Hemostasis and the diseased liver

A study on hemostatic disorders in liver disease and liver transplantation

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Hemostase en de zieke lever

Een studie naar hemostase afwijkingen bij leverziekten en levertransplantaties

Proefschrift

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List of abbreviations

α_2-AP	α_2-antiplasmin
ALAT	Analine aminotransferase
aPTT	Activated partial thromboplastin time
ASAT	Aspartate aminotransferase
AT-III	Antithrombin-III
DDAVP	1-Desamino-8-D-Arginine vasopressine
DIC	Disseminated intravascular coagulation
EACA	ϵ-aminocaproic acid
ECLT	Euglobulin clot lysis time.
ELISA	Enzyme-linked immunosorbent assay.
FbDP	Fibrin degradation products
FgDP	Fibrinogen degradation products
FFP	Fresh frozen plasma
HLT	Heterotopic partial liver transplantation
NT	Normotest
OLT	Orthotopic liver transplantation
PAI	Plasminogen activator inhibitor
PC	Packed cells
PHR	Partial hepatic resection
PT	Prothrombin time
RBC	Red blood cells
SF	Soluble fibrin
t-PA	Tissue-type plasminogen activator
TAT	Thrombin-antithrombin-III complex
TT	Thrombotest
UW	University of Wisconsin
VVB	Veno-venous bypass

Chapter 1

Hemostasis in liver disease and liver transplantation

a review of literature

Contents

1.0 Introduction

The liver plays a major role in the control of hemostasis and as a result hemostatic disturbances are detected in a majority of the patients with liver disease [1,2]. The incidence of bleeding in patients with liver cirrhosis has been estimated to be 32% and is sometimes life-threatening [3]. Bleeding may occur from esophageal varices or ulcers or may be due to hemostatic disorders. Variceal bleeding appeared not to be related to impairment of hemostasis [4]. The hemostatic problems in liver disease are both complex and multifactorial and depend on the balance between hepatic synthesis and clearance of coagulation and fibrinolysis proteins and their inhibitors, the presence or absence of dysfibrinogenemia and a thrombocytopenia or -pathy. Despite extensive research in this field, the pathogenesis of hemostatic disorders in liver disease is still not fully elucidated. In this review on hemostasis in liver diseases, the following issues will be discussed:

- The normal hemostasis system
- Hemostasis & liver disease
- Hemostasis & (liver) surgery
- Hemostasis & liver transplantation

1.1 The normal hemostatic mechanisms

Under normal conditions, blood circulates through the vasculature without appreciable thrombus formation or hemorrhage [2,5]. The hemostatic mechanism is a dynamic balance between coagulation (fibrin formation) and fibrinolysis (fibrin resolution) [6]. It primarily protects the integrity of the vascular system, so that in case of injury the vascular walls are healed and their functions restored and secured. Secondly fibrin provides a matrix for tissue repair. Regulation of the hemostasis process ultimately depends upon interrelations between the vascular endothelium, the platelets in the circulating blood as well as the processes of blood coagulation and fibrinolysis.

If vessel wall injury occurs, platelets adhere to the subendothelial collagen[3]. During adhesion of platelets, several factors are secreted by the platelets, which induce platelet aggregation. During this process of platelet adhesion and aggregation the coagulation cascade is activated. Activation of coagulation factors

proceeds in an explosible fashion by a series of consecutive enzyme reactions with multiple feed-back loops (figure 1). Ultimately thrombin is generated with the conversion of fibrinogen to fibrin monomers, which polymerize and are cross-linked by factor XIII to form a stable clot. The conversion of prothrombin into thrombin is catalysed by activated factor X, in the presence of calcium ions and a negatively charged phospholipid. Traditionally two independent coagulation pathways are distinguished; the extrinsic (cellular) cascade and the intrinsic (humoral) cascade, which both results in activation of factor X. Data are now available that this distinction may be not completely correct, as many interrelation exist between the two systems.

The extrinsic coagulation pathway is initiated by complex formation between tissue factor, exposed by the injured cells, and factor VII. This complex activates factor X in the presence of factor Va, calcium and phospholipids.

The intrinsic pathway proceeds via activation of factors XII, XI, and IX. The initiating mechanism is still a matter of discussion, but negatively charged surfaces play an important role in this process. Prekallikrein and high molecular weight kininogen are also involved. Factor VIIIa acts as co-factor of factor IXa and enhances the rate of factor X activation by orders of magnitude. An alternative pathway of activation of factor IX is provided by the factor-VII-tissue-factor-calcium complex, that is also capable of factor X activation via activation of factor IX.

Obviously the induction of coagulation proceeds in highly buffered system, in which the effect of activated components is balanced by the presence of high concentrations of inhibitory proteins, such as antithrombin-III, heparin cofactor-II, protein C and protein S, which exert their regulating influence at different levels during the process.

Fibrin, the end product of blood coagulation has only a temporary function. During tissue repair, fibrin is proteolytically degraded by plasmin. The process of breakdown of fibrin is called fibrinolysis. If significant amounts of free plasmin are present also the degradation of fibrinogen may occur (fibrinogenolysis). Plasmin is formed from the inactive zymogen plasminogen through proteolysis by plasminogen activators. Endogenous plasminogen activators are, tissue-type

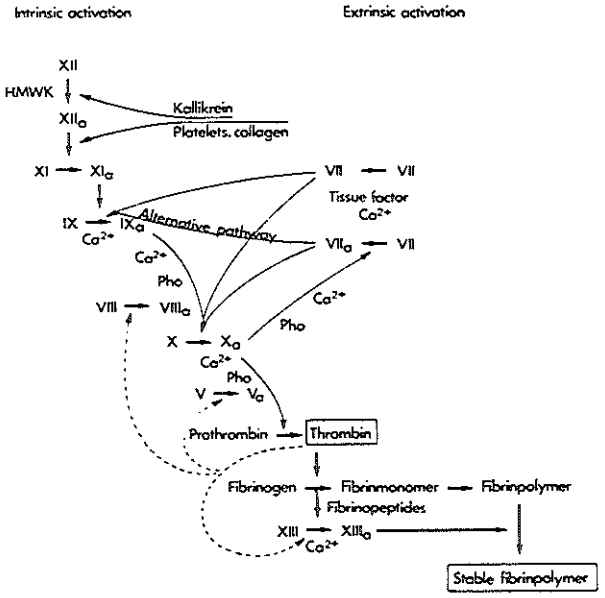

Fig 1 Schematic presentation of the coagulation cascade. A more extensive description is given in the text. HMWK = High molecular weight kininogen

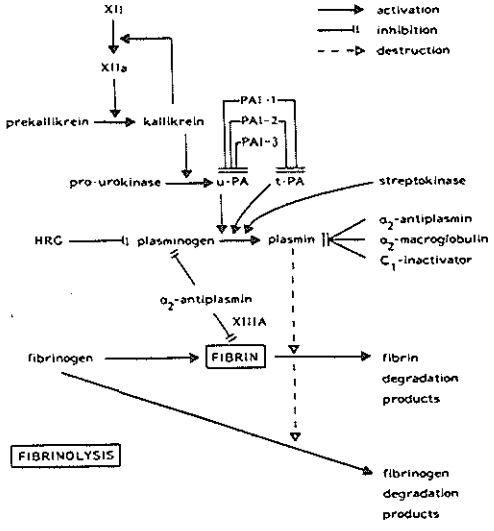

Fig 2 Schematic presentation of the fibrinolytic system. A more extensive description os given in the text. HRG = Histidine-rich glycoprotein, PAI = plasminogen activator inhibitor, t-PA = tissue-type plasminogen activator inhibitor, u-PA = urokinase-type plasminogen activator inhibitor.

plasminogen activator (t-PA), pro-urokinase, urokinase and a factor XII-dependant activator.

The normal coagulation and fibrinolytic systems are depicted in figure 1 & 2.

1.2 Hemostasis and liver disease

Liver disease is often associated with abnormalities in the hemostatic system, including a low platelet count, low levels of clotting factors and increased levels of fibrin(ogen) degradation products [2,7,8]. Many of these abnormalities can be attributed to the impairment of liver function itself; low levels of coagulation proteins can result from decreased synthesis or loss to extravascular spaces [9], a decreased platelet count to pooling in the spleen or alcohol-related bone marrow suppression [10,11] and high levels of fibrin(ogen) degradation products to impaired hepatic clearance or to degradation of fibrinogen in ascites [9]. In addition, an acquired dysfibrinogenemia has been described in 50-78% of patients with liver cirrhosis [12,13]. Moreover, several investigators have suggested that disseminated intravascular coagulation (DIC) and/or increased fibrinolysis contribute to the hemostatic abnormalities in liver disease [14].

1.2.1 Disseminated intravascular coagulation

Disseminated intravascular coagulation is a pathological stimulation of the coagulation cascade resulting in intravascular coagulation, consumption of coagulation proteins and platelets, which may lead to a hemorrhagic state [13]. A compensatory fibrinolysis develops. The similarity between coagulation defects in liver cirrhosis and DIC has prompted several investigators to suggest that DIC contributes to the coagulopathy of cirrhosis [15-18]. Assessing its contribution to hemorrhage is difficult since it is superimposed on a coagulation system already compromised by undersynthesis of key factors and -inhibitors. The pathogenesis of DIC in liver disease is multifactorial and includes the release of tissue thromboplastin from necrotic liver cells [16], the absorption of endotoxins from the intestine into the portal system [19,20], reduced clearance of activated coagulation proteins and reduced synthesis of their inhibitors (e.g. antithrombin-III). The concept of DIC as an important feature in liver failure is supported by some

pathophysiological data such as:

(1) The increased catabolism of radiolabelled fibrinogen, plasminogen and prothrombin and the reversal of this condition by heparin administration [17,21-23]

(2) Administration of antithrombin-III to patients with liver cirrhosis and patients with antithrombin-III deficiency normalized the half-life of labelled fibrinogen [24], thus suggesting that low levels of antithrombin-III in liver disease contributed to the development of DIC.

(3) The observed failure of replacement therapy in normalizing the clotting parameters unless heparin is simultaneously administered [25].

Others have refuted these evidences; the reversal of the reduced half-life of radiolabelled fibrinogen was not valid, since the injected fibrinogen was contaminated with other radiolabelled proteins and the heparin effect was uninterpretable because the radioactive count of the labelled fibrinogen was at times of measurement too close to the background count [26]. An autopsy series of 184 cases of patients with acute and/or chronic liver disease demonstrated the presence of microthrombi in more than three organs in only four patients, suggesting that the remaining 180 patients did not have DIC [27]. Others, in turn, have questioned such findings, suggesting that either fibrinolysis during life or postmortem fibrinolysis may impair detection of microthrombi [28,29].

Whether or not DIC occurs in severe liver insufficiency and whether this mechanism plays a significant role in the pathogenesis of the hemorrhagic syndrome still remains a controversial issue. There is however, general agreement that patients with liver disease are more susceptible to DIC than patients with an intact liver function [15,30]. When a cirrhotic patient becomes septic or hypotensive, DIC may develop rapidly.

1.2.2 Fibrinolysis

Accelerated fibrinolysis is a recognized complication in liver disease, which may contribute to the bleeding complications in these patients. Accelerated lysis of incubated blood clots from cirrhotic individuals was already reported by Goodpasture in 1914 [31], and subsequently confirmed by others [32-35]. Accelerated

fibrinolysis is often multifactorial in origin. The liver is not only an important site of clearance of circulating plasminogen activators, but also synthesizes the main plasmin inhibitor α_2-antiplasmin [35]. Patients with liver disease are known to be prone to activation of their fibrinolytic system upon specific stimuli, such as physical stress, venous stasis, nicotinic acid and 1-desamino-8-D-arginine vasopressin (DDAVP) infusion and exercise [36]. Bleeding has been shown to be related to periods of enhanced fibrinolysis. Francis et al have showed that augmented fibrinolytic activity as measured by the whole blood clot lysis time, predisposed to soft tissue hemorrhage after trauma [37,38]. They also found a trend towards increased intracranial bleeding in patients with enhanced fibrinolysis. The increase in fibrinolytic activity was however not found by all investigators [39-41]. In most of these studies accelerated fibrinolysis was found in some, but not all patients [42].
Although increased fibrinolytic activity in liver disease occurs as a distinct entity, part of it may also be due to secondary fibrinolysis as a response to DIC [14,43].

1.2.3 Thrombocytopenia and -pathy.

Liver disease is frequently associated with platelet abnormalities, that may be either quantitative or qualitative. Thrombocytopenia has been reported to occur in 37 to 77 % of patients with liver cirrhosis [44,45]. It may be caused by decreased survival because of splenic sequestration [46,47]; consumption in DIC [14]; platelet associated IgG in chronic active hepatitis [48,49] or antibodies in alcoholic cirrhosis [50]. Furthermore impaired production may be an associated factor, due bone marrow depression. Bone marrow depression can be observed for various reasons;

- Folate deficiency, resulting from low dietary intake and lowered storage capacity of the liver [44,51].
- Viral hepatitis, which is a rare but life-threatening complication [52].
- Toxic effect of heavy alcohol ingestion [53-56]

Platelet dysfunction may be observed in approximately 50 % of the patients with chronic liver disease [44]. Platelet function may be abnormal either due to a reduction in the numbers of the larger, more hemostatically active platelets [57] or an altered cholesterol/phospholipid ratio, leading to reduced availability of archidonic acid for prostaglandin synthesis [58].

1.3 Hemostasis and (liver) surgery

1.3.1 Surgery in general

Surgery in general imposes on the patient a situation of hypercoagulability, probably through a combination of tissue damage and the release of hormones and cytokines, which affect hemostasis [59,60]. In response fibrinolysis is activated by a rise in t-PA activity. Patients with impaired liver function requiring surgical procedures are especially at increased perioperative risk for hemostatic complications as the abovementioned hemostatic changes are superimposed on an already compromised hemostatic system and therefore impose on these patients a greater hazard for bleeding or thrombotic complications.

It is furthermore known from literature that surgery and particularly general anesthesia, which reduces the hepatic blood flow by 30-50% during induction, exacerbates pre-existent liver failure [61].

1.3.2 Surgery on the liver

Surgical procedures on the liver generally include liver suture after trauma or liver resection. Liver trauma may be complicated by sequelae after immediate bleeding. Late bleeding may also occur, whereby the leakage of bile (containing fibrinolytic activity) at the site of trauma may play a role. Complications to the trauma, such as bile peritonitis may also in themselves impose hemostatic changes.

A limited resection of the liver is associated with hemostatic changes, similar to those encountered during surgery in general (see above), and thus may serve as an example of the ordinary response to surgery [60]. Marked alterations in hemostasis occur with an extensive resection of the liver, but clinical hemostatic problems are seldom encountered [60]. The functioning liver tissue must be reduced for more than 90% before serious derangement of liver function develops. However, because the frequent coexistence of liver cirrhosis in patients requiring liver resection, a preoperative assessment of the hepatic reserve is important [62]. When partial hepatectomy is contemplated, the severity of the underlying disease as well as the ability of the liver to regenerate must be carefully considered. An indicator of the hepatectomy tolerance may be the Child-Pugh classification.

1.4 Hemostasis in liver transplantation

1.4.1 Historical background

The first known efforts at experimental orthotopic liver transplantation of the liver were made by Cannon of Los Angeles in 1956 [63]. It was a very short report, without title and description of methods. The animals did not survive the operation. In 1958 the first programs for orthotopic transplantation of the canine liver were initiated [64-66]. The first attempt to replace a human liver was made at the University of Colorado on March 1 in 1963 by Starzl. The patient died as did 4 others during the next seven months [67,68]. In 1963 and 1964 other unsuccessful attempts at liver replacement were reported from Boston and Paris [69,70]. Although these achievements sparked worldwide interest, a long-term survivor was not obtained until 1967 [71]. One-year survival rates were poor (approximately 30%), until 1980 when cyclosporin was introduced as the principal immunosuppressive agent [72]. Cyclosporin (as well as technical advances and improved patient management) ushered in survival rates of 65% from 1980 to 1984 and currently many centers report one-year survival rates of 80% or better [73]. Liver transplantation is nowadays an accepted treatment for end-stage liver disease.

