POSSIBLE ROLE OF EXTRACELLULARLY RELEASED PHAGOCYTE PROTEINASES IN COAGULATION DISORDER DURING LIVER TRANSPLANTATION

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Orthotopic liver transplantation is frequently associated with a complex coagulation disorder, influencing the outcome of the procedure. In this respect, disseminated intravascular coagulation (DIC) had been suggested to be of causative importance for bleeding complications after reperfusion of the liver graft. In 10 consecutive patients undergoing orthotopic liver transplantations, we studied the occurrence of two phagocyte proteinases of different origin in the graft liver perfusate and in systemic blood during the operation, as well as their effects on hemostasis. As compared with plasma samples taken at the end of the anhepatic phase, highly significant increases of cathepsin B and thrombin-antithrombin III complexes (TAT), as well as highly significant decreases in antithrombin III, protein C, and C_1-inhibitor were observed in graft liver perfusate. Von Willebrand factor and fibrinogen were slightly decreased, whereas the elastase-alpha$_1$ proteinase inhibitor complexes (EPI) were elevated. In plasma the activity of cathepsin B remained unchanged during the prereperfusion phases, but immediately after revascularization of the graft this cysteine proteinase increased. The EPI showed a gradual increase in plasma during the preanhepatic and anhepatic phases but a more pronounced increase in the reperfusion phase. In parallel with the rise in these two proteinases TAT increased and the activities of antithrombin III and C_1-inhibitor in plasma decreased after reperfusion. At 12 hr after revascularization plasma levels of TAT, antithrombin III, and C_1-inhibitor had returned to the prereperfusion ranges, whereas cathepsin B and EPI were significantly above the baseline levels. These observations are consistent with the hypothesis that extracellularly released lysosomal proteinases may play a role in the development of a DIC-like constellation, including thrombin formation after revascularization of the liver graft. For the first time we could prove the occurrence of phagocyte proteinases in graft liver perfusate and evaluate the importance of these proteinases for the understanding of the pathophysiology leading to bleeding complications in patients undergoing orthotopic liver transplantation.

Orthotopic liver transplantation (OLT)* has become an established method in end-stage liver diseases (*1*). In spite of refined surgical techniques and improved perioperative management bleeding remains a critical problem (*2*). Previous studies have suggested hyperfibrinolysis (HF) and disseminated intravascular coagulation (DIC), predominantly in the late

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* Abbreviations: AT III, antithrombin III; C_1I, C_1-inhibitor; DIC, disseminated intravascular coagulation; EPI, elastase = alpha$_1$ proteinase inhibitor; HF, hyperfibrinalysis; OLT, orthotopic liver transplantation; TAT, thrombin = antithrombin III complexes; vWF, von Willebrand factor.

anhepatic phase and after reperfusion, to be of causative importance for bleeding complications during OLT, influencing blood transfusion requirements and clinical outcome of the procedure (*3–5*). Whereas HF may be due to an increased release and reduced clearance of activators of the fibrinolytic system (*4, 5*), the mechanisms leading to DIC are poorly understood (*3, 6*). In this respect proteinases extracellularly released from phagocytes of the liver graft may enter the circulation and play a role in the deterioration in hemostasis.

In the present study we demonstrate for the first time the occurrence of the cysteine proteinase cathepsin B from macrophages and the serine proteinase elastase from polymorphonuclear granulocytes in the graft liver perfusate and evaluate the role of these proteinases in the disturbances of hemostasis observed during OLT.

MATERIALS AND METHODS

Patients. Ten consecutive patients undergoing primary OLT at the University Hospital Rudolf Virchow in Berlin between August 19 and November 23, 1989 were enrolled in this prospective study. OLT was performed by a standard technique, using a venovenous bypass (*7, 8*). Intraoperative blood loss was compensated for by the transfusion of packed RBC (median 6.5; range 4–26 units) and fresh-frozen plasma (7; 1–39) but neither platelets nor concentrates of hemostatic factors.

