Soluble Thrombomodulin Antigen as a Marker for Endothelial Damage During Liver Transplantation


Orthotopic liver transplantation (OLT) is now being performed with improved survival rates in a high number of patients suffering from different liver diseases. Despite better control of blood loss, hemorrhages still occur, particularly after reperfusion. Problems arising from defective hemostasis during major abdominal surgery are a major risk for patients with terminal chronic liver disease. Liver transplantation, most often performed in such patients, differs from operations because the recipients start with a diseased liver and end up with a healthy liver, the function of which, however, is compromised by preservation damage.

Thrombomodulin (TM), an integral glycoprotein on the surface of endothelial cells, serves as a receptor for thrombin. Thrombin bound to TM greatly reduces procoagulatory and platelet-stimulating effects but activates the zymogen, protein C. Activated protein C together with protein S inactivates two blood coagulation cofactors, factor Va and factor VIIIa, and indirectly stimulates fibrinolysis. Thus, TM plays an important role as an anticoagulant protein on the blood vessel wall. Immunohistochemically, TM has been found to be mainly present on endothelial cell surfaces of blood and lymphatic vessels in all organs except the brain. A smaller from of TM, the soluble thrombomodulin (sTM), has been isolated from human blood and urine. The structure of sTM is not known but is thought to be similar to the soluble protein obtained after proteolytic modification of TM with elastase—a cleaved form of tissue TM with loss of part of the transmembrane domain, and the cytoplasmatic tail. Therefore, sTM in plasma appears to be derived from injured endothelial cells or to be proteolytically cleaved from TM by proteases.

In vitro, sTM has been shown to be a marker of endothelial damage and several previous clinical studies have shown that plasma levels of sTM are increased in various diseases associated with endothelial cell damage or proteolytic activity on the endothelial cell surface, including DIC, adult respiratory distress syndrome (ARDS), thromboembolic disease, thrombotic thrombocytopenic purpura, diabetes mellitus with microangiopathy, systemic lupus erythematosus (SLE), and chronic myelogenous leukemia. Because they are usually associated with vascular endothelium alterations, TM plays an important role as an anticoagulant protein on the blood vessel wall. However, in the context of liver transplantation, the understanding of pathophysiology of TM in the coagulation-fibrinolysis equilibrium is still in its infancy. There are only few reports on sTM in liver transplantation. In orthotopic liver transplantation, both platelet and leukocyte activation as well as prothrombin activation are suspected of being caused by damaged endothelial cells in the grafted liver. In this study, plasma sTM levels as an endothelial marker were measured in the course of 11 consecutive liver transplantation. Samples were taken at nine different time points perioperatively as well as the perfusate released from the graft outflow vein during the flushing procedure.

MATERIALS AND METHODS

A total of 11 patients were enrolled in this study. They consisted of four males and 7 females, with an average age of 15.1 years (range, 2 to 67 years). Eight pediatric cases were living-related-donor liver transplantation; the remaining three adult patients received cadaveric allograft transplantations. The most common indication for liver transplantation was biliary atresia (7 patients), followed by postnecrotic cirrhosis (2 patients), glycogen storage disease (1 patient), and Budd-Chiari syndrome (1 patient). Orthotopic liver transplantation was carried out by established surgical techniques, without a venovenous bypass. Packed red blood cells, whole blood, and fresh frozen plasma, but neither platelets nor concentrates of hemostatic factors, were substituted to compensate for intra- and postoperative blood loss. Belzer UW-CSS solution (DuPont) was used during cold storage of the graft liver.

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This work was supported by Grant NSC86-2314-B183-019 from the National Science Council, Taiwan, Republic of China. Address reprint requests to Chao-Long Chen, MD, Liver Transplant Program, Chang Gung University and Chang Gung Memorial Hospital, Kaohsiung Medical Center, 123, Ta-Pei Road, Niao-Sung County, Kaohsiung, Taiwan.
Blood samples were taken from the arterial line at the following time points:

A. Preanhepatic
   T1: After induction of anesthesia and before the start of surgery
   T2: 5 Minutes before the beginning of the anhepatic phase

B. Anhepatic
   T3: 15 Minutes after the beginning of the anhepatic phase
   T4: 5 Minutes before reperfusion

C. Postanhepatic
   T5: 5 Minutes after reperfusion
   T6: 15 Minutes after reperfusion
   T7: 60 Minutes after reperfusion

In addition, a sample of perfusate released from the liver graft during the flushing procedure was also collected.

D. Postoperative
   T8: Posttransplant day 1
   T9: Posttransplant day 3

Blood samples were drawn into 0.11 mol/L sodium citrate (9:1), placed immediately on melting ice, and centrifuged within 30 minutes (2000g, 15 minutes). Plasma was kept frozen at −70°C until tested. Plasma TM antigen was measured using a micro-enzyme-linked immunosorbent assay (ELISA) (Diagnostica Stago, Paris, France); a micro-ELISA plate coated with monoclonal antibodies bound sTM in the sample.

RESULTS
Soluble Thrombomodulin Measurements

The mean sTM level before the operation in the 11 patients was 17.16 ng/mL. It was 2.1 times of the normal controls (range 4.05 to 13.32 ng/mL). During the preanhepatic phases, no significant change in mean TM levels occurred. It was slightly elevated at the end of the anhepatic phase, but immediately after graft reperfusion there was a significantly higher level—1.95-fold—in relation to sample T3 (15 minutes after the beginning of the anhepatic phase), with a P value of .001. The mean level of sTM in perfusate after revascularization of the graft was significantly above (27.4-fold) the level before reperfusion (Fig 1).

Postreperfusion sTM and Early Liver Enzyme Release

There was a correlation of arterial postreperfusion sTM level with maximum GPT within the first 24 postoperative hours.

DISCUSSION

The higher level of sTM before operation as compared with the normal population results from repetitive tissue damage and regeneration as well as decreased hepatic sTM clearance in patients with end-stage liver disease. The increase of sTM during the anhepatic phase may reflect further reduced clearance in the absence of the liver.

The peak levels of sTM after reperfusion are caused at least in part by a sudden release from the graft liver. Probably, endothelial cell damage in the graft during cold ischemic time and reperfusion injury plays a major role. A high level of elastase protease inhibitor (EPI) complexes, cathepsin B, thrombin-antithrombin complexes, and tumor necrosis factor after reperfusion have been reported,19 and a contributing role of extracellularly released phagocyte proteases in endothelial cell damage and degradation of membrane-bound TM in capillaries of the graft liver is thus possible. Endothelial cell damage in the host could also cause an increase in plasma TM levels.

Because endothelial cell damage in reperfusion injury is believed to have an impact on the primary graft function, sTM values should reflect early liver enzyme release. Our results also showed a good correlation of arterial postreperfusion sTM level with maximum GPT within the first 24 postoperative hours. However, in all patients, both the GPT levels and the sTM levels returned to normal in 2 weeks.

CONCLUSIONS

The higher plasma level of sTM in patients with end-stage liver disease and the accumulation of sTM in plasma after anhepatic phases during OLT suggest that the liver plays an important role in TM clearance in humans. The high levels
of sTM in the plasma and perfusate in the early reperfusion phase during OLT strongly indicate the massive release of sTM into the bloodstream immediately following reperfusion. It reflects considerable endothelial cell damage to the graft liver vascular bed upon reperfusion of the graft. As high postoperative sTM levels are associated with high liver enzyme release, this study emphasizes the importance of endothelial damage in the pathogenesis of reperfusion injury in a clinical setting.

REFERENCES