The liver can also be transplanted as an extra (auxiliary) organ at an ectopic site. This concept originated from work by Welch and coworkers [74] and is theoretically attractive because the recipient liver is left in situ, avoiding the laborious removal of the cirrhotic liver, which is often cemented to the diaphragm and posterior abdominal wall, owing to previous inflammation [75]. The first HLT in man was performed in 1964 [76], one year after the first human OLT by Starzl [77]. Of the 50 patients, who underwent operations for auxiliary heterotopic liver transplantation, only 2 survived more than a year. An auxiliary liver transplantation that truly prolonged life was first achieved in 1972 [78]. In 1980 the 29-month survival of an adult recipient was reported in Paris [79]. As OLT became increasingly successful, the interest in HLT waned. The reasons for failure of HLT included a lack of space in the peritoneal cavity for the graft, atrophy of the graft due to a lack of portal-blood inflow, and venous congestion of the graft [80]. After extensive experimental work, a human HLT program was started in Rotterdam [81]. The main features of the technique used are a graft reduced in size by partial hepatectomy

and provided with an arterial and portal blood inflow while venous drainage was established in an area with low pressure. The HLT program has given to date good clinical results in end-stage liver disease [81-83]. In a report describing the results of a comparative study between OLT and HLT, no difference was found in morbidity and mortality between OLT and HLT, although patients treated with HLT were in a more advanced stage of their disease as compared with the patients who underwent OLT. Successful transplantation resulted in a rapid regeneration of the liver graft with atrophy of the host liver [84]. In one patient transplanted for (sub)acute liver failure, regeneration of the host liver has been reported [83].

1.4.2 Liver transplantation and blood loss

In spite of all refined surgical techniques, improved perioperative management and immunosuppressive regimen, massive blood loss remains a serious problem in liver transplantation. In the first published report of the European Liver Transplant Registry, bleeding complications were the most frequent cause of death during the operation and in the first postoperative week [85]. There is a well recognized correlation between the amount of red blood cells transfused intraoperatively and the subsequent postoperative morbidity and mortality [86,87].

A major difference between OLT and HLT is that the recipient's liver is left in situ during HLT. As this may have a serious impact on the perioperative blood loss, the causes for blood loss in OLT and HLT will be discussed separately.

1.4.2.1 Orthotopic liver transplantation

Conventionally OLT is divided into three stages. Stage I (preanhepatic stage) begins with the induction of anesthesia and ends with the occlusion of blood flow to the patient's liver. Stage II (anhepatic phase) continues until the donor liver is reperfused by the patient's circulating blood. Stage III (postreperfusion period) is from the moment of reperfusion until closure of the surgical incision. In table 1 reasons for perioperative blood loss according to the intraoperative stages are summarized.

Stage I: Most patients come to surgery with varying degrees of coagulopathy associated with end-stage liver disease. In adult patients it was found that the

Table 1

Reasons for perioperative blood loss according to the operative phases

Stage I (preanhepatic period)	Preoperative coagulopathy Removal of the host liver - Previous abdominal surgery - Portal hypertension with collaterals - Presence of ascites Renal function
Stage II (anhepatic period)	Development fibrinolysis and/or DIC - Lack of clearance of activated fibrinolytic and coagulation factors - Activation during VVB
Stage III (postreperfusion period)	Hyperfibrinolysis - Release of t-PA from the graft - Release of humoral substances that activate fibrinolysis (cytokines) - Disseminated intravascular coagulation - Release of thromboplastic material - Local clotting activation on damaged endothelium Release of heparin(-like) substances Trapping of platelets in graft Graft failure

synthesis function of the host liver, as reflected by preoperative coagulation profile, is significantly correlated with intraoperative blood loss and survival [86,88,89]. Blood loss and survival are also associated with the diagnosis of liver disease, as patients with a parenchymal liver disease tend to have more severe clotting abnormalities than patients with a cholestatic liver disease [87,90,91]. In attempts to optimize the preoperative hemostatic functions, preoperative transfusion of plasma products and platelet concentrates as well as plasmapheresis have been performed [92,93]. Although these measures led to a reduction of the use of blood products, they have not been examined in a controlled study. The presence of DIC in donors did not adversely affect graft function or patient outcome [94].

Others have related pretransplant recipient parameters to intraoperative blood loss and found that a urinary sodium excretion of <10 mmol and a serum sodium of < 132 mmol/l were highly predictive of blood loss of > 10L [88].

Furthermore excessive surgical blood loss may occur during the removal of the host liver, especially in patients with previous hepatobiliary surgery [88]. Previous abdominal surgery, resulting in adhesions has been recognized as a risk factor for severe bleeding and a high incidence of mortality in adults [72,87,95,96]. In addition bleeding from collaterals may occur in patients with portal hypertension [97]. The presence of ascites could also be considered as an important risk factor for high intraoperative blood requirements [98].

Stage II and III: Hemostatic changes specifically associated with the transplantation procedure itself may develop. The most striking abnormalities occur late in the anhepatic period and become more marked after reperfusion [99]. These disturbances appeared to be a major contributing factor to a high blood loss. In the past decennia many studies have examined the pathogenesis of the hemostatic changes in orthotopic liver transplantation. Signs of hyperfibrinolysis (a), DIC (b) or both have been reported [90,100-105].

(a) Fibrinolysis

Several authors have speculated that increased fibrinolysis is the most important factor in the origin of hemorrhage [106,107]. An acute fibrinolytic reaction was the most prominent finding in the first recipient of OLT [67,108]. In this patient uncontrollable bleeding developed immediately after revascularization of the hepatic graft and led to death a few hours later. A marked shortening of the euglobulin clot lysis time and rapid clot lysis was demonstrated with the thrombelastogram. Since then, fibrinolysis has been recognized during liver transplantation. In recent series severe fibrinolysis was found in 80% of the patients undergoing OLT [90,101,106].

Recent studies have demonstrated that the increased fibrinolytic activity is related to increased levels of tissue-type plasminogen activator [101,109]. This enzyme is a important activator of the fibrinolytic system. Under normal physiologic conditions it is present in the circulation at low levels (5 to 10 ng/ml) [110]. It is released from endothelial cells into the circulation upon several stimuli, such as stress, venous

stasis, vaso-active agents, anoxia and acidosis [111-113]. It is cleared by the liver with a short half-life of 3-5 minutes [114,115]. Increased levels of t-PA during OLT could result from a combination of increased endothelial release and decreased hepatic clearance. Despite extensive research, the mechanism of increased t-PA activity during orthotopic liver transplantation is still not fully elucidated. In several studies t-PA levels were found to increase during the anhepatic period [99,116], which has been ascribed to lack of hepatic clearance. Haemodynamic changes during the veno-venous bypass, have been suggested as an alternative explanation for the increased t-PA activity during the anhepatic period. These changes may induce t-PA release from the vascular endothelium, via components, such as catecholamines, histamine, serotonin, bradykinin and vasopressin [116]. It has, for instance, been known for a long time that fibrinolytic activity is increased during cardiopulmonary bypass [117].

Others make a strong case for an "explosive" increase in t-PA activity after reperfusion and have suggested that t-PA is released from the ischemically damaged graft upon reperfusion [106,118]. The demonstration that activated protein C neutralizes plasminogen activator inhibitor (PAI) and thus has a pro-fibrinolytic effect, has suggested a possible (additional) role of protein C in the increased fibrinolysis [119].

It is still a point of discussion whether fibrinolysis is mostly primary in origin or secondary to DIC. Differentiation between primary and secondary fibrinolysis has been difficult, mainly due to inappropriate tests [101,106]. Recently Porte et al measured thrombin-antithrombin-III complexes, an indirect indicator of thrombin generation, during OLT and found that these complexes increased steadily towards the end of the operation, whereas the increase in t-PA activity was limited to the anhepatic and postreperfusion period. This suggested a thrombin-independent t-PA release [101].

(b) Disseminated intravascular coagulation

Already in the 1960s, it was suggested that DIC contributed to the coagulation abnormalities encountered during liver transplantation [28,102,103]. A simultaneous decrease in platelets, fibrinogen, antithrombin-III and clotting factors and the

elevation of fibrin degradation products has been observed, akin to findings in DIC. Although improvements in graft preservation techniques, surgical and anesthetic procedures and a more stringent substitution scheme have led to a reduction of the coagulation changes, it is still controversial whether DIC contributes to the bleeding problems or not. Theoretical pathogenetic mechanisms for DIC include the lack of clearance of (clot-promoting) activated clotting factors during the anhepatic period and the release of thromboplastic material and/or humoral substances from the revascularized graft upon reperfusion. Furthermore local intravascular coagulation can be activated by exposed subendothelium of the ischemically damaged graft.
The presence of DIC was made plausible by experiments where samples were taken from both the arterial inflow and venous outflow of the recirculated liver graft and an arterio-venous gradient of platelets was found across the graft [120,121]. In turn, attempts to counteract this phenomenon by administration of heparin have not been successful and even have led to uncontrollable bleeding [103,122]. Furthermore, histopathologic examinations of the liver grafts did not identify microthrombi. Others, however have questioned such findings, suggesting that postmortem fibrinolysis may impair detection of microthrombi [28,123].
Recent insights in the humoral regulation of hemostasis have suggested a role for humoral substances released from the graft in the development of DIC; graft endothelial damage leads to the attraction of inflammatory mediators, such as macrophages and polymorphonuclear granulocytes [124,125]. These mediators are stimulated to produce cytokines (lysosomal proteinases), which, released from the graft upon revascularization, may actively interfere with coagulation and fibrinolysis [126]. It was shown that pretreatment with cyclosporin, an inhibitor of cytokine release, ameliorated the hemostatic changes after reperfusion in pigs, as measured by prothrombin times, platelet counts and fibrinogen levels [127]. Riess et al have demonstrated the occurrence of phagocyte proteinases from different cellular origin (cathepsin B from macrophages and elastase from polymorphonuclear granulocytes) in the liver graft perfusate and correlated the plasma levels of these proteinases with the coagulopathy in OLT [128]. Cathepsin B was envisaged as in indicator for the simultaneous extracellulary release of other proteinases.

(c) Heparin or heparin-like factors

An isolated prolongation of the thrombin time has been described, that could be (partially) neutralized by protamine sulfate, suggesting the presence of heparin [120,129,130]. The question arises as to the nature and source of the heparin, which is exerting the heparin effect. It has been ascribed to endogenous heparin or heparin-like substances released form donor livers or to exogenous heparin in the preservation fluid that is released into the recipient after reperfusion after sequestration into the graft during preservation. Studies reporting the endogenous release of heparin are based on studies performed in dogs. These animals are especially susceptible to release of these substances during a period of shock or acute phase reactions [131,132]. Therefore the results do not necessarily apply to humans. Presently the use of heparin in the recipient or preservation fluid is avoided [104], but a "heparin effect" may still be observed after graft recirculation. This can easily be treated with protamine sulfate and suggests that the release of endogenous heparin or heparin-like substances may play a role in the bleeding tendency of some patients [133].

(d) Thrombocytopenia

A decrease in platelet count is one of the changes that are uniformly seen after reperfusion of the donor liver, despite the transfusion of platelets [28,134]. Although thrombocytopenia may be a sign of DIC, it is often seen as an isolated phenomenon. Experimental studies suggest a role of the transplanted liver in the origin of thrombocytopenia [28,135]. Simultaneous measurement of platelets in the arterial inflow and venous outflow revealed a reduction up to 55% across the graft. Infusion of radiolabeled autologeous platelets in four patients, who underwent OLT, provided evidence for sequestration in the graft [120]. However, there is still no certainty on the pathophysiological mechanism of the fall in platelets. Local intravascular coagulation on the damaged endothelium of the graft [104], extravasation of platelets into the spaces of Disse [28] and increased phagocytosis of platelets by Kupffer cells have been suggested [136]. Others found the changes of platelet function of greater importance than changes in platelet numbers, giving transient prolongation of bleeding time in dogs after OLT, while platelet count

remained in the low normal range [137]. A process of reversible platelet aggregation in association with an increased activity of the reticuloendothelial system after graft reperfusion, was suggested ,later fold by a release of non-functioning platelets into the circulation. Therefore, although it is most likely that the donor liver plays a role in the origin of the thrombocytopenia after reperfusion, its pathophysiology remains unsolved.

1.4.2.2 Heterotopic auxiliary liver transplantation

Auxiliary transplantation of a liver in a heterotopic position is theoretically attractive because the host liver is left in situ, thus avoiding the laborious removal of the cirrhotic liver, the associated blood loss, the subsequent anhepatic period and fall in cardiac output [81]. The limitation of the surgical trauma may lead to a speedier recovery of the patient and failure of the graft does not necessarily lead to death or emergency retransplantation. Furthermore, in case of potential reversible liver damage, regeneration of the host liver can be awaited [83]. Hemostatic changes during HLT can be expected to be less severe than during OLT for the following reasons; firstly, the anhepatic period is avoided and the host liver can (to a greater or lesser extent) participate in the clearance of t-PA and other hemostasis factors. As no veno-venous bypass is used, the associated hemostatic changes are avoided. Secondly, although graft reperfusion is similar for OLT and HLT, the released hemostatic proteins and/or humoral substances may be cleared by the host liver. Sparse information on the influence of HLT on hemostasis was obtained from initial animal studies in the 1960s and 1970s [120,129], but in those days heparin was routinely used, which has confused the issue. As mentioned previously, a clinical heterotopic liver transplantation program was started in Rotterdam in 1986 [81]. Hemostatic studies in the first patients demonstrated that hemostatic changes were less severe than in OLT and were not observed until after reperfusion of the graft [138,139]. This suggested that the host liver in HLT protects the recipient from hemostatic deterioration.

1.4.3 Management of hemostasis during liver transplantation

Massive amounts of packed erythrocytes, cryoprecipitate, fresh frozen plasma and

platelets are often needed to control bleeding during liver transplantation, considerably more than during most other surgical procedures [140]. During the past decades, the dramatic increase in liver transplantations has led to a better understanding of the coagulation problems encountered during the procedure. Concomitantly, the search for improved techniques to control bleeding and limit the usage of blood products has intensified. The advent of the cell-saver was an important step forward. However the question of how much coagulation testing needs to be done to guide replacement of blood products during liver transplantation remains to be answered [119]. Some transplant centers use only conventional coagulation tests (prothrombin times, activated partial thromboplastin time and platelet count) at regular intervals during transplantation, others perform complete coagulation surveys including clotting factor assays and euglobulin clot lysis times, and others do complete surveys along with thrombelasthographic monitoring. A difficulty in the intraoperative management of liver transplantation has been actively monitoring the coagulation system and determining the appropriate treatment during dynamic blood volume changes. The results of routine clotting tests are generally available about 20 minutes after a sample of blood has been collected and specific factor assays take considerably longer. In contrast to these various tests, the thrombelasthography (TEG) monitors the coagulation process continuously and rapidly in the operation theatre. The use of the TEG was first suggested by von Kaulla et al.[100] and Howland et al.[93]. Kang et al. reported that the use of TEG was reliable and rapid, and was associated with a 33% reduction of blood and fluid infusion volume, whereas blood coagulability was maintained without an increase in the number of blood products used [90]. As the effect of the TEG was not demonstrated in a randomized study, but a historical control group was used, these results have been questioned by others,.

If enhanced fibrinolysis occurs, therapy with synthetic fibrinolysis inhibitors may be a solution. Although antifibrinolytic treatment appears beneficial, it has only been used sporadically. Already in the early series recipients were treated with epsilon aminocaproic acid (EACA) and the bleeding was controlled [141]; however a hypercoagulable (hypofibrinolytic) state developed in the postoperative period and the recipients died of extensive venous thrombosis and multiple pulmonary

emboli [100]. Based on these findings it was suggested that administration of EACA could be harmful in transient fibrinolysis. Later Kang et al demonstrated that the judicious use of a small dose EACA (1g), when its efficacy was confirmed in vitro by thrombelastography of EACA-treated blood, effectively treated the severe fibrinolysis associated with liver transplantation without clinical thrombotic complications [106].

Aprotinin is an inhibitor of human plasmin, kallikrein and to some extent urokinase and can in theory inhibit the development of fibrinolysis [142]. The toxicity of aprotinin is extremely low and even high doses are well tolerated [143]. Since the application of high doses aprotinin in open heart surgery led to a significant reduction of transfusion requirements of about 70 %, the effectiveness of its application on fibrinolysis during liver transplantation has been investigated [144,145]. It was found that high dose aprotinin inhibited hyperfibrinolysis during OLT and reduced blood transfusion requirement [146,147]. Prophylactic use of aprotinin is advocated. Continuous infusion of aprotinin appeared to be advantageous over bolus application [147].

Currently, the use of heparin during liver transplantation is generally avoided as even small amounts of heparin may lead to severe hemorrhage. Occasionally, a "heparin effect" may be observed after graft recirculation, presumably due to the release of endogenous heparin or heparin-like substances. This can easily be treated with protamine sulfate [133].