Samples of systemic blood were collected from an arterial line after induction of anesthesia (sample 1), 5 min before (2) and 10 min after (3) removal of the liver, 5 min before (4) as well as 5 min (5), 15 min (6), 1 hr (7) and 12 hr (8) after reperfusion of the liver graft. In addition, a sample of the perfusate released from the liver graft was taken from the graft liver vein at the end of the flushing procedure, when the graft liver was flooded with the recipient's systemic blood.

Blood was collected in plastic syringes prefilled with 1/10 volume of 0.13 M/L trisodium citrate and centrifuged at 3000 ×g for 20 min. The plasma was separated and immediately frozen at −70°C until testing.

Assays. Fibrinogen was estimated by a clotting assay according to Clauss (*9*). The activities of antithrombin III (AT III), protein C, and C_1-inhibitor (C_1-I) were measured using kits from Behringwerke, Marburg, Germany. Antigen levels of von Willebrand factor (vWF; Boehringer Mannheim, Mannheim, Germany), thrombin-antithrombin III complexes (TAT; Behring Werke, Marburg, Germany) and elastase in complex with alpha-1-protease inhibitor (EPI, as previously described [*10*]) were determined by ELISA. Cathepsin B was measured by its enzymatic activity against the fluorogenic substrate Z-Phe-Arg-NMec (*11*).

The Wilcoxon rank-sum test and two-sample test were used to test the significance of differences within and between groups, respectively. *P* values of ≤0.05 and ≤0.01 were considered to be significant and highly significant, respectively.

RESULTS

In the perfusate vWF (NS) and fibrinogen were lower than in the corresponding plasma specimens collected 5 min before reperfusion (Table I). The activities of AT III, protein C, and C_1-I showed a highly significant reduction in the perfusate. In contrast, levels of TAT, as well as EPI (NS) and cathepsin B were increased.

In the course of the operation procedure, plasma levels of cathepsin B remained unchanged until the reperfusion phase (Fig. 1). At that time a highly significant further increase immediately after revascularization was evident. Thereafter cathepsin B in plasma remained significantly elevated as compared with the prereperfusion levels until the end of the observation period. Plasma levels of EPI slowly rose until the end of the anhepatic phase, but showed a significant increase after reperfusion. Having reached the maximum 60 min after revascularization, plasma concentrations of EPI decreased, but did not reach the baseline level (sample 1) during the study period.

TABLE 1. Some parameters of hemostasis (median, range) in graft liver perfusate as compared with systemic blood sampled 5 min before reperfusion of the graft in 10 OLT

Parameter	Systemic blood	Perfusate	*P* value
Fibrinogen (g/L)	1.9; 1.1–2.7	1.5; 0.5–2.2	0.018
vWF (%)	307; 94–405	275; 36–446	0.222
AT III (%)	70; 35–89	38; 26–56	0.004
C-1-I (%)	77; 55–107	66; 27–92	0.006
Protein C (%)	24; 8–54	12; 0–27	0.003
TAT (ng/ml)	30; 15–270	105; 10–324	0.005
EPI (ng/ml)	322; 231–766	584; 136–904	0.102
Cathepsin B (U/L)	0.2; 0.1–0.2	30.2; 7.0–75.8	0.002

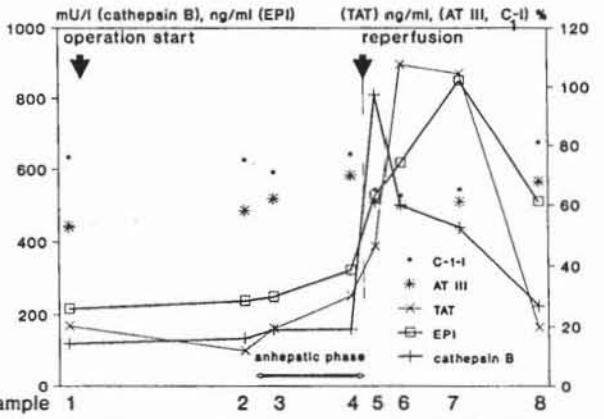

FIGURE 1. Some parameters of hemostasis (medians) in the course of 10 OLT.

