RBCs.17 D-Dimer levels were measured with the Dimer test enzyme immunoassay (ELA) kit (American Diagnostica, Greenwich, CT). Levels of t-PA antigen16 and urokinase-type plasminogen activator (u-PA) antigen16 were measured by monoclonal antibody–based, enzyme-linked immunosorbent assay. t-PA activity was determined by amidolytic activity in the presence of plasminogen and soluble fibrin toward the chromogenic substrate H-0-norleucyl-hexahydroxytosyl-lysine-p-nitroanilide diacetate hydrochloride (Spectrozyme PL, American Diagnostica). Specific t-PA-associated activity was determined after subtraction of background activity and neutralization with anti-t-PA antibody.20

### RESULTS

The clinical characteristics of the eight patients studied are summarized in Table 1. The patients' ages ranged from 19 to 58 years. A preoperative coagulopathy of variable severity was present in each patient except no. 6. Each patient had significant symptomatic liver disease.

On the basis of measured parameters of coagulation obtained during surgery, the patients fell into three groups. Three patients (nos. 4, 5, 6) had minimal evidence of fibrinolysis (Table 2). Two patients (nos. 2, 3) had moderate fibrinolysis (Table 3). Three additional patients (nos. 1, 7, 8) developed severe fibrinolysis (Table 4). Figure 1 shows the operative course of a representative patient (no. 5) with minimal evidence of fibrinolysis. This patient showed stable levels of fibrinogen, α2-antiplasmin, and plasminogen and no elevation of t-PA antigen levels or activity. FDP and D-dimer levels were not elevated.

In contrast, the operative course of a representative patient (no. 8) with severe fibrinolysis is shown in Fig 2. This patient developed marked consumption of α2-antiplasmin, degradation of fibrinogen, elevation of FDP and D-dimer levels, and a decline in functionally active plasminogen levels. These abnormalities were most pronounced during the anhepatic phase of surgery. Associated with the fibrinolysis was a marked elevation of t-PA antigen levels and t-PA activity.

Results of coagulation testing for patients with minimal, moderate, and severe fibrinolysis are shown in Tables 2, 3,
and 4, respectively. In each table the operation is divided into three phases. Phase 1 (average, 3.7 hours) extends from the beginning of surgery until clamping of the hepatic vessels. The second phase of surgery is the anhepatic phase (average, 83 minutes) during which the liver is surgically removed from the patient. Phase 3 (average, 5.0 hours) begins with revascularization of the new graft and extends until the end of surgery.

In patients with severe fibrinolysis (Table 4), coagulation was most abnormal during the anhepatic period. During this phase the median fibrinogen level fell to 13% of the original level. This was associated with a 32-fold rise in the median level of FDPs, consumption of α2-antiplasmin to 5% of normal, and a median rise in t-PA activity to 40-fold above normal. After revascularization of the new graft, systemic fibrinolysis progressively improved. By the end of surgery, t-PA activity had returned to normal. Similar but less severe abnormalities occurred in the patients with moderate fibrinolysis (Table 3). The patients with systemic lysis demonstrated levels of t-PA activity during the anhepatic phase that ranged from 120 to 930 IU/mL (normal, 0 to 20 IU/mL) with corresponding levels of t-PA antigen from 30 to 416 ng/mL (normal, 5 to 10 ng/mL). All five patients were found to have substantially elevated levels of FDPs with peak titers associated with the marked decline in fibrinogen during the anhepatic period. D-Dimer levels, however, accounted for <1% of the FDP levels, thus lending further evidence for systemic fibrinogenolysis.

In contrast, patients with minimal lysis (Table 2) had stable levels of fibrinogen that were 95% of original during the anhepatic phase. Levels of α2-antiplasmin and plasmin-
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Fibrinogen were also stable during surgery, and neither t-PA activity nor antigen levels became elevated. FDP and D-dimer levels were uniformly low except in one patient at one sampling time.

Levels of u-PA antigen were measured in parallel with t-PA antigen. In contrast to t-PA, elevation of u-PA did not occur in patients with systemic lysis (Table 4). Moreover, u-PA antigen levels did not increase in any patient during the anhepatic phase of surgery. Instead, levels of u-PA antigen progressively declined during the procedures.