1.5 Hemostasis after liver transplantation

Bleeding is by far the main cause of death, not only during transplantation, but also in the first postoperative week and it is the second main cause within the first 30 days [85]. As the liver is the major site of synthesis and clearance of hemostasis proteins, recovery of hemostasis depends on the functional integrity of the transplanted liver upon revascularization. Graft dysfunction may lead to a life-threatening hypocoagulability in the immediate postoperative period, sometimes with devastating consequences [84,120]. Little data exists regarding the hemostatic recovery after OLT. Critical events occur in the first few days after liver replacement, that may have an impact on the survival of the graft. Stahl et al. found an imbalance

between procoagulant and anticoagulant mechanisms, indicating a sustained prothrombotic state, that may contribute to the risk of hepatic artery thrombosis [148]. Using a regimen of low-dose heparin and fresh frozen plasma infusion, no thrombosis were encountered. Two years later Velasco et al. confirmed that after OLT a hypercoagulable state developed as a result of a delayed recovery of the anticoagulant and fibrinolytic systems as compared to the procoagulant system [149]. None of their patients, however, developed vascular thrombosis, despite the fact that no regimen of low dose heparin and fresh frozen plasma was used. The authors suggested that the hypercoagulable state perse not always has clinical consequences [150]. Blood flow is one of the three factors of Virchows triad (the others being hypercoagulability and vessel wall injury), and the importance of adequate blood flow in preventing thrombosis is well illustrated by animal experiments [45]. The combination of venous stasis and hypercoagulability seems to be required for formation of a thrombus. It is well possible that an additional blood flow retardation due to false surgical techniques increases appreciably the risk of thrombosis of the hepatic artery and hepatic vein. Overzealous correction of clotting defects and polycythemia caused by overtransfusion have been reported to also contribute to the risk of thrombosis of a hepatic artery or portal vein [118,151,152].
A theoretical advantage of HLT is that the residual function of the host liver could contribute to the postoperative recovery of hemostasis. Failure of the graft does not necessarily lead to bleeding complications, death or emergency retransplantation.

1.6 Aims of the study

In this thesis studies on hemostatic disorders in liver cirrhosis and liver transplantation have been described. Aims of the work were to further investigate;

1. Whether (low-grade) DIC occurs in liver cirrhosis applying new quantitative tests, measuring thrombin-antithrombin III complex, soluble fibrin and d-Dimer.

2. The mechanism underlying increased fibrinolysis during OLT by comparing fibrinolytic activity in OLT, HLT and PHR.

3. The mechanism underlying the coagulation changes in liver transplantation and whether they are associated with DIC, by measuring thrombin-antithrombin-III complex and conventional DIC parameters during orthotopic and heterotopic liver transplantation and partial hepatic resections.

4. The effects of different long-term graft preservation on hemostasis during porcine orthotopic and heterotopic liver transplantations and whether HLT protects the recipient from the effects of long-term preserved grafts on hemostasis.

5. To determine the origin of the heparin(-like) effect observed after reperfusion of the liver graft. (Whether heparin, given to the donor shortly before the hepatectomy, can be retrieved in the recipient after graft reperfusion.)

6. Whether there is a difference between OLT and HLT regarding postoperative hemostatic recovery and whether there is a contribution from the host liver in postoperative synthesis of hemostasis factors.

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Chapter 2

Disseminated intravascular coagulation in liver cirrhosis

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Abstract

We measured thrombin-antithrombin-III complex (TAT), soluble fibrin (SF) and D-Dimer levels in 51 patients with liver cirrhosis to determine whether these tests provide new evidence for the presence of disseminated intravascular coagulation (DIC) in liver cirrhosis.

TAT levels (median, range) were increased in the patient group (4.2 μg/l, 1.8-60.0) compared to the reference group (2.0 μg/l, 1.5-7.6). SF levels (0 nmol/l, 0-80) were also increased in the patients as compared to the controls (0 nmol/l, 0), but there was no correlation between TAT and SF levels ($r=0.23$, $p<0.98$). TAT levels did not correlate with AT-III levels ($r=-0.36$, $p<0.49$), but there was an inverse correlation between SF and AT-III ($r=0.60$, $p<0.001$)). If AT-III levels were above 0.30 U/ml, SF levels remained low, whereas SF levels were increased in patients with AT-III levels below 0.30 U/ml. These findings suggest that if sufficient AT-III is present, thrombin formation is adequately controlled, whereas at low levels of AT-III, thrombin escapes inactivation by AT-III and may act upon fibrinogen leading to the formation of SF and a low-grade DIC. SF levels correlated well with D-Dimer levels ($r=0.55$, $p<0.001$), which is consistent with DIC and secondary fibrinolysis.

In conclusion: (1) Thrombin formation is increased in liver cirrhosis, as indicated by increased TAT levels in 21 of 51 patients. (2) The plasma concentration of AT-III appears to be of major importance for the development of DIC. The present study provides evidence for DIC in severe liver cirrhosis when AT-III levels are less than 0.30 U/ml.

Introduction

Liver disease is often associated with abnormalities in the hemostatic system, including a low platelet count, low levels of clotting factors and increased levels of fibrin(ogen) degradation products (FDP) [1-4]. Since the liver plays a central role in the synthesis and clearance of most hemostatic proteins, many of these abnor-

malities can be attributed to impairment of liver function. However several investigators have suggested that disseminated intravascular coagulation (DIC) contributes to the hemostatic abnormalities in liver disease, inasmuch as hemostatic disturbances in liver disease are very similar to those in DIC [5,6]. Possible pathogenetic mechanisms for DIC are the release of procoagulant factors from necrotic hepatocytes or the absorption of endotoxin from the intestine into the portal system [7-9]. Evidence for DIC was supplied by kinetic studies with radiolabeled fibrinogen, plasminogen and prothrombin, that showed a reduced half-life of these proteins, which tended to be reversed by heparin [10-13]. Administration of antithrombin-III (AT-III) to patients with cirrhosis and patients with AT-III deficiency normalized the half-life of labeled fibrinogen [14]. This suggested that low levels of AT-III contributed to the development of DIC. However the concept of DIC in cirrhosis has not received universal support. Most of the changes in coagulation and fibrinolytic factors can also be explained by decreased synthesis of the liver or by loss to extra-vascular spaces, whilst a decreased platelet count may be explained by pooling in the spleen. High levels of FDP may result from impaired clearance or from degradation of fibrinogen in ascites [6,15,16]. The increase in the half-life of labeled fibrinogen after heparin was considered to be the most reliable evidence for DIC. However in the original studies the injected fibrinogen was contaminated with other labeled proteins and the heparin effect was uninterpretable, because the radioactive count of the labeled fibrinogen was at the times of measurement too close to the background count [17]. For these reasons the presence of DIC in liver cirrhosis is still open to debate.

DIC is a dynamic condition with ongoing thrombin generation, disseminated fibrin deposition with secondary activation of the fibrinolytic system. Once produced thrombin cleaves fibrinopeptide A and B from fibrinogen, converting it into fibrin monomer, soluble fibrin (SF) and finally cross-linked fibrin. In turn thrombin is rapidly inactivated by antithrombin-III, via the formation of thrombin-antithrombin-III (TAT) complexes. The fibrinolytic system is activated at the same time, leading to the break-down of cross-linked fibrin, with release of specific degradation products (D-Dimers).

Recently quantitative tests have been developed that enable us to quantitate TAT,

SF and D-Dimer levels in plasma. In the present study we applied these tests in a group of patients with stable liver cirrhosis of different severity to determine whether they provide new evidence for the presence of a low-grade DIC in compensated liver cirrhosis.

Patients materials and methods

Patients

The clinical material for this study was provided by 51 consecutive outpatients with stable cirrhosis, visiting the Department of Internal Medicine for routine control (table 1). Patients with a coexistent infection or other conditions that could predispose to DIC and patients who had received blood products within two weeks before blood sampling, were excluded from the study. The male/female ratio of the patients was 1.3 and the median age was 51 years (range 22-73). The liver cirrhosis was histologically proven. The etiology of the liver cirrhosis is summarized in table 1. Patients were divided according to the Child-Pugh classification; 22 were

Table 1

Etiology of the cirrhosis in all studied individuals.

Nature of the cirrhosis	All patients n=51	Child A n=22	Child B n=12	Child C n=17
Alcohol abuse	20	9	5	6
Viral hepatitis	15	6	3	6
Autoimmune hepatitis	9	5	3	1
Primary biliary cirrhosis	5	2	1	2
Miscellaneous*	2	0	0	2

*α1-antitrypsin deficiency, primary sclerosing cholangitis
The patients are categorized according to the Child-Pugh classification[18]

classified as Child A , 12 as Child B, and 17 as Child C [18]. None of the patients had a history of hemostatic complications.

A control group consisted of 12 apparently healthy laboratory volunteers (male/female = 1/1 ; median age 33 years, range 25-51).

Materials

Blood samples were obtained between 9.00 and 11.00 am by venepuncture and were anticoagulated by one-tenth volume of ice-cold sodium citrate 0.11 mol/l. The first 10 ml of blood were discarded. The samples were immediately placed on melting ice. Plasma was prepared by centrifugation (30 min at 2000g, 4°C), snap-frozen in small aliquot and stored at -70°C.

Assays

AT-III activity was determined using the substrate HD-CHA-But-Arg-pNA obtained from Behringwerke [19] and fibrinogen was measured according to Clauss [20]. The prothrombin time was determined according to Quick, with home made brain thromboplastin [21]. Factor V was measured using factor-V deficient plasma (Behringwerke) using a prothrombin time technique. Levels of α2-antiplasmin were assayed according to Friberger et al. [22].

Thrombin time was measured by adding 200 μl thrombin to 200 μl prewarmed plasma [23].

TAT levels were determined with a sandwich-type enzyme immunoassay, using rabbit antibodies to thrombin as catching antibody and (peroxidase conjugated) rabbit antibodies to AT III as tagging antibody (Behringwerke, Marburg) [24].

SF levels were determined using "Coaset fibrin monomer" (Kabi, Amsterdam, The Netherlands). This assay is based on the potent stimulation of tissue-type plasminogen activator (t-PA) activity by fibrin. Conversion of plasminogen to plasmin, catalysed by t-PA, is determined with a chromogenic peptide substrate for plasmin and the generated colour is proportional to the concentration of SF in the standard sample being assayed. The characteristics of the assay have been published elsewhere [25].

D-Dimer levels were determined using an ELISA with monoclonal antibodies to

fibrin degradation product D-D as catching antibody and rabbit antibody to fibrin degradation product D as tagging antibody (Diagnostica Stago, Asnieres, France). Latex agglutination of D-Dimer was measured by the D-Di test (Diagnostica Stago, Asnieres, France). This test uses a monoclonal anti-D-Dimer antibody on latex beads. There was an excellent correlation between the D-Dimer latex agglutination and ELISA values ($r=0.80$, $p<0.0001$).

Statistical analysis

The Wilcoxon test and the Spearman rank correlation test were used for statistical analysis. A p-value of less than 0.05 was considered to be significant.

All values are presented as medians and range together with the 10th and 90th percentiles.

Table 2

Levels of prothrombin time (PT), antithrombin-III (AT-III), factor V, Fibrinogen, α_2-antiplasmin (α_2-AP) and platelet count in Child A, B and C cirrhosis and the control group. Levels are given as median, range with the 10th and 90th percentile under it.

Variables	Reference values	Normals	Child A	Child B	Child C
PT sec	15-19	16.5 (13-18) (15-18)	19 (17-23) (17-22)	21 (17 24) (17-24)	24 (17-30) (19-29)
AT-III U/ml	0.85-1.20	0.96 (0.79-1.27) (0.83-1.06)	0.65 (0.35-1.01) (0.50-0.86)	0.61 (0.35-0.93) (0.41-0.89)	0.29 (0.17-0.69) (0.18-0.47)
Factor V U/ml	0.50-1.50	0.95 (0.68-1.00) (0.80-1.00)	0.86 (0.19-1.20) (0.46-1.20)	0.74 (0.27-1.20) (0.38-1.08)	0.41 (0.21-0.87) (0.24-0.82)
Fibrinogen g/l	1.6-2.8	2.4 (1.4-4.9) (1.6-3.3)	2.6 (1.2-4.0) (1.6-3.1)	3.2 (1.2-5.9) (1.4-4.9)	1.7 (0.6-3.8) (0.8-3.4)
α_2-AP u/ml	0.85-1.20	0.95 (0.86-1.18) (0.88-1.14)	0.81 (0.57-1.01) (0.64-0.89)	0.73 (0.49-1.00) (0.51-0.95)	0.45 (0.21-0.63) (0.22-0.55)
Platelets $x10^9/l$	130-350	180 (155-198) (160-272)	80 (45-330) (53-166)	161 (61-320) (69-226)	85 (14-345) (39-294)

Results

The values (median and 10th and 90th percentiles) for the routine coagulation tests prothrombin time, antithrombin-III, factor V, α2-antiplasmin, fibrinogen and platelet count in patient with liver cirrhosis, subdivided according to Child-Pugh, and in the control group are summarized in table 2. The prothrombin time, antithrombin-III, factor V and α2-antiplasmin levels became worse with increasing severity of the disease. Fibrinogen was only reduced in Child C cirrhosis and the platelet count varied widely within the Child-Pugh classification.

The individual levels of TAT, SF and D-Dimer, subdivided according to Child-Pugh, are depicted in figure 1. TAT levels (median and 10th and 90th percentiles) were increased in the patient group (4.2 μg/l, 2.2-14.5) as compared to the reference

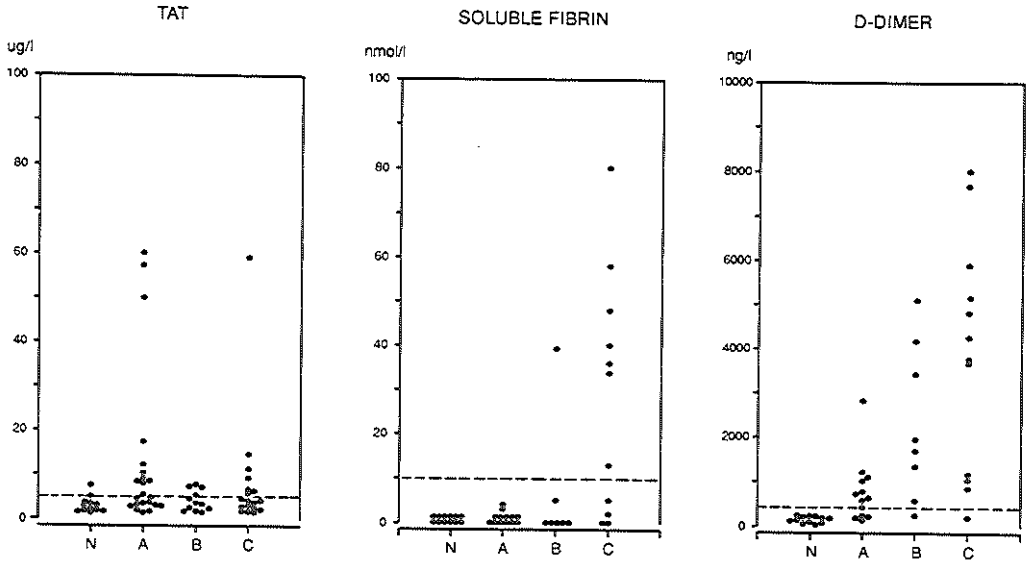

Fig 1. Individual plasma levels of thrombin-antithrombin-III complex (TAT), soluble fibrin (SF) and D-Dimer levels in patients with Child A, B and C liver cirrhosis compared to those of a control group. The dotted lines indicate the upper limit of normal.