TAT slowly increased during the anhepatic phase but showed a highly significant rise with maxima 15–60 min after reperfusion as compared with the values at the end of the anhepatic phase, followed by a decrease to the baseline level 12 hr after reperfusion.

Activities of AT III and C_1-I remained unchanged until the beginning of the anhepatic phase. Thereafter a slight increase—significant for AT III—was observed. Reperfusion of the liver resulted in a significant decrease in both protease inhibitors as compared with sample 4, with minimum levels 15–60 min after reperfusion. At the end of the observation period prereperfusion levels were reached again.

DISCUSSION

In earlier studies of hemostasis in OLT we and others found signs of increased fibrinolytic activity—as measured by euglobulin lysis time, thrombelastography, or plasmin-antiplasmin complexes—and increases in different types of plasminogen activators peaking in the late anhepatic and very early reperfusion phases (*4, 5*). On the other hand laboratory signs of DIC—as measured by increases in TAT, fibrinmonomers, as well as decreases in AT III, fibrinogen, and protein C—rapidly develop after revascularization and persist for hours (*3, 4, 6*). The increase in oozing at the operation area and the occurrence of severe bleeding both observed exclusively postreperfusion prompted us to study the role of the graft liver perfusate in the derangement of the hemostatic balance in OLT more extensively.

Our results for the first time demonstrate the presence of the cysteine proteinase cathepsin B from macrophages and of the serine proteinase elastase from polymorphonuclear granuloytes in liver graft perfusate, and correlate the plasma levels of these proteinases with the coagulopathy in OLT.

Flushing of the liver graft does not remove all of the conservation fluid as shown by the decrease in the coagulation proteins vWF and fibrinogen in the perfusate as compared with the amount of the same proteins in the systemic circulation. On the other hand highly significant reductions in the activities of the proteinase inhibitors AT III, protein C, and C_1-I, and an increase in TAT—highly significant, too—were measured in the perfusate as compared with the systemic circulation (Fig. 1, sample 4). These findings suggest prothrombin activation in the graft liver circulation.

The release of cathepsin B, an effective thiol endopeptidase without an adequate inhibitor in human plasma (*11*) from the graft is not only reflected by its excessive elevation in the perfusate, but also results in a significant and sustained increase in cathepsin B activity in postreperfusion plasma. In addition to a possible direct role of cathepsin B in the activation and consumption of plasma factors in patients undergoing OLT, this proteinase may be envisaged as an indicator for the simultaneous extracellulary release of other lysosomal proteinases from macrophages.

The slow increase of EPI during the anhepatic stage may either reflect a decreased clearance of the enzyme-inhibitor complex or an ongoing inflammation response of PMN-granulocytes entering the operation field (*12*). On the other hand the rise in plasma levels of EPI after perfusion of the graft indicates a release of elastase from PMN granulocytes entering the preserved liver or—less likely—from granulocytes sticking in liver graft vessels. Elastase cleaves several coagulation factors and inhibitors in vitro (*13*), but in healthy individuals $alpha_1$-proteinase inhibitor controls the proteolysis by elastase. In severely pathologic states this inhibitor may be rendered ineffective by oxygen radicals released together with elastase by PMN granulocytes (*14*). The increase in EPI occurring in patients with severe trauma or sepsis had been correlated not only to the decrease of plasma factors, such as antithrombin III, clotting factor XIII, and $alpha_2$-macroglobulin, but also to the clinical severity and outcome of the disease (*11*, *15*).

The increase in the two lysosomal proteinases measured is closely paralleled by the plasma levels of TAT (+ fibrinmonomers and D-dimers; not shown), as well as by decreasing activities of AT III and C_1-I after revascularization of the graft, suggesting intravascular thrombin generation in the early reperfusion period.

Thus systemic proteolysis by the extracellularly released lysosomal proteinases of different cellular origin elastase and cathepsin B—or other mediators released in parallel from the same cells—is probably involved in the pathophysiology of intravascular activation and consumption of coagulation factors, which may result in a DIC-like state during the reperfusion phase in OLT.

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