The intraoperative transfusion requirement for each patient is shown in Table 5. Patients with fibrinolysis required substantially greater blood product support during surgery. To better understand potential differences between patients with and without fibrinolysis, we reviewed the intraoperative course of each patient. A summary of the results of serial intraoperative measurements of BP, duration of hypotension during the anhepatic phase, arterial blood pH, and total graft ischemia time is shown in Table 5. The five patients with t-PA-associated fibrinolysis were characterized by periods of shock and acidemia not observed in the patients without systemic fibrinolytic activation. The times of peak t-PA elevation corresponded closely to the times of the lowest measured arterial blood pH during surgery. The three patients who did not exhibit elevation of t-PA activity were not hypotensive during the anhepatic phase.

DISCUSSION

Although orthotopic liver transplantation is known to be associated with a predisposition to an intraoperative hemostatic defect and frequent massive hemorrhage, the pathophysiology of this coagulopathy has not yet been fully characterized. Previous studies by von Kaulla et al did describe a marked shortening of the euglobulin lysis time characterized. Previous studies by von Kaulla et al did characterize the importance of coagulopathy during liver transplantation. Fibrinolysis, intravascular coagulation, and the persistence of exogenous heparin used at that time for graft perfusion were proposed; however, further clarification of the fibrinolytic abnormalities were not obtained.

More recently Lewis et al studied intraoperative coagulation changes in 67 adult first-time liver transplant recipients. A shortening of the euglobin lysis time was found in 85% of cases, with rapid lysis (45 minutes) in 64%. Mean factor VIII:C levels declined from 1.97 U/mL to 0.63 U/mL.

In this study we measured alterations in blood coagulation by using currently available assays for specific components of the fibrinolytic system. Although the number of patients in our study was small, the data suggest that three factors need to be present in combination for pathological fibrinolysis to be manifested in patients undergoing orthotopic liver transplantation. Increased plasma levels of t-PA, decreased hepatic clearance of t-PA by the surgically absent or severely ischemic liver, and surgical generation of fibrin for t-PA activation were each present in the patients with moderate to severe fibrinolysis. In contrast, patients with minimal fibrinolysis (Table 2) showed no evidence of increased levels of t-PA despite an equivalent period of decreased t-PA clearance (anhepatic period) and a shared surgical stimulus for the formation of fibrin. Experience with infusions of recombinant t-PA for the treatment of ischemic heart disease where plasma levels of t-PA can reach 5 to 10 µg/mL has typically not been associated with such profound fibrinolysis and blood loss. However, the importance of high levels of fibrin, such as would be present with extensive surgical manipulation, in potentiating fibrinolysis after infusion of recombinant t-PA has been recently demonstrated in a rabbit model. It is possible that the absence of similarly high levels of fibrin in patients with acute myocardial infarction treated with t-PA represents a less potent stimulus for systemic activation of plasminogen by t-PA, even when it

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Table 5. Intraoperative Characteristics of Eight Adult Patients Undergoing First-Time Liver Transplantation

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Blood Use in OR</th>
<th>Hypotension During Anhepatic Phase</th>
<th>Arterial Blood pH</th>
<th>Total Graft Ischemia Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBC</td>
<td>FFP</td>
<td>Pts</td>
<td>Cryo</td>
</tr>
<tr>
<td>Minimal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>14</td>
<td>18</td>
<td>0</td>
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<tr>
<td>5</td>
<td>10</td>
<td>19</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>17</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>50</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>149</td>
<td>99</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>61</td>
<td>80</td>
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<tr>
<td>7</td>
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<td>131</td>
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<td>20</td>
</tr>
<tr>
<td>8</td>
<td>205</td>
<td>89</td>
<td>68</td>
<td>34</td>
</tr>
</tbody>
</table>

Abbreviations: OR, operating room; FFP, fresh-frozen plasma; Pts, platelets; Cryo, cryoprecipitate.
is present at ten- to 20-fold higher levels than those measured during the anhepatic phase of liver transplantation.

Because all liver transplant patients undergo both an anhepatic phase and also develop some degree of fibrin formation during the surgical procedure, the stimuli for increased release of t-PA into the circulation would appear to be the most important factor in the development of the lytic state during this surgery. It is unknown why some liver transplant patients develop increased t-PA activity and others do not. Although plasminogen activator inhibitor (PA-I) may play a role in patients with low levels of t-PA release during surgery, our measurements of t-PA activity document that the high levels of t-PA seen in some patients exceed inhibition by PA-I. Although it is unclear which factors stimulate excess t-PA release during surgery, these data would suggest that the development of shock and acidemia or its treatment with vasopressors may be important. Desamino-o-arginine vasopressin (DDAVP) is known to promote the release of t-PA from vascular endothelial cells. Whether pharmacological doses of vasopressor agents used to treat shock in our patients could also promote t-PA release is not certain. Previous studies of patients undergoing massive transfusion in the setting of trauma and major surgery have demonstrated the importance of shock in the development of disseminated intravascular coagulation. The findings of this study suggest that shock may also play a relevant role in the development of fibrinolysis during liver transplant surgery.