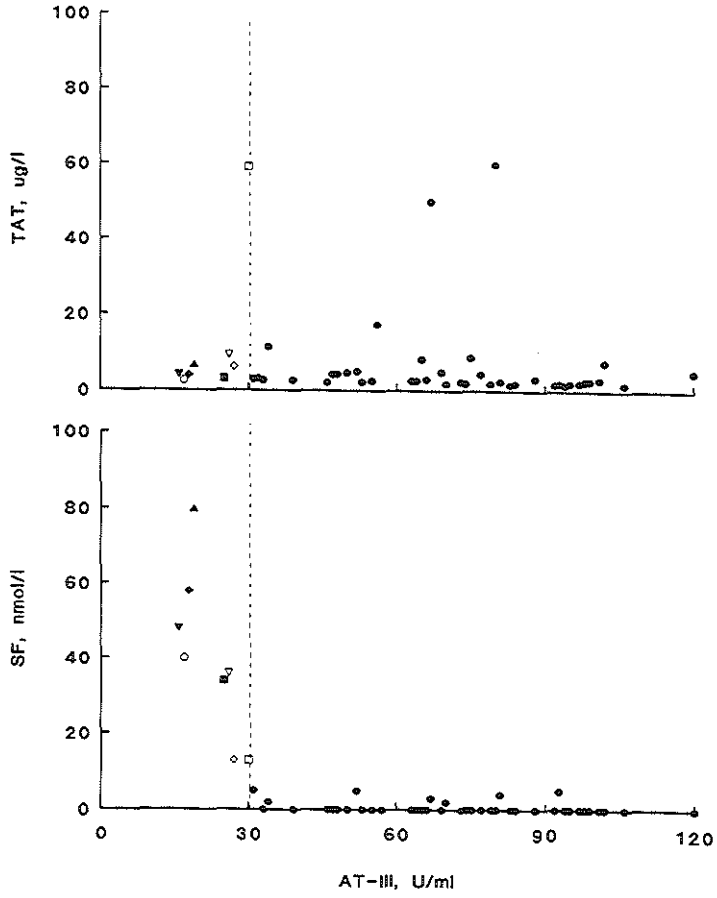

Fig.2 Levels of thrombin-antithrombin-III complex (TAT) and soluble fibrin (SF) of all studied individuals as a function of AT-III. The individuals with antithrombin-III levels of less than 0.30 U/ml have been depicted with separate symbols.

group (2.0 μg/l, 1.6-5.0), but there was no significant difference between Child A, Child B and Child C cirrhosis respectively. One control subject had a slightly elevated TAT level. Median SF (0 nmol/l, range 0-40) and D-Dimer (1188 ng/l, range 210-5184) levels were also increased in the patient group and as compared to the control group (0 nmol/l, 0 and 164 ng/l, 95-232, resp.). In contrast to TAT, SF and D-Dimer levels showed an apparent relation with the Child classification.

There was no correlation between TAT and SF levels ($r=0.23$, $p<0.98$) or TAT and D-Dimer levels ($r=0.28$, $p<0.84$). SF levels correlated significantly with D-Dimer levels ($r=0.55$, $P<0.001$).

TAT and SF levels have been plotted as a function of AT-III in figure 2. There was no correlation between TAT and AT-III levels ($r=-0.36$, $p<0.49$), but an inverse correlation between SF levels and AT-III ($r=-0.60$, $p<0.001$). TAT levels were sometimes high in patients with AT-III levels above 0.30 U/ml. SF levels were normal at-III levels higher than 0.30 U/ml, but were raised when patients had AT-III levels below 0.30 U/ml.

D-Dimer levels also increased with decreasing AT-III levels. The D-Dimer levels were found to be raised at higher AT-III levels than was seen for SF. All patients with elevated SF levels (the patients with AT-III levels of less than 0.30 U/ml) had increased levels of D-Dimer.

D-Dimer levels also correlated well with α2-antiplasmin levels ($r=-0.55$, $P<0.001$), indicating a relation with primary fibrinolysis.

There was no relation between SF levels and the thrombin times ($r=0.36$, $p<0.11$). Prolonged thrombin times were associated both with normal and increased SF levels.

Discussion

Several mechanisms have been postulated to be involved in the pathogenesis of the hemostatic abnormalities in chronic liver disease. One of the hypothesis proposes a role for low-grade DIC. In this study routine coagulation tests were abnormal in most patients with liver cirrhosis and they were strongly dependant on the severity of the liver disease, as indicated by their relation with the Child-Pugh classification. As the coagulation changes in liver cirrhosis are very similar to those described in DIC, a different approach towards the diagnosis of DIC in liver cirrhosis is needed than in other conditions. DIC is a dynamic process with ongoing thrombin formation and secondary fibrinolysis. When thrombin is formed in the

circulation, part of it will be bound to antithrombin-III to form TAT. Increased TAT levels have been reported in conditions involving activation of the coagulation pathway, such as deep venous thrombosis and DIC [26]. In the present study increased TAT levels were found in 21 out of 51 patients with liver cirrhosis, suggesting that increased thrombin formation, and thus a low-grade DIC, is present in many patients with liver cirrhosis. In liver cirrhosis raised TAT levels may be due to increased thrombin formation, but could also be caused by impaired hepatic clearance of TAT. If impaired hepatic clearance is the main cause of increased TAT levels in cirrhosis, one would expect a relationship between TAT levels and the severity of the cirrhosis. In the present study we found no relation between TAT and the Child-Pugh classification.

If thrombin escapes inactivation by antithrombin-III, it splits fibrinopeptides A and B from fibrinogen, converting it into fibrin monomers, SF polymers and finally cross-linked fibrin. The presence of increased levels of SF, as we found in Child C cirrhosis, therefore indicates that thrombin has acted on fibrinogen and thus is evidence of DIC. However surprisingly, TAT levels did not correlate with SF levels, despite the fact that both are considered to be indicators of an activated coagulation. This phenomenon can be explained by the key role of AT-III in the regulation of the coagulation. Whereas TAT levels did not correlate with antithrombin-III, there was an inverse correlation between SF and antithrombin-III. If AT-III levels are above 0.30 U/ml SF levels remain low, despite extremely high TAT levels in some cases, indicating that increased thrombin formation in these patients was adequately controlled by AT-III. However, as soon as antithrombin-III levels fall below 0.30 U/ml, as in Child C cirrhosis, thrombin escapes inactivation by AT-III and may act upon fibrinogen leading to the formation of SF, and thus to a low-grade DIC.

Since DIC is characterized by thrombin formation with secondary fibrinolysis, we also quantitated D-Dimer levels as an index of fibrinolysis. We found that all patients with elevated levels of SF also had increased levels of D-Dimer. This is compatible with the diagnosis DIC with secondary fibrinolysis. In some patients D-Dimer levels were increased although normal SF levels were observed. One possible explanation is that in some cases liver cirrhosis is also associated with primary fibrinolysis, due

to impaired hepatic clearance of t-PA [27]. Increased levels of D-dimer does not remit the distinction between fibrinolysis secondary to thrombin formation and primary fibrinolysis. The good correlation between D-Dimer and α2-antiplasmin levels favours the presence of primary fibrinolysis.

The results presented above suggest that SF is a useful tool for the recognition of a prethrombotic state in liver cirrhosis. However an alternative explanation for the increased levels of SF has to be considered; liver disease has been associated with an acquired dysfibrinogenemia, characterized by impaired fibrin polymerization and prolonged thrombin times [28]. It could be theorized that the impairment of fibrin polymerization leads to elevated levels of SF. Thrombin time measurement in these patients was not of much help, since thrombin times were prolonged both in patients with normal and elevated soluble fibrin levels. For the following reason we think that the SF levels are not influenced by delayed fibrin polymerization; The SF assay used here, is based on the potent stimulation of t-PA by fibrin [29]. The conversion of plasminogen to plasmin, catalysed by t-PA, is determined with a chromogenic peptide substrate for plasmin and the generated colour is proportional to the concentration of SF. Impairment of fibrin polymerization has been shown to reduce t-PA stimulation to the levels of fibrinogen [30]. Therefore the elevated levels of SF, as measured in 8 patients, are not due to delayed polymerization, but increased activation of coagulation.

In animal experiments it has been demonstrated that in addition to TAT also SF and D-Dimer are cleared by the liver with short half-lives (7 min, 3 and 10 hours respectively) [31-33]. Therefore an influence of impaired clearance on the levels in question cannot be excluded.

The results of this study show that thrombin formation may be increased in patients with liver cirrhosis, as indicated by the increased levels of TAT, irrespective of the severity of the disease. However the concentration of antithrombin-III in the circulation could be of major importance for the development of a low-grade DIC, since very low levels of antithrombin-III were associated with an elevated concentration of SF. TAT levels by themselves are of little value for the diagnosis of DIC in liver cirrhosis. SF levels however may be a useful tool for the diagnosis of a low-grade DIC in liver cirrhosis.

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Chapter 3

Increased tissue-type plasminogen activator activity in orthotopic but not in heterotopic liver transplantation: the role of the anhepatic period

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Abstract

The major cause of the increased tissue-type plasminogen activator (t-PA) activity during orthotopic liver transplantation (OLT) is still unclear. Both lack of hepatic clearance of t-PA in the anhepatic period and increased endothelial release from the graft upon reperfusion have been proposed as the major causes. Heterotopic liver transplantation (HLT) avoids the resection of the host liver and is a useful model to help differentiate between these two possibilities. In this study the fibrinolytic system was evaluated in 10 OLTs, 18 HLTs and a control group of 10 partial hepatic resections (PHRs).

A marked increment in t-PA activity, from 0.2 to 5.2 IU/ml ($p<0.02$), was observed during the anhepatic period of OLT, which rapidly normalized after reperfusion. In contrast t-PA activity levels remained normal in HLT and PHR. In OLT as well as HLT there was no increase in t-PA activity following reperfusion. The first venous hepatic outflow after reperfusion did not contain elevated t-PA activity levels. Plasma degradation products of fibrin and fibrinogen increased during the anhepatic period of OLT (from 2.60 to 8.80 μg/ml ($p<0.008$) and from 0.40 to 1.60 μg/ml ($p<0.04$) respectively) and remained elevated thereafter. In HLT and PHR these levels remained low.

In conclusion. The lack of hepatic clearance during anhepatic period is probably the most important factor in the evolution of increased t-PA activity during OLT.

Introduction

Orthotopic liver transplantation (OLT) is now an accepted means of therapy for patients with acute and chronic liver failure [1,2]. However this procedure may be associated with large losses of blood, which are, in part, due to profound changes in hemostasis. The pathogenesis of these changes is still not completely understood. As a consequence the management of hemostasis during the operation is not optimal, causing an increased morbidity and mortality in liver

transplant patients.

Enhanced fibrinolytic activity has been implicated as a major cause of bleeding during OLT, especially during the anhepatic and early postreperfusion period [3,4]. The mechanism underlying the increased fibrinolysis is not yet well defined. Both the lack of hepatic clearance of tissue-type plasminogen activator (t-PA) during the anhepatic period and the release of t-PA from either the vascular endothelium or hepatocytes upon reperfusion have been suggested as causes of hyper-fibrinolysis [5-7].

Liver transplantation can also be performed with auxiliary heterotopic liver transplantation (HLT). In HLT the diseased host liver is left in situ and the graft is transplanted in a heterotopic position. The anhepatic period is thus avoided and the host liver can still, to a greater or lesser extent, participate in the clearance of t-PA and other hemostasis proteins during the transplantation. HLT thus may provide us with a useful model to differentiate between the effects of graft reperfusion and the anhepatic period on the hemostatic changes. However, the clinical results of HLT in the past have been poor. The reasons for the failure included a lack of space in the peritoneal cavity for the graft, atrophy of the graft due to a lack of portal-blood inflow, and venous congestion of the graft. We have overcome these problems, by using a graft reduced in size by partial hepatectomy and provided with arterial and portal blood inflow while venous drainage was established inn an area with low pressure. The HLT program in our institution, has given to date good clinical results in both acute and chronic liver disease [8,9].

In this study we compared the fibrinolytic activity during OLT and HLT, in order to attain a better insight into the mechanism of increased t-PA activity during OLT. As it has been described that surgery itself may also stimulate fibrinolysis [10], we studied a reference group of 10 partial hepatic resections (PHR), to establish the extent of fibrinolytic activity induced by the surgical trauma itself, including manipulation of the liver.

Patients, materials and methods

Patients

This study was performed on a consecutive series of 10 patients undergoing OLT, 18 patients undergoing HLT and 10 patients undergoing PHR. The causes of the liver disease and the Child-Pugh score [11] are summarized in table 1.

Surgical technique

OLT was performed by a standard technique [12]. The veno-venous bypass was used in 5 cases. The surgical technique of HLT has been described in detail elsewhere [8]. Segments II and III of the left lobe of the liver and the gallbladder were removed in bench surgery and the graft was placed in the right subhepatic region. Three vascular anastomoses were made: The suprahepatic vena cava of the graft was placed end-to-side to the infrahepatic vena cava of the recipient; the portal vein was was placed end-to-side to that of the recipient, and the hepatic artery, with an aortic patch, was placed end-to-side to the infrarenal aorta of the recipient. Finally, the common bile duct was anastomosed to a Roux-and-Y jejunal loop.

Table 1

Diagnosis and Child-Pugh score (median and range) in 10 patients undergoing OLT and 18 undergoing HLT

Diagnosis	OLT	HLT	
Fulminant hepatitis	4	4	
Hepatitis B	2	5	
Autoimmune hepatitis	0	1	
PBC/PSC*	3	6	
Carcinoma	1	0	
miscellaneous	0	2	
Child-Pugh score**	8 (7-10)	12 (8-15)	P<0.05

*Primary biliary cirrhosis/sclerosing cholangitis

**Child-Pugh score was calculated only in chronic liver disease.

Perioperative management

Pre-, peri- and postoperative management was identical for both transplantation procedures and has been described previously [8]. Selective bowel decontamination with nonabsorbable antibiotics was started before surgery and continued until the biliary and other catheters were removed. Anticoagulants (heparin initially and phenprocoumon thereafter) were administerd for 3 months after surgery. The immunosuppressive regimen conststed of antithymocyte globulin, prednisolone and azathioprine during the first week and then prednisolone and cyclosporine.

Prior to the operation, substitution therapy with fresh frozen plasma (FFP), cryoprecipitate and platelets was given, in order to improve preoperative hemostatic function. Intraoperative blood loss was compensated for by the transfusion of erythrocyte concentrate and fresh frozen plasma (FFP) in a scheme of 1:1. Additional FFP, platelets or cryoprecipitate were given depending on the laboratory results; 6 units of platelets were given when the platelet count fell below $60x10^9$/l, 4 units of cryoprecipitate were given when fibrinogen levels fell below 1.3 g/l, and 4 units of FFP were given when the prothrombin time was longer than 22 seconds. No antifibrinolytic agents or heparin were used. Blood loss was estimated by measuring the amount of aspired blood from the operation field (and the weight of the surgical swabs.

OLT was divided into three stages. Stage I begins with the induction of anesthesia and ends with the occlusion of blood flow to the patient's liver. Stage II (anhepatic phase) continues until the donor liver is reperfused by the patient's circulating blood. Stage III is from the moment of reperfusion until closure of the surgical incision. For comparison it was decided that in HLT stage II starts 120 minutes into surgery. Stage I and III are the same as in OLT. In PHR stage II continues from the start of the resection of the liver until the completion of the resection, followed by stage III, which continues until closure of the surgical incision.

Blood samples were collected according to the following schedule: immediately after induction of anesthesia, 30 minutes before stage II (II-30), 5 minutes into stage II (II+5), 15 minutes before stage III (III-5), and 5 to 10 minutes, 30, 60 and 120 minutes into stage III (III+5, 30, 60 and 120 respectively). In addition, a blood sample was taken from the graft liver vein, at the moment the graft was reperfused

with the recipient's systemic blood (first venous hepatic outflow). The samples were collected from an unheparinized arterial line in plastic tubes and were anticoagulated with one-tenth volume cold trisodium citrate 0.11 mol/l. The samples were immediately placed on melting ice and centrifuged (30 min., 2800g, 4°C) within 20 minutes. Plasma was snap-frozen and stored at -70°C until testing.

Assays

Tissue-type plasminogen activator (t-PA) activity was assayed according to Verheijen et al. [13]. t-PA antigen levels were measured using an enzyme-linked immunosorbent assay (Biopool IMULSE™ t-PA, Umea, Sweden). Plasminogen acitvator inhibitor (PAI-1) antigen levels were determined by an ELISA (Biopool TINTELIZA, Umea, Sweden). EIA kits for the specific determination of fibrin degradation products (FbDP) and fibrinogen degradation products (FgDP) were obtained from Organon Technica, Turnhout, Belgium. The characteristics of these EIAs have been published before [14].

Statistical analysis

Statistical analysis was performed using non-parameteric tests. P-values of < 0.05 were considered to be significant. All values are given as medians.

Table 2

Preoperative hemostasis parameters (median and range) in patients undergoing OLT and HLT.

	Reference	OLT	HLT
APTT sec	22-45	35.5 (28-76)	39 (28-64)*
PT sec	15-19	23.5 (16-82)	23 (17-46)*
Factor V U/ml	0.50-1.50	0.42 (0.03-1.57)	0.38 (0.19-1.76)*
AT-III U/ml	0.85-1.20	0.45 (0.07-1.21)	0.37 (0.10-1.28)*
Fibrinogen g/l	1.6-2.8	2.2 (0.7-6.5)	1.3 (0.8-5.8)*

*p NS

Results

A preoperative coagulopathy, related to end-stage liver disease, was present in both OLT and HLT (table 2). Patients undergoing HLT had lower preoperative levels of hemostasis factors than patients undergoing OLT, but the difference was not significant. The Child-Pugh score (table 1), however was significantly higher in patients treated with HLT than in patients undergoing OLT ($p<0.05$).

The median levels of t-PA activity during OLT, HLT and PHR are shown in figure 1. No enhanced t-PA activity was observed after the induction of anesthesia, as judged by normal t-PA activity levels. In OLT the t-PA activity levels rose sharply during the anhepatic period (stage II), from a median of 0.2 IU/ml to 5.2 IU/ml ($p<0.02$) at the end of the anhepatic period, and normalized rapidly after reperfusion. The change in t-PA activity during the reperfusion (from III-5 to III+5) was not significant. In contrast, t-PA activity levels remained within the normal range (0-1 IU/ml) throughout surgery in HLT and PHR (figure 1). t-PA activity was not elevated in the blood collected from the hepatic vein upon reperfusion (median 0.0 IU/ml, range 0.0-0.85). t-PA activity levels in OLTs using the veno-venous bypass and OLTs without using the veno-venous bypass were not significantly different.

The concentrations of t-PA antigen followed a similar course to t-PA activity levels. During OLT t-PA antigen levels almost doubled from 11.5 ng/ml, at the start of the operation, to 23.7 ng/ml at the end of the anhepatic period ($p<0.04$), and returned to starting levels within an hour after reperfusion. In HLT and PHR, t-PA antigen levels remained unchanged througout surgery.

PAI-1 antigen levels, in contrast, decreased in OLT during stage II, from 33.5 ng/ml to 16.1 ng/ml ($p<0.1$), and steadily increased to 57.1 ng/ml towards the end of the transplantation ($p<0.02$). In HLT PAI-1 antigen levels rose slightly during stage II, from 23.9 ng/ml to 30.5 ng/ml ($p<0.2$) and increased further to 52.2 ng/ml at the end of the surgical procedure ($p<0.001$).

The concentrations of fibrin degradation products (FbDP), reflecting the breakdown of fibrin, are depicted in figure 2. FbDP levels increased considerably during the anhepatic period of OLT from 2.6 to 8.8 μg/ml ($p<0.008$) and remained elevated after reperfusion. In contrast FbDP levels did not alter significantly in HLT or in PHR

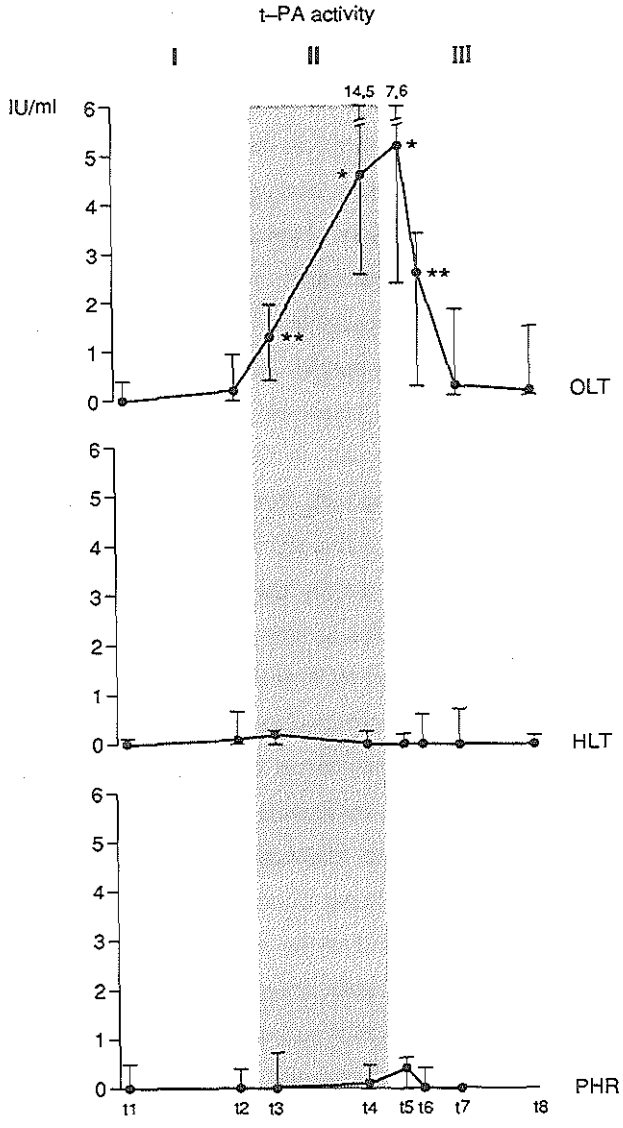

Fig 1 Median concentrations and quartiles of t-PA activity in 10 patients undergoing OLT, 18 patients undergoing HLT and 10 patients undergoing PHR.
I: Induction of anesthesia.
II: Start anhepatic phase (OLT), 120 minutes into surgery (HLT) or start of liver resection (PHR)
III: Reperfusion (OLT and HLT), and completion of resection (PHR)
t1: start, t2: II-30, t3: II+5, t4:III-5, t5: III+5, t6: III+30, t7: III+60, t8: III+120
** $p<0.05$, * $p<0.0001$, difference between OLT and HLT or PHR, respectively.

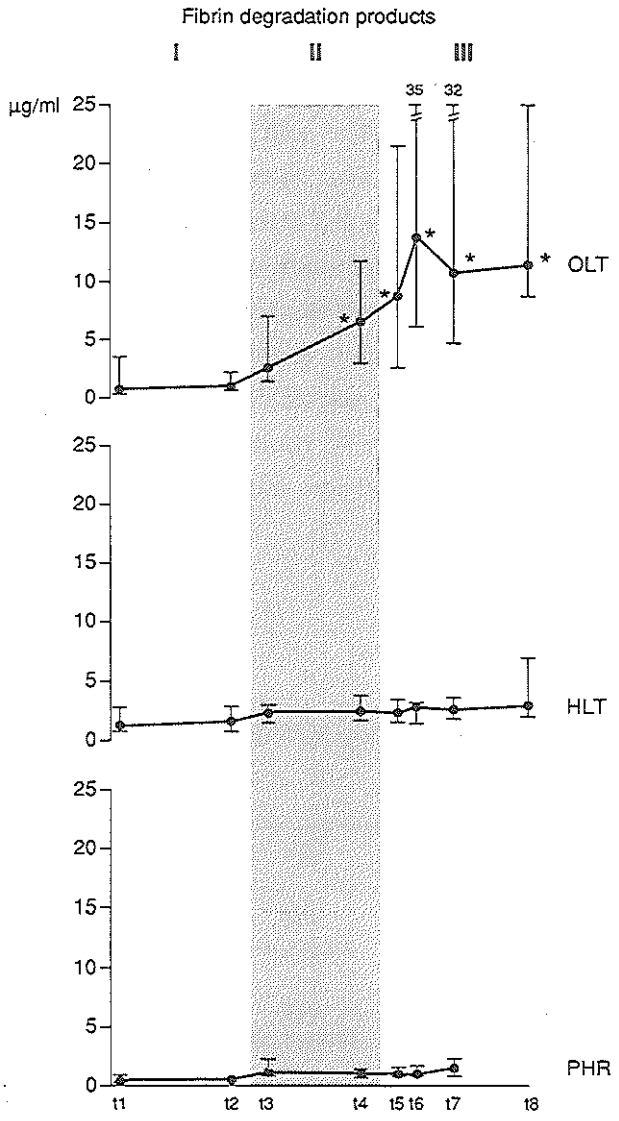

Fig 2 Median levels and quartiles of fibrin degradation products in 10 orthotopic, 18 heterotopic liver transplantions and 10 partial hepatic resections

I: Induction of anesthesia.

II: Start anhepatic phase (OLT), 120 minutes into surgery (HLT) or start of liver resection (PHR)

III: Reperfusion (OLT and HLT), and completion of resection (PHR)

t1: start, t2: II-30, t3: II+5, t4:III-5, t5: III+5, t6: III+30, t7: III+60, t8: III+120

* $p<0.01$, difference between OLT and HLT or PHR, respectively.

(figure 2). The concentrations of fibrinogen degradation products (FgDP), reflecting the breakdown of fibrinogen, followed a similar, though less pronounced, course. FgDP levels increased from 0.40 to 1.60 μg/ml during stage II of OLT ($p<0.04$), and remained at this level thereafter. In HLT, FgDP levels did not rise above 0.75 μg/ml, which was reached at the end of the operation.

The intraoperative use of blood products is shown in table 3. The mean use of red blood cells was higher in OLT as compared to HLT, but the difference was not significant. A breakdown of blood replacement according to the stage of the surgical procedure revealed no statistically significant difference in blood use for OLT and HLT in stages II and III. However, there was a significant correlation ($r=0.50$, $p<0.02$) between blood use and t-PA activity during stage II within the OLT group.

Table 3

Intraoperative blood use in patients undergoing OLT and HLT respectively

		OLT	HLT
Blood loss (L) (estimated)	mean	16.7	8.8*
	median (range)	7.5 (4.5-80)	6.0 (1-28)
RBC[1] (U)	mean	24.5	18.9*
	median (range)	13.3 (8.2-89)	13.8 (9.4-44)
FFP (U)	mean	24.4	21.0*
	median (range)	15 (4-88)	20.5 (4-49)
Cryoprecipitate (U)	mean	5.1	8.1*
	median (range)	3 (0-20)	8 (0-20)
Platelets (U)	mean	17.3	28.3*
	median (range)	12 (0-48)	24.5 (0-64)

[1]Includes packed red blood cells and autologous blood
*p NS

Discussion

The major cause of increased t-PA activity during OLT is still unclear. Both lack of hepatic clearance of t-PA in the anhepatic period and increased release of t-PA from the graft upon reperfusion have been suggested as possible causes. Furthermore it has been described that surgery itself may stimulate fibrinolysis. In this study we compared hemostatic parameters during OLT, HLT and PHR, firstly to differentiate between the single effect of reperfusion on fibrinolysis and that of the anhepatic period and secondly to establish the extent of fibrinolytic activity induced by the surgical trauma itself.

The results obtained in this study show clearly that the lack of t-PA clearance during the anhepatic period is essential for the development of a fibrinolytic state during OLT: a marked increase in t-PA activity was observed during the anhepatic period of OLT, whereas no increase in t-PA activity was seen in HLT. Neither in OLT nor in HLT was there a significant increase in t-PA activity immediately following reperfusion. PAI-1 antigen levels in OLT decreased concomitantly with the increase of t-PA activity. Reduction of PAI-1 is presumably due to the quenching of t-PA activity. In accordance to the t-PA results, fibrin degradation products were markedly elevated during OLT, but not during HLT and PHR. A limited degree of fibrino*geno*lysis, as occurs under strong fibrinolytic conditions, was reflected by the slight increase of fibrinogen degradation products during OLT.

Whereas the increase in t-PA activity was limited to the anhepatic period, fibrin and fibrinogen degradation products remained elevated over a longer period. The latter can be explained by the mechanism of action of t-PA. It is known from in vitro experiments that t-PA has a selectivity for plasminogen activation at the site of fibrin formation and becomes incorporated in newly formed hemostatic clots [15]. Plasmin so formed is not easily accessible to α_2-antiplasmin, and results in premature lysis and consequently delayed bleeding from fresh wounds [16]. Hence the clinical effect of t-PA extends over a longer period than can be measured systemically.

Hemodynamic changes during the veno-venous bypass, have been suggested as an alternative explanation for the increased t-PA activity during the anhepatic period. These changes may induce t-PA release from the vascular endothelium, via

components, such as catecholamines, histamine, serotonin, bradykinin and vasopressin [17]. It has, for instance, been known for a long time that fibrinolytic activity is increased during cardiopulmonary bypass [18]. However, no difference in t-PA activity was observed between OLTs in which the veno-venous bypass was used and in those where it was not. This makes increased endothelial t-PA release during the veno-venous bypass a less likely candidate as the cause of change in t-PA activity.

Previous investigators have reported an increase in t-PA activity following reperfusion and have attributed this phenomenon to release of t-PA from the ischemically damaged endothelium of the liver graft. In the present study, however, there were no indications for t-PA release from the graft; neither during OLT nor during HLT was a significant increase in t-PA activity seen following reperfusion. Even if one thinks that the peak t-PA levels were missed at sampling at 5-10 minutes after reperfusion, the lack of increase of fibrin degradation products after reperfusion in HLT, militates against an abundant t-PA release from the graft. Moreover t-PA activity was not elevated in samples, collected from the hepatic vein at the moment the graft was reperfused. Possibly, the recent improvements in graft procurement and preservation, which have reduced the ischemical damage to the graft, are responsible for the reduction of t-PA release from the graft.

Finally the extent of fibrinolysis induced by hepatic surgery, with manipulation and extensive trauma to the vascular bed, seems negligible, as no appreciable increase in t-PA activity was observed during PHR.

Although increased t-PA activity levels were only observed during OLT and not during HLT, this did not lead to a significant difference in blood loss between both procedures as measured by blood transfusion requirements. However, blood loss is influenced by multiple factors, which could have neutralized the single effect of hyperfibrinolysis during OLT.

In conclusion: our results confirm that an enhanced fibrinolysis occurs during OLT. In contrast, fibrinolysis does not play an important role during HLT. The most important factor in the evolution of the enhanced fibrinolysis seems to be the lack of hepatic clearance of t-PA during the anhepatic period. However definite proof of our hypothesis awaits the direct demonstration of reduced clearance of t-PA.

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Chapter 4

Intravascular coagulation in liver transplantation Is it present or not?

A comparison between orthotopic and heterotopic liver transplantation

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Abstract

It is still not clear whether disseminated intravascular coagulation (DIC) contributes to the hemostatic disturbances in orthotopic liver transplantation (OLT). Theoretically the lack of hepatic clearance of procoagulant factors during the anhepatic period and the release of thromboplastic material from the graft might trigger DIC. During heterotopic liver transplantation (HLT) the host liver is left in situ and procoagulant factors may still be cleared; DIC, if present, may not occur until after reperfusion. The aim of the present study was to gain more insight into the underlying mechanism of the coagulation changes during liver transplantation by comparison of OLT and HLT. Thrombin-antithrombin-III complexes (TAT), an indicator of thrombin generation, fibrin degradation products (FbDP) and routine clotting times were assayed in 12 OLTs, 18 HLTs and in a control group of 10 partial hepatic resections (PHR).

TAT increased dramatically after reperfusion in OLT and HLT. Peak levels were not significantly different. In contrast, FbDP levels increased only in OLT, to a maximum of 13,8 μg/ml. Routine clotting times changed mildly and similarly in both OLT and HLT.

Conclusions: Graft reperfusion triggers excessive thrombin formation, but there are no other signs of subsequent DIC. Any thrombin formed is probably rapidly inhibited by antithrombin-III. The rise in FbDP during OLT is the result of increased fibrinolysis, which occurred only in OLT and not in HLT.

Introduction

Orthotopic liver transplantation is with increasing frequency becoming an effective means for treating patients with end-stage acute and chronic liver failure [1,2]. In spite of refined surgical techniques and improved perioperative management, bleeding remains a critical problem [3]. Profound changes in hemostasis occur, especially during the anhepatic and reperfusion period. The mechanism underlying these

changes is still incompletely understood. It has been demonstrated that hyperfibrinolysis is a major cause, related to increased levels of tissue-type plasminogen activator (t-PA) [4,5]. The simultaneous decrease in platelets, fibrinogen, antithrombin-III and clotting factors and the elevation of fibrin degradation products, has prompted investigators to suggest that disseminated intravascular coagulation (DIC) is also implicated [6,7]. Theoretically lack of clearance of (clot-promoting) activated clotting factors during the anhepatic period and the release of thromboplastic material from the revascularized graft may induce DIC. Furthermore local intravascular coagulation can be activated by exposed subendothelium of the ischemically damaged graft. The assessment of DIC during liver transplantation is very difficult as during any surgical procedure coagulation changes occur, akin to findings in DIC [8,9]. The presence of DIC was made plausible by experiments where samples were taken from both the arterial inflow and venous outflow of the recirculated liver graft and an arteriovenous gradient of platelets was found across the graft [10,11]. Attempts to counteract this phenomenon by the administration of heparin have not been successful and have even led to uncontrollable bleeding [12]. Histopathologic examinations of the liver grafts did not identify microthrombi, although this also does not rule out DIC since thrombi could be lysed in situ [13,14]. Therefore the role of DIC in liver transplantation is still open to debate.