The complete absence of any hepatic clearance of t-PA is perhaps unique to liver transplantation. The importance of hepatic blood flow on fibrinolytic activation was demonstrated in the same rabbit model used to study the effects of fibrin on the activation of fibrinolysis after t-PA infusion; however, a marked reduction in hepatic blood flow to 12% of normal was required to double the circulating half-life of t-PA, thus suggesting that a decreased hepatic clearance of t-PA might only be clinically relevant in situations involving severe interruption of hepatic blood flow. The importance of the hepatic circulation in protecting liver transplant patients from the systemic effects of endogenously released t-PA is further suggested by the rapid improvement in pathological fibrinolysis that parallels the decline in circulating t-PA levels after revascularization of a viable donor liver. Primary graft failure, technical problems with reperfusion, and persistent hepatic ischemia have all been clinically recognized to be associated with continued hemorrhage during liver transplantation. A persistent inability of the damaged graft to clear circulating t-PA may in part explain these clinical observations.

Finally, the presence of surgically induced fibrin formation alone is not likely to be a sufficient stimulus for the fibrinolysis observed in these patients because even extensive surgical procedures are generally not accompanied by such a profound degree of fibrinolysis. Thus it seems clear that the unique combination of increased plasma levels of t-PA, decreased clearance of t-PA, and availability of fibrin can coexist in the surgical setting of hepatic transplantation and together would appear capable of stimulating the systemic activation of fibrinolysis.

The pathological fibrinolysis observed in some patients during liver transplant surgery not only destabilizes fibrin clot formation but may also have detrimental effects on platelet function during surgery. Recent work has suggested that plasmin is able to disrupt glycoprotein Ib on the platelet surface, however, the exact role of platelet dysfunction during liver transplant surgery remains to be defined.

In contrast to the marked elevation of t-PA that occurred during the anhepatic phase of surgery, u-PA antigen levels did not follow a similar pattern. Although there was a mild-to-moderate increase in some patients at the beginning of surgery, the levels of u-PA tended to decrease during surgery and were uninfluenced by the anhepatic phase or periods of shock and acidosis. These data not only document a dissociation in the physiological release of t-PA and u-PA but also demonstrate that acute pathophysiological changes in systemic fibrinolytic activity are more influenced by t-PA than u-PA during liver transplantation.

It should be emphasized that orthotopic liver transplantation is a technically demanding surgical procedure. Vascular abnormalities and surgical difficulties can compound both disseminated intravascular coagulation and altered fibrinolysis. Although our study involves a relatively small number of patients, it clearly suggests that fibrinolytic activation mediated in large part by t-PA does occur in some patients during liver transplantation. We propose that these patients release excessive t-PA that is not cleared from the circulation because of both severe liver failure and surgical interruption of hepatic blood flow during the anhepatic phase of the operation. These increased levels of t-PA are further stimulated by readily available fibrin, which results not only in clot-specific fibrinolysis but eventually the development of a systemic lytic state. The net result of this process would logically be inadequate hemostasis with the potential for generalized bleeding.

Such a mechanism, if supported by further studies, could have both prognostic and therapeutic implications for the use of pharmacological inhibitors of the fibrinolytic system, particularly during the anhepatic phase. Studies are currently underway to investigate not only the factors promoting the release of t-PA during liver transplantation but also the potential for specific pharmacological inhibition of fibrinolysis during surgery.

It is not clear to what extent such pathophysiology may occur in clinical settings other than liver transplantation. Certainly patients with severe trauma or other surgical conditions characterized by a high availability of fibrin, elevated circulating t-PA levels, and severe hepatic ischemia could be likely candidates for t-PA-associated fibrinolysis and its predisposition to bleeding. Recognition of this process coupled with the availability of assays to better characterize changes in the fibrinolytic system should provide a basis for the improved understanding of similar disorders in other clinical settings.

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REFERENCES

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WH Dzik, CF Arkin, RL Jenkins and DC Stump