Liver transplantation can also be performed by auxiliary heterotopic liver transplantation (HLT), whereby the diseased host liver is left in situ and the graft is placed in a heterotopic position. The clinical results of HLT were initially discouraging, mainly due to technical problems. However after extensive experimental studies a clinical HLT program was started in our institution and shows to date good clinical results [15,16]. In HLT, the anhepatic period is avoided and the host liver can still account for the clearance of procoagulant factors. Therefore (DIC) coagulation changes, if any, may not occur until after reperfusion of the graft. Comparison of OLT and HLT could give more insight into the mechanism of coagulation changes.

Central to the process of DIC is (uncontrolled) thrombin formation. An indirect marker of thrombin generation is the thrombin-antithrombin-III complex (TAT) [17]. In the present study TAT levels were measured during OLT and HLT, to investigate

whether (and when) excessive thrombin generation occurs during liver transplantation. Because the surgical trauma itself also triggers thrombin formation, we measured TAT during partial hepatic resection (PHR), to assess the contribution of the surgical trauma to the elevation of TAT.

Patients, materials and methods

Patients

We studied 12 patients undergoing OLT, 18 patients undergoing HLT and 10 patients undergoing PHR. All procedures were done at the University Hospital Dijkzigt-Rotterdam, The Netherlands. The clinical characteristics of the patients are summarized in table 1.

Prior to transplantation, the liver graft was maintained in Euro-Collins preservation solution in 12 cases of HLT, whereas the UW solution was used in the other cases.

Surgical technique

OLT was performed by a standard technique [18]. The veno-venous bypass was used in 5 cases. The surgical technique of HLT has been described in detail elsewhere [19]. Segments II and III of the left lobe of the liver and the gallbladder were removed in bench surgery and the graft was placed in the right subhepatic

Table 1
Diagnosis in 12 patients undergoing OLT, 18 undergoing HLT and 10 undergoing PHR

Diagnosis	OLT	HLT	PHR
Fulminant hepatitis	4	4	0
Hepatitis B	2	5	0
Autoimmune hepatitis	0	1	0
PBC/PSC*	3	6	0
Carcinoma	2	0	8
miscellaneous	1	2	2

*Primary biliary cirrhosis/sclerosing cholangitis

region. Three vascular anastomoses were made: The suprahepatic vena cava of the graft was placed end-to-side to the infrahepatic vena cava of the recipient; the portal vein was was placed end-to-side to that of the recipient, and the hepatic artery, with an aortic patch, was placed end-to-side to the infrarenal aorta of the recipient. Finally, the common bile duct was anastomosed to a Roux-and-Y jejunal loop.

Perioperative management

Pre-, peri- and postoperative management was identical for both transplantation procedures and has been described previously [19]. Intraoperative blood loss was compensated for by the transfusion of red blood cell (RBC) concentrate and fresh frozen plasma (FFP) in a scheme of 1:1. Additional FFP, cryoprecipitate and platelets were given depending on laboratory results. No antifibrinolytic agents or heparin were given.

Methods

Conventionally OLT is divided into three stages. Stage I begins with the induction of anesthesia and ends with the occlusion of blood flow to the patient's liver. Stage II (anhepatic phase) continues until the donor liver is reperfused by the patient's circulating blood. Stage III is from the moment of reperfusion until closure of the surgical incision. For comparison it was decided that in HLT stage II starts 120 minutes into surgery. Stage I and III are the same as in OLT. In PHR, stage I also begins with the induction of anesthesia and stage II continues from the start of the resection of the liver until completion of the resection, followed by stage III, which continues until closure of the surgical incision.

Blood samples for hemostatic analysis were collected from an unheparinized arterial line into plastic tubes and were anticoagulated with one-tenth volume cold trisodium citrate 0.11 mmol/l. The samples were immediately placed on melting ice and centrifuged (30 min., 2800g, 4°C) within 20 minutes. Plasma was snap-frozen and stored at -70°C until testing.

Blood samples were collected according to the following schedule: immediately after induction of anesthesia, 30 min before stage II, 15 min into stage II, 15 min

before stage III, and 5 to 10, 30, 60 and 120 min after the beginning stage III. Levels of TAT-complex and (conventional) DIC screening tests were measured. The latter included fibrin degradation products (FbDP), prothrombin time (PT), fibrinogen levels , antithrombin-III levels (AT-III) and platelet count. DIC was defined as a substantial decrease in platelet count and fibrinogen associated with a fall in AT-III, a prolongation of PT and elevation of FbDPs

Assays

TAT-complex levels were quantitated with a sandwich-type enzyme-linked immunosorbent assay, using rabbit antibodies to thrombin as tagging antibody and rabbit antibodies to antithrombin-III as catching antibodies [17]. EIA kits for the specific determination of fibrin degradation products (FbDP) and fibrinogen degradation products (FgDP) were obtained from Organon Technica, Turnhout, Belgium. The characteristics of these EIAs have been published before [20]. The PT was performed using routine procedures. Reagent used was Thromborel-S (Behring Diagnostica, Amsterdam, The Netherlands). AT-III activity was measured (according to Abilgaard) [21], using an amidolytic method with the synthetic peptide substrate S-2238 (Coatest^{R} heparin, Kabi, Amsterdam, the Netherlands) and expressed in U/ml (normal plasma=100 U/ml). Fibrinogen was measured according to Clauss [22], using thrombin from Dialab, Leusden, The Netherlands.

Table 2

Preoperative hemostasis parameters (median and range) in patients undergoing OLT and HLT.

	Reference	OLT	HLT
APTT sec	22-45	35.5 (28-76)	39 (28-64)
PT sec	15-19	23.5 (16-82)	23 (17-46)
Factor V U/ml	0.50-1.50	0.41 (0.03-1.57)	0.38 (0.19-1.76)
AT-III U/ml	0.85-1.20	0.44 (0.07-1.21)	0.37 (0.10-1.28)
Fibrinogen g/l	1.6-2.8	2.2 (0.7-6.5)	1.4 (0.8-5.8)
α2-AP U/ml	0.85-1.20	0.55 (0.12-1.01)	0.46 (0.27-1.0)

The difference between OLT and HLT was never significant.

Statistical analysis

Statistical analysis was performed using non-parametric tests. A p-value of less than 0.05 was considered significant.

Results

A preoperative coagulopathy, related to end-stage liver disease was present in both OLT and HLT (table 2). There was no significant difference regarding these preoperative hemostatic factors between OLT and HLT. The Child-Pugh score [23], reflecting the severity of the liver cirrhosis, was significantly higher in patients treated with HLT as compared to those who underwent OLT ($p<0.05$).

The courses of TAT (median) levels during OLT, HLT and PHR are depicted in figure 1. In stage I, TAT levels were not significantly different between OLT, HLT and PHR. However, during stage II, TAT levels increased appreciably in OLT, as compared to HLT ($p<0.02$) and PHR ($p<0.002$). Reperfusion of the graft (stage III) was followed by a sharp increase of TAT levels in both OLT and HLT ($p<0.05$). In contrast TAT levels remained unchanged during stage III of PHR. The absolute increase in TAT after reperfusion was similar for OLT and HLT as is shown in figure 1. TAT levels were not significantly different between OLT and HLT during stage III.

The courses of fibrin degradation products (FbDP), reflecting the breakdown of cross-linked fibrin, are also shown in figure 1. FbDPs increased significantly in stage II ($p<0.008$) of OLT, and remained elevated after reperfusion, whereas they did not alter significantly during HLT and PHR. Fibrinogen degradation products (FgDP), reflecting the breakdown of fibrinogen, followed a similar, though less pronounced, course. In OLT, FgDP levels increased from 0.39 μg/ml at the start of the operation towards to 1.90 μg/ml after reperfusion ($p<0.04$), and descreased till 1.65 μg/ml at the end of the operation. In HLT and PHR, FgDP levels did not alter significantly. Maximum level (0.75 μg/ml) was reached at the end of the operation

The courses of the other conventional DIC parameters, prothrombin time, fibrinogen, AT-III and platelet levels, are shown in figure 2. Starting levels of PT, fibrinogen and AT-III were not significantly different between OLT and HLT. Platelet

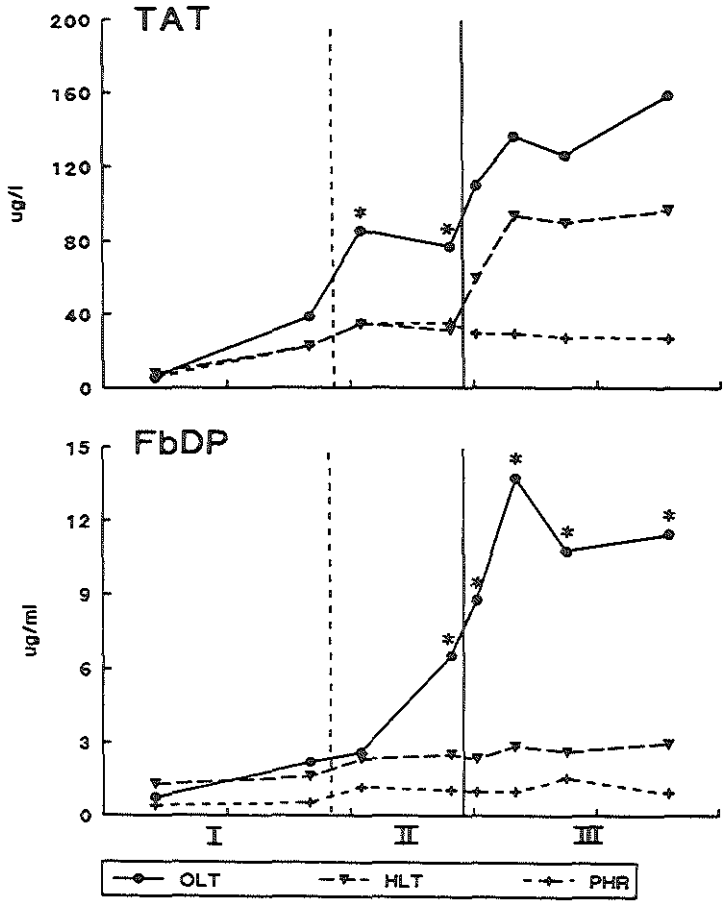

Fig. 1 Median levels of thrombin-antithrombin-III complex fibrin degradation products (FbDP) in orthotopic (OLT) and heterotopic liver transplantation (HLT) and partial hepatic resection (PHR). *p<0.05 OLT compared to HLT and PHR .

levels were at the start significantly lower in HLT as compared to OLT ($p<0.01$). During stage II, levels of PT, fibrinogen, AT-III and platelets did not change significantly in OLT and HLT. After reperfusion, these parameters changed only slightly and returned toward baseline value at the end of the operation. During the

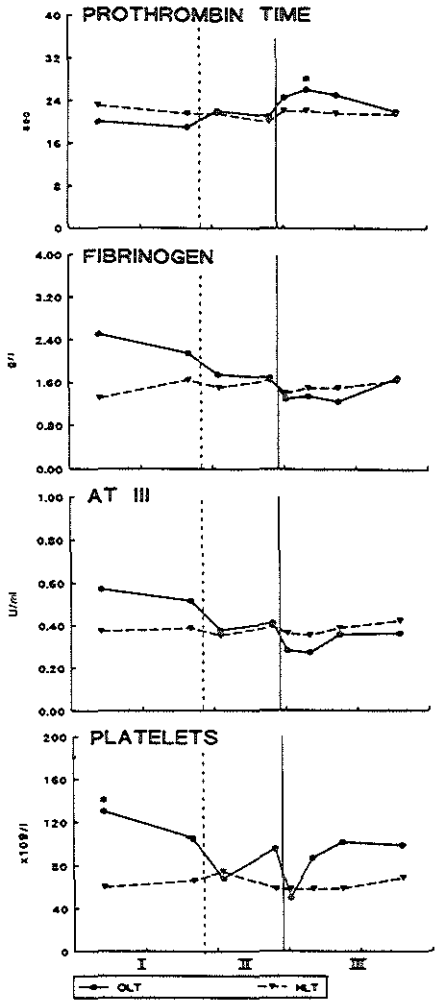

Fig. 2 Median levels of prothrombin time, fibrinogen, antithrombin-III and platelets count in patients undergoing OLT and HLT. *p<0.05 OLT compared to HLT.

entire operation, levels of PT, fibrinogen, AT-III and platelets were not statistically different between OLT and HLT. The PT levels immediately after and 30 minutes after reperfusion ($p<0.03$ and $p<0.02$, respectively).

There was no correlation between TAT and antithrombin-III ($r=0.002$, p ns)

The intraoperative use of blood products is shown in table 3. A breakdown of blood replacement according to the stage of the surgical procedure revealed no statistically significant difference between OLT and HLT during stage II and III.

Table 3

Intraoperative blood use (median, range) in patients undergoing OLT and HLT , subdivided according to the operative phases.

Phase		I	II	III
RBC* (u)	OLT	6.0 (3.0-9.2)	4.0 (1.5-48.6)	3.1 (2.0-32.6)
	HLT	3.0 (2.0-8.0)	5.0 (1.6-24.0)	7.8 (3.0-16.6)
FFP (u)	OLT	1.5 (0.0-12.0)	3.0 (0.0-52.0)	6.0 (2.0-28.0)
	HLT	8.0 (0.0-14.0)	5.0 (0.0-24.0)	8.0 (4.0-36.0)
Cryo (u)	OLT	0.0 (0.0-5.0)	0.0 (0.0-8.0)	3.5 (0.0-10.0)
	HLT	2.0 (0.0-10.0)	0.0 (0.0-8.0)	4.0 (0.0-15.0)
Platelets	OLT	0	0 (0-12)	12 (0-36)
($x10^9$/l)	HLT	0 (0-17)	0 (0-32)	16 (0-32)

*RBC includes packed red blood cells and modified whole blood.

Discussion

The origin of hemostatic disorders during liver transplantation is still not fully elucidated. Increased fibrinolysis plays a major role, but it is still not clear whether DIC is implicated. TAT levels, as indirect measure of thrombin formation, were determined during OLT and HLT and a control group of PHR, in an effort to elucidate whether DIC occurs during liver transplantation.

In OLT, TAT levels increased consistently, with a dramatic increase afterreperfusion. Increased levels of TAT prior to reperfusion most probably are the result of the combination of increased thrombin formation during the laborious removal of the

cirrhotic liver and the lack of hepatic clearance of TAT. The most marked rise in TAT, however, occurred following reperfusion of the graft, both in OLT and HLT. These observations strongly suggest that an excessive amount of thrombin is formed during reperfusion. There are several possible explanations for thrombin generation upon reperfusion; firstly, the endothelial cells are susceptible to cold hypoxia and may be damaged during preservation [24,25]. Free oxygen radicals have been implicated in this mechanism of graft injury [26]. The injured endothelium of the graft may lead to platelet aggregation upon reperfusion and consequently to activation of the coagulation system [27]. Secondly, tissue damage leads to the activation of inflammatory cells, such as macrophages [28,29] These are stimulated to produce cytokines, which may affect coagulation [29,30]. Recently it has been shown that pretreatment with cyclosporin, a powerful inhibitor of the function of macrophages, ameliorated the hemostatic changes after reperfusion in pigs, as measured by prothrombin times, platelet count and fibrinogen levels [31]. Surgery itself causes a relatively small increment in TAT levels, as TAT levels increased only slightly in PHR. This is most probably a sign of local clotting activation on the wound surfaces.

Although elevation of TAT reflects increased thrombin formation, it is not yet clear, whether this actually develops into the DIC syndrome. DIC is a dynamic condition with ongoing fibrin formation and secondary fibrinolysis. FbDP levels, as parameter of fibrinolysis, were found to increase in concert with TAT levels in OLT, but not in HLT. As the amount of thrombin generation in OLT and HLT was comparable, the difference in FbDP levels between both procedures suggests that the increase in FbDP in OLT is not inherent to DIC. Most probably FbDP levels are due to primary fibrinolysis. It has recently been demonstrated that increased fibrinolysis is present during OLT and not during HLT [32,33]. Moreover, other parameters of DIC, such as PT, fibrinogen, AT-III and platelet count, did not change as dramatically as TAT levels, both in OLT and HLT, and returned already towards baseline, when TAT levels were still high. These findings, especially the lack of increase of FbDPs in HLT, indicate that, although thrombin formation is increased after reperfusion, the subsequent development of DIC is mild or absent. This phenomenon has also been reported in patients with liver cirrhosis and has been explained by the key role of

AT-III in the regulation of coagulation; If AT-III levels are sufficiently high, any thrombin formed is very rapidly inhibited by AT-III [34]. This leads to increased levels of TAT, but prevents thrombin acting on fibrinogen and thus the development of DIC. However if AT-III reach very low levels (less than 0.30 U/ml), thrombin escapes the control of AT-III and intravascular coagulation may ensue. Whereas some have reported beneficial effects of AT-III supplementation on the reduction of DIC, others failed to observe such an effect [7,35-37]. Possibly, in the latter studies AT-III levels did not reach levels low enough to allow the development of DIC.

In conclusion: the present study provides evidence for increased thrombin formation during reperfusion of the graft, both in OLT and HLT. However there were no signs of the subsequent development of DIC. Any thrombin formed is most probably so rapidly inhibited by AT-III that it does not cause measurable coagulation. The concentration of AT-III could be of importance in the prevention of intravascular coagulation.

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Chapter 5

The effects of long-term graft preservation on intraoperative hemostatic changes in liver transplantation

A comparison between orthotopic and heterotopic transplantation in the pig

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Abstract

We compared hemostatic changes during OLT and HLT after various periods of graft storage, to investigate whether the host liver in HLT protects the recipient from hemostatic deterioration induced by severe graft storage damage. In particular, the mechanism of fibrinolytic deterioration was investigated. The effect of prostaglandin E_1 (PGE_1) on these parameters was also studied.

Material and methods: 69 pigs underwent either OLT (N=32) or HLT (N=37) with a graft stored for 2 hr (N=31), 24 hr (N=16), 48 hr (N=7), or 72 hr (N=15). PGE_1 was given intravenously to both donor and recipient animals and was added to the preservation and flushing solutions. Fibrinolysis (euglobulin clot lysis time, t-PA activity and α_2-antiplasmin) and coagulation parameters (activated partial thromboplasmin time, prothrombin time, fibrinogen and platelet count) were measured at several intervals during transplantation.

Statistics: Univariate non-parametric tests were used for analysis of coagulation and fibrinolysis parameters. For the three main variables- i.e., the type of transplantation, the use of PGE_1, and the preservation time, multiple regression analysis was performed.

Results: Fibrinolytic activity increased during the anhepatic period of OLT. Graft reperfusion was followed by a rise in t-PA in both OLT and HLT. In HLT, t-PA quickly returned to normal, whereas a continuous increase was found in OLT. The coagulation parameters, in turn, remained unchanged during the anhepatic period and deteriorated in OLT compared to HLT. The duration of graft storage was directly related to the severtiy of the hemostatic changes, although this effect was more apparent in OLT than in HLT.

Conclusions: Changes in hemostasis are more pronounced in OLT than in HLT. This suggests that the host liver protects the recipient from the effects of graft storage damage, even after long preservation times. Early postreperfusion fibrinolytic activity was presumably due to t-PA release from the graft both in OLT and HLT. The further rise t-PA in OLT might be caused by the release of cytokines from the graft, that subsequently evoke endothelial t-PA release. In HLT, t-PA and cytokines may be cleared by the native liver. No positive or negative effect of PGE_1 on coagulation or fibrinolysis parameters was noticed.

Introduction

Orthotopic liver transplantation (OLT) has become an accepted method to treat patients with end-stage chronic liver disease. However, the procedure is frequently complicated by severe changes in hemostasis. Both increased fibrinolysis [1,2] and disseminated intravascular coagulation [3,4] have been implicated in playing a role. The most striking abnormalities occur late in the anhepatic period and become more marked after reperfusion of the graft [5,6]. Earlier studies have demonstrated that the severity and duration of hemostasis abnormalities were mainly related to the quality of the donor liver [7,8]. Release of activated hemostasis factors and/or humoral substances from the graft, that may interfere with hemostasis have been suggested to play a role.

Auxiliary heterotopic liver transplantation (HLT) has been proposed as an alternative to hepatic replacement. In HLT, the host liver is left *in situ* and the graft is transplanted in a heterotopic position. The anhepatic period is avoided and the function of the host liver retained. Hence, substances released from the allograft at reperfusion might be cleared and the deterioration of hemostasis less severe. Recently, we demonstrated that changes in fibrinolysis after reperfusion are more severe and sustained in OLT than in HLT after 2 hours of simple cold graft storage [9]. This suggested that the native liver in HLT protects the recipient from the changes induced by preservation damage. We were now interested in wether the host liver in HLT also protects the recipient from the effects of long-term preserved grafts as well as the mechanism of hemostatic deterioration. We have thereby concentrated on fibrinolysis and included, addtionally to previous studies, t-PA activity measurement.

Graft protection by addition of prostaglandin E_1 (PGE_1) to the flush solution has been demonstrated in human liver transplantation [10]. Furthermore, succesful liver transplantations have been reported after long-term simple cold storage with University of Wisconsin (UW) solution without addition of prostaglandins: for 24 hours and more in man [11] and for 48 hours in dogs [12]. It is not known if cytoprotective agents give additional protection. Therefore we also studied the effects on hemostasis of adding of $PGE_1$to the preservation fluid

Methods

Sixty-nine female Yorkshire pigs were randomly allocated to OLT (N=32) or HLT (N=37). Thirty-one grafts were transplanted after 2 hr of cold storage, 16 after 24 hr, 7 after 48 hr and 15 after 72 hr. In the 2-hr experiments, Eurocollins' solution was used for graft storage because UW solution was not yet available. In the long-term preservation experiments (24-hr, 48-hr, and 72-hr), UW solution was used. Sixteen transplantations in the different preservation groups were performed using PGE_1 (Table 1). Body weights of the donor and recipient animals were similar, about 25 Kg. Histocompatibility was matched by the mixed lymphocyte culture-test [13].

Surgical technique

Donor and recipient operations were performed as described earlier [14]. In OLT, a venovenous heparin-coated iliacoportajugular bypass was introduced [15].

Blood loss was estimated from the amount of liquid sucked away from the surgical field and collected in Buleaux bottles during the transplantation. Depending on the amount of blood loss, 500-1000 ml donor blood was given to the recipient. Usually, this was necessary around 30 minutes after graft reperfusion. Anesthetic techniques were the same for all animals.

Prostaglandin

When PGE_1 was given, this was started 60 min before donor hepatectomy by an intravenous infusion rate of 100 ng/kg/min [16]. In addition, 1 mg/L PGE_1 was added to the UW solution [10]. The recipient animal was treated with a similar infusion as the donor, starting about 60 min before the recirculation of the graft. Before implantation, the graft was rinsed via the portal vein with two liters of cold (4°C) 0.9 % saline to which 1 mg/L PGE_1 was added. PGE_1 was supplied by The Upjohn Company, The Netherlands.

Table 1.

Number of animals in the various preservation groups.

	Experimental group:								TOTAL:
	2 hr[a]		24 hr		48 hr		72 hr		
	OLT	HLT	OLT	HLT	OLT	HLT	OLT	HLT	
PGE_1+	0	0	4	4	1	2	1	4	16
PGE_1-	16	15	4	4	1	3	5	5	53
TOTAL	16	15	8	8	2	5	6	9	69

[a] Duration of liver preservation.

Blood samples

For comparison, the moments of measurement were synchronized, relative to the moment of recirculation. Blood samples were drawn around every important operative step according to the following schedule: Starting value; ± 1 hour before reperfusion (R-60), 5 minutes before recirculation (R-5), 5 minutes after recirculation (R+5), 30 minutes after recirculation, during the reconstruction of the arterial anastomosis (R+30), 60 minutes after recirculation (R+60) and 90 minutes after recirculation (closure) (R+90). Additionally, on reperfusion, the first 30 ml of perfusate were drawn from the infrahepatic vena cava of the graft (FR). These first hepatic outflow samples (FR) after reperfusion were also subjected to hemostasis studies.

In OLT, R-5 is a measurement in the anhepatic phase. In HLT, there is no real anhepatic phase, but during the portal vein anastomosis there is a period of partial clamping of the portal vein. In the pig this often implies (almost) complete clamping. Blood samples (20 ml) were taken from an arterial line. Eighteen ml of blood was divided equally into two polystyrene test tubes, containing 1 ml ice-cold trisodium

citrate 0.11 mol/l and immediately placed on melting ice. Plasma was collected after centrifugation (2800 g, 4°C, 20 min), snap-frozen and stored in small aliquots at -70°C until used. Two ml blood was also collected into 0.045 ml 15% sol 6.75 mg EDTA.

A normal plasma pool was prepared from equal volume samples obtained from 10 animals immediately after induction of anaesthesia. This pool was defined as having 100% of normal coagulation and fibrinolysis proteins.

Hemostasis studies

Tissue-type plasminogen activator (t-PA) was assayed according to Verheijen et al. [17] using plasminogen, t-PA stimulator and S-2251 (from Kabi Diagnostica, Woerden, The Netherlands) and anti (porcine-heart) t-PA immunoglobulin (from Biopool AB, Umea, Sweden). To determine the euglobulin clot lysis time (ECLT), standard euglobulin fractions of plasma were prepared at pH 5.9 with a plasma dilution of 1:10 [18]. Precipitates were redissolved in Tris/Tween buffer (0.1 M Tris/HCl, containing 0.1% Tween 80 [v/v] pH 7.5) and to 0.2 ml aliquots of the dissolved euglobulin fractions 0.1 ml portions of calcium-thrombin solution ($CaCl_2$ 25 mmol/l and thrombin 10 NIH U/l) were added to induce clot formation. The lysis time of the clot was recorded. The disappearance of air bubbles was regarded as the endpoint of lysis. α_2-Antiplasmin (α_2-AP) activity was measured according to Friberger et al. [19] using Coatest® antiplasmin (Kabi Diagnostica, Woerden, The Netherlands). Fibrinogen was measured according to Clauss [20] using thrombin from DiaLab, Leusden, The Netherlands.

Routine clotting times, including the activated partial thromboplastin time (aPTT), reflecting the intrinsic coagulation pathway and the prothrombin time (PT), reflecting the extrinsic pahtway, were performed according to standard procedures. Reagents used were Actin® (Baxter Dade AG, Düdingen, Switzerland) for the aPTT and Thromborel-S® (Behring Diagnostica, Amsterdam, The Netherlands) for the PT.

Statistics:

Data were subjected to computerized statistical analysis (PATFILE statistical package). Continuous variables are given as median (mean, ± standard error of

the mean) and analyzed by nonparametric tests: in case of independent samples the Mann-Whitney test was used, and in case of paired samples the Wilcoxon rank-sum test. The coagulation changes were also analyzed in a multiple regression model (SPSS/PC+) using the three main variables in this study: the type of transplantation, the use of PGE_1 and the preservation time. The relative influence of these variables on a coagulation parameter is represented by the T-value (ratio between B and Standard Error of B) together with the significance of T.

Results

All median values are given in table 3. Changes from time to time, grouped according to the hemostasis parameters are elaborated on in the following paragraph. Data are presented separately for each of the three main variables in this study: the type of transplantation, the effect of graft preservation time, and the effect of PGE_1.

Type of transplantation

Fibrinolysis parameters: The changes in t-PA activity were reciprocal to those in ECLT. During OLT, t-PA activity levels rose significantly during the anhepatic period ($P<0.02$), and increased sharply after reperfusion ($P<0.05$) till the end of the operation. In contrast, during HLT, t-PA activity levels remained low before reperfusion, increased temporarily after reperfusion ($P<0.01$) and normalized again thereafter. The proportional increase in t-PA activity was similar in HLT and OLT immediately after reperfusion. At all sampling points, except R-60 and R+5, the t-PA activity in OLT was significantly higher than in HLT (at R-5: $T=-2.20$, $P=0.03$ and at R+30 through R+90: T between -2.79 and -3.12, $P<0.01$).

The t-PA activity levels were higher in the first venous outflow of the graft (FR) in HLT (1.22 IU/ml, median value) compared to OLT (0.36 IU/ml, $P<0.02$), while this difference was not yet present in the portal vein blood entering the graft ($T=1.06$, $P=0.32$).

α_2-AP levels declined significantly from R-60 through R+5 ($P<0.001$), and remained

Table 2.

Hemostatic parameters at the various operative moments: values are given as median (95% confidence limits of the mean).

Parameter:	R-60[a]	R-5	FR	R+5	R+30	R+60	R+90
t-PA (IU/ml)							
OLT	0.07 (0.05-1.30)	0.33 (0.35-1.01)	0.36 (0.34-1.35)	0.61 (0.46-1.89)	1.08 (1.05-0.43)	1.36 (0-9.59)	1.86 (0.25-8.22)
HLT	0.05 (0.04-0.13)	0.12 (0.09-0.24)	1.22 (1.23-4.36)	0.56 (0.53-1.20)	0.22 (0.34-1.24)	0.50 (0.30-1.09)	0.26 (0.09-0.05)
ECLT (min)							
OLT	>180 >180	>180 (127-168)	121 (100-144)	126 (97-147)	105 (79-132)	134 (65-168)	115 (67-158)
HLT	>180 >180	>180 (179-181[b])	119 (79-137)	170 (119-161)	>180 (138-173)	>180 (139-181[b])	>180 (159-181[b])
α_2-AP (U/ml)							
OLT	99 (84-102)	64 (53-67)	52 (40-53)	43 (41-53)	49 (38-54)	55 (35-67)	57 (49-65)
HLT	99 (90-103)	73 (67-82)	66 (53-71)	52 (49-63)	60 (52-66)	62 (55-69)	69 (62-76)
aPTT (sec)							
OLT	16 (15-17)	18 (18-20)	33 (28-50)	25 (27-61)	31 (33-73)	35 (31-97)	32 (7-145)
HLT	16 (15-16)	16 (11-35)	34.4 (37-78)	25 (23-59)	24 (21-39)	25 (25-40)	21 (17-33)
PT (sec)							
OLT	13 (13-14)	14 (14-16)	20 (19-33)	18 (17-21)	21 (19-28)	25 (21-29)	23 (20-27)
HLT	14 (13-15)	15 (12-24)	23 (24-38)	17 (12-37)	17 (17-19)	19 (17-20)	19 (18-21)
Fibrinogen (g/l)							
OLT	1.55 (1.36-1.92)	0.80 (0.75-0.99)	0.45 (0.34-0.58)	0.60 (0.45-0.72)	0.64 (0.48-0.75)	0.65 (0.41-0.78)	0.70 (0.58-0.86)
HLT	1.40 (1.34-1.62)	0.85 (0.71-1.02)	0.59 (0.45-0.74)	0.72 (0.59-0.88)	0.70 (0.64-0.94)	0.72 (0.63-0.91)	0.81 (0.67-1.15)
Platelets (*10^9/ml)							
OLT	392 (338-432)	315 (264-346)	228 (199-294)	220 (176-282)	171 (153-245)	99 (66-230)	[c]
HLT	370 (327-469)	291 (275-430)	191 (158-296)	223 (199-308)	170 (169-253)	150 (125-267)	

[a] Abbreviations according to Table 1.
[b] Upper 95%-confidence limit exceeds 180 min.
[c] No samples available.

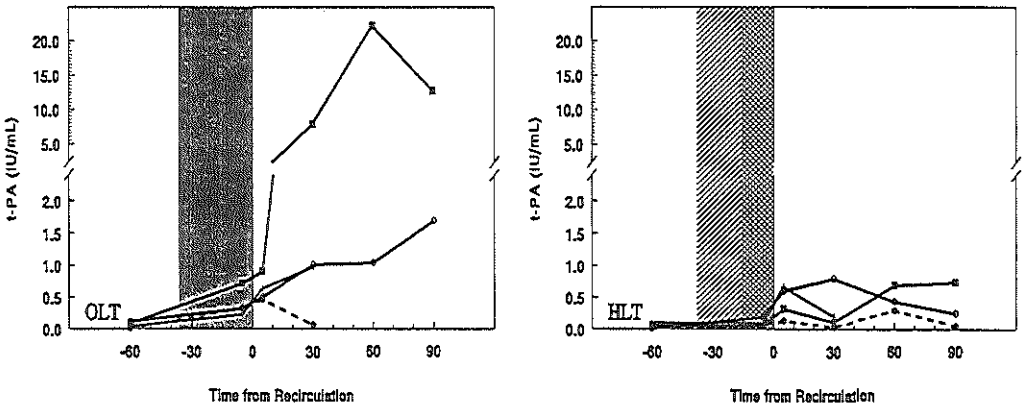

Fig 1. Tissue-type plasminogen activator (t-PA) activity levels (median) during liver transplantation. The horizontal axis gives the time in minutes to or from the moment of graft recirculation.
Symbols: (OLT) Orthotopic Liver Transplantation is illustrated on the left and (HLT) Heterotopic Liver Transplantation on the right. Preservation time: +——+ : 2 Hr; o——o : 24 Hr; ⊛——⊛ : 48 Hr (line is interrupted as this group consists of only seven animals); ▫——▫ : 72 Hr. Shaded areas: ▩ is the anhepatic period in OLT; in HLT ▨ is the period in which the subhepatic inferior vena cava is partially clamped and ▩ in which also the portal vein is (partially) clamped.

almost unchanged thereafter. α_2-AP levels were lower in OLT than in HLT in the postreperfusion period from R+30 through R+90 (T between 2.08 and 3.37, $P<0.05$).

Coagulation parameters: The PT and aPTT increased slightly in the period before reperfusion and increased significantly thereafter ($P<0.001$). Before reperfusion and immediately thereafter, there was no significant difference between OLT and HLT for these tests, but in the postreperfusion period, at R+30 and R+60, the PT ($T=-3.48$ and $T=-3.55$, $P=0.001$) and aPTT ($T=-3.83$ and $T=-4.11$, $P<0.001$) were prolonged in OLT compared to HLT.

Fibrinogen levels practically halved before reperfusion ($P<0.001$) without reaching a significant difference between both types of transplantation ($P=0.98$). After

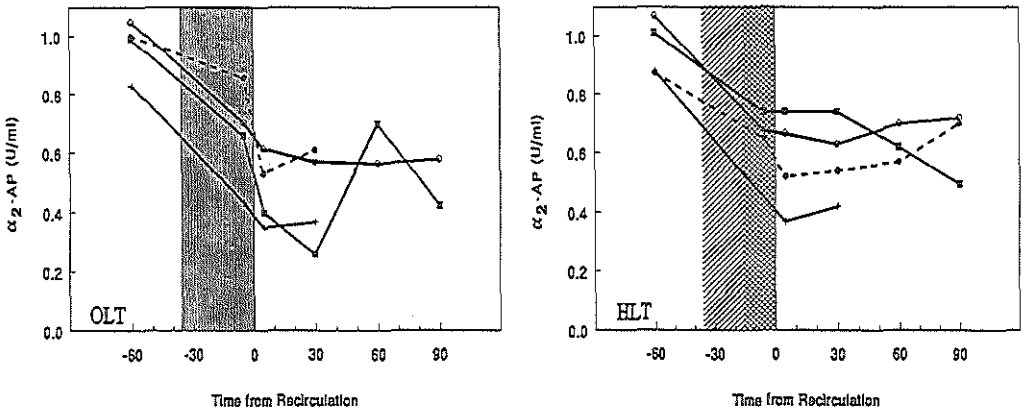

Fig. 2. α_2-Antiplasmin (α_2-AP) levels during liver transplantation. Axes and symbols as in Figure 1.

reperfusion, fibrinogen levels remained stable and at R+5 and R+30 the fibrinogen level in OLT was significantly lower compared to HLT (T=2.05 and T=2.09, P<0.05).

Graft storage time

Fibrinolysis parameters: The preservation time had an evident effect on the fibrinolysis parameters, although not yet apparent at R+5 (for t-PA: T=0.45, P=0.66). Especially at R+60 and R+90, the t-PA activity (figure 1), the ECLT and α_2-AP deteriorated with increasing preservation times. The cold storage time had some effect on t-PA activity and ECLT before reperfusion, although these did not reach statistical significance in the multiple regression analysis (T=-0.88, P=0.39 and T=-0.86, P=0.39).

The first venous outflow (FR) from the 2-hr and 24-hr preserved grafts contained high levels of t-PA activity. No increased t-PA levels could be demonstrated in the first venous outflow from the 48-hr and 72-hr preserved grafts.

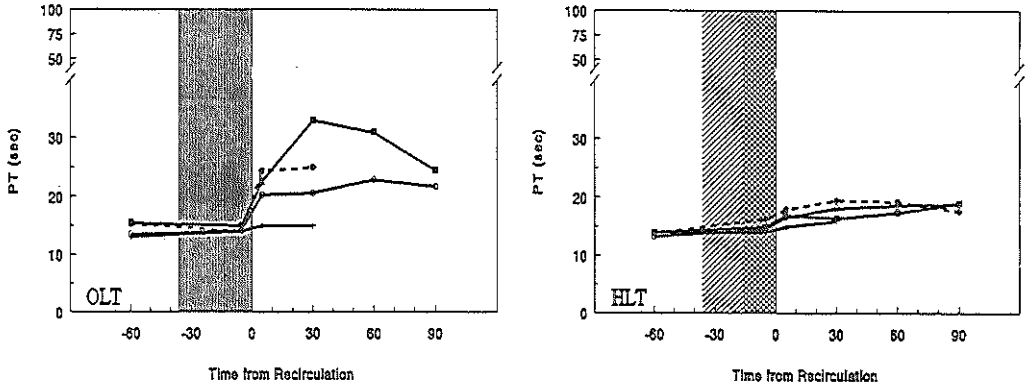

Fig. 3. Prothrombin Times (PT) in during liver transplantation. Axes and symbols as in Figure 1.

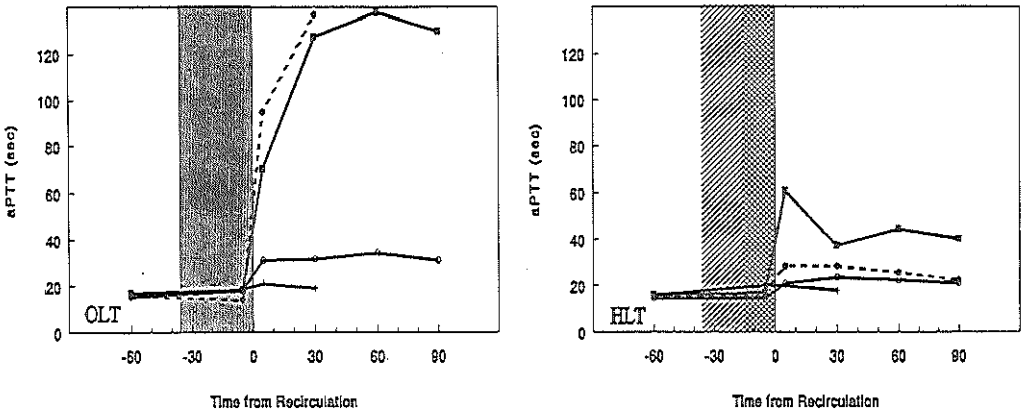

Fig. 4. Activated Partial Thromboplastin Times (aPTT) during liver transplantation. Axes and symbols as in Figure 1.

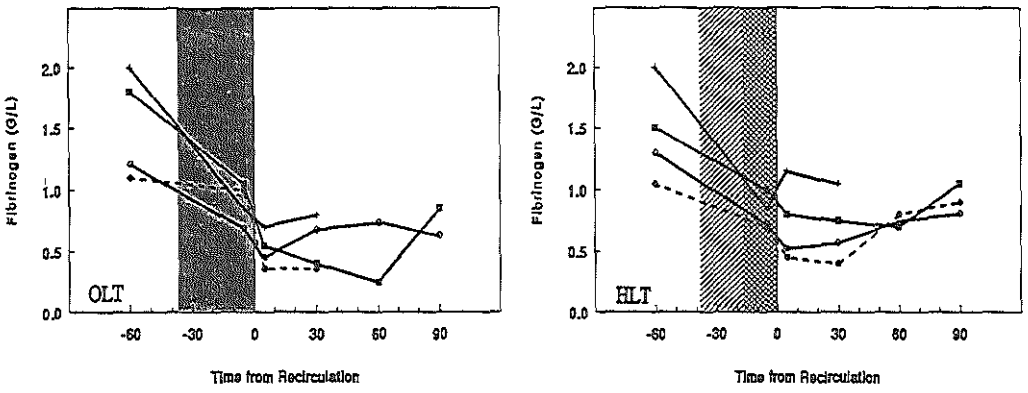

Fig. 5. Fibrinogen levels during liver transplantation. Axes and symbols as in Figure 1.

Coagulation parameters: The multiple regression analysis for the effect of the graft storage time revealed a clear influence on the PT and aPTT levels (Figures 2 and 3, respectively). This was evident in the period after reperfusion, starting at R+5 ($T=2.38$, $P=0.02$ and $T=3.88$, $P<0.001$) and continuing till R+30 for PT ($T=3.17$, $P<0.01$) and R+60 for aPTT ($T=3.93$, $P<0.001$).

In contrast, fibrinogen levels were not significantly influenced by graft storage time. At R+5 and R+30 a longer ischemic period correlated with a decreased fibrinogen level, but the difference did not reach significance.

The preservation time had an evident effect on the platelet count (all T-values below -3.0 and $P<0.005$).

Use of PGE_1

Niether coagulation not fibrinolysis tests were affected by PGE_1 administration at any of the measured points (data not shown).

Table 3

Median blood loss and blood transfusion during the transplantation

	2 hr		24 hr		48* hr		72 hr	
	OLT	HLT	OLT	HLT	OLT	HLT	OLT	HLT
Blood loss								
-median	400	500[a]	850	425[b]	160	1125	1100	925[b]
-lowest	250	250	400	100		700	600	200
-highest	1800	1200	2200	700		1500	2000	1500
Blood Transfusion								
-median	650	600	1000	600[b]	750	1100	600	1000[b]
-lowest	450	400	200	500		900	450	375
-highest	2000	1100	1200	1200		1250	1000	1500

[a] The blood loss in the 2 hr experiment is lower compared to the longer preservation groups ($p=0.03$).
[b] Significantly different from OLT.
* Only one animal represented

Blood loss and survival

The amount of blood loss and blood transfusion in the different groups are given in table 3. In the multiple regression analysis blood loss was significantly controlled by the type of transplantation ($T=-2.33$, $p=0.02$) and the preservation time ($T=2.88$, $p<0.01$). Because none of the animals survived OLT after 72-hr graft storage, many never received a second unit of blood. This explains why it appeared that HLT required more blood transfusion in the 72-hr experiment despite the higher blood loss in OLT.In the multiple regression the duration of preservation was a significant determinant of survival ($T=-3.59$,$p<0.001$). Survival was not significantly different in the 2-hr preservation experiments. However, in the 24-hr and 48-hr experiments, survival was significantly longer after HLT compared to OLT ($p<0.05$). Survival in the extended preservation experiments was dismal: In OLT all animals died the day of the operation after 48-hr and 72-hr graft preservation (three of which died within

Table 4

Causes of death after liver transplantation.

	2 hr		24 hr		48 hr		72 hr	Total
	OLT	HLT	OLT	HLT	OLT	HLT	OLT	HLT
Total	16	15	8	8	2	5	6	969
Hemorrhage	3	1	4	2	2	5	3	626
Portal vein block[a]						3	1	4
Heart failure			1	1				13
Cholangitis	1		1					2
Volvulus	3	2		1				6
Rejection[b]	2							2
Technical		4						15
Sacrificed	3	5						8
Others[c]	2	2		1				5
Unknown	2	1	3	2				8

[a] Total intrahepatic thrombosis of the portal venous system
[b] Only if rejection was thought to be the direct cause of death
[c] Pulmonary sepsis (n=3),pneumothorax (n=1), peritonitis (n=1).

30 minutes after repefusion) and in HLT all animals after 72-hr graft preservation. Three animals survived more than 3 weeks after HLT of a 24-hr preserved graft. Two more animals survived after HLT for two days, one in the 24-hr group and one in the 48-hr group. In table 4 the causes of mortality are categorized.

Discussion

The results from this study clearly show that the host liver in HLT also protects the recipient from the deleterious effects of long-term preserved grafts on hemostasis. We found a dramatic and sustained increase in t-PA activity after reperfusion in OLT, while the increase in t-PA in HLT was limited and transient. In OLT, the deterioration of t-PA activity was directly related to the duration of graft storage. In

HLT this effect was much less evident, which demonstrated that the protective effect of the host liver on the development of hyperfibrinolysis also holds true with longer graft storage times. There are several explanations for the increase of t-PA after reperfusion. First, endothelial cells, an important source of t-PA, are more susceptible to cold hypoxia and reperfusion than parenchymal cells [21,22]. Free oxygen radicals have been implicated in this mechanism of graft injury [23,24]. The damaged endothelium may release t-PA directly into the circulation. Another theory is that the tissue damage leads to the attraction of inflammatory cells, including macrophages [25,26]. When activeted, these cells are stimulated to produce cytokines (interleukin-1, tumor necrosis factor), which may actively interfere with coagulation and fibrinolysis [27]. Recently, it has been shown that pretreatment with cyclosporine, a powerful inhibitor of the function of macrophages, ameliorated the hemostatic changes after reperfusion in pigs, as measured by prothrombin times, platelet counts, and fibrinogen levels [28]. The initial increase in t-PA activity in HLT may be explained by a too abundant release of t-PA to be instantaneously cleared by the host liver.

Comparison of t-PA activity levels in the portal vein inflow and the venous outflow revealed a gradient across the graft, which indicated that t-PA is also actively released by the graft. It was found that t-PA levels in the first hepatic outflow of the graft were higher in HLT than in OLT. A possible explanation is that the partial resection of the liver graft in HLT is responsible for this. This extra handling could make endothelial cells more liable to release t-PA [29]. It was notable that the high t-PA activity levels were only found in the first hepatic outflow from the 2-hr and 24-hr cold storage grafts and not in the 48-hr and 72-hr groups. Possibly, the endothelial cells in the latter group are damaged to such an extent that they no longer contain t-PA. Their contents, including t-PA, may have leaked into the preservation fluid, which is subsequently washed out during flushing before reperfusion. Despite this t-PA depletion, subsequent contact activation of the blood with the injured graft endothelium may evoke systemic t-PA release as suggested above. Probably, the same process occurred in HLT, but now the cytokines may be cleared by the host liver.

Fibrinogen levels, in turn, showed a serious decline in the *pre*reperfusion period in

both OLT and HLT. There was no difference between OLT and HLT, which suggestes that the changes are related to the surgical procedure in general and not to the anhepatic period. Previous investigators have suggested that fibrino*geno*lysis during the use of an external bypass might also contribute to the decrease in fibrinogen levels [15,30]. The latter is however likely to be of minor influence, as in HLT no signs of hyperfibrinolysis were present nor was a shunt used. Although part of the decline can be ascribed to hemodilution, there has to be another explanation as the hematocrit, a measure of hemodilution, did not equally decrease. Most likely a surgically induced activation of the coagulation system and extravasation of hemostasis proteins to the extravascular space are involved [29].

Similar to the fibrinolytic parameters, reperfusion induced a serious deterioration in coagulation parameters in OLT, whereas the changes in HLT remained limited. There was an apparent influence of the graft storage time. Presumably release of procoagulant factors from the preserved graft or local clotting activation on the damaged endothelium had initiated a consumption coagulopathy, as has been previously described. The present observations, however, show that HLT has also a protective effect on coagulation changes.

Remarkable effects of PGE_1 were found in preventing warm ischemic and reperfusion injury [31] in both experimental [32] and clinical [10] studies. We therefore implemented a trial of PGE_1 in our experiment with long-term preservation. As determined by fibrinolytic and coagulation tests, our study provides no evidence of a protection against hemostatic disturbances. As opposed to the multitude of positive studies on PGE_1, it is difficult to explain why we found no protective effect at all. Besides having included not enough animals to detect small differences, there is probably only a narrow margin of therapeutic efficacy, if it exists, between trivial and irreversible damage. Apparently, the upper limit is surpassed with 24 hr preservation of the porcine liver. In addition, prostaglandins can be assumed to counteract a physiological protective mechanism that secludes marginally functioning areas in the graft. This may well prove useful in retrieving reversibly injured parenchyma [33] but in contrast, it could promote the entrance into the bloodstream of deleterious substances originating from injured cells.

In conclusion, it was demonstrated that in pigs changes in hemostasis after long-

term graft storage are less dramatic in HLT compared to OLT. This suggests that HLT also protects the recipient from the effects of long-term preserved grafts. Addition of PGE_1 to the preservation fluid had no effect on the hemostatic changes in both OLT and HLT. Although these experiments are performed in the presence of a healthy host liver (although deprived from portal blood after the transplantation), while in the clinical situation the host liver is diseased, some remaining liver function is always present. The results are indicative of the benifical role of the (remnant function of the) host liver on hemostasis.

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Chapter 6

Donor-heparin may appear in the recipient after reperfusion of the liver graft

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Abstract

The release of heparin has been mentioned as one of the causes of hypocoagulability after reperfusion of the liver graft. It has been ascribed to endogenous heparin released from the donor liver or to exogenous heparin in the preservation fluid that is released into the recipient after sequestration into the graft during preservation. The aim of this study was to investigate whether systemic administration of heparin to the donor before the hepatectomy contributes to the appearance of heparin in the recipient after reperfusion.

We studied 20 patients undergoing an auxiliary heterotopic liver transplantation; 15 donors had received heparin immediately before circulation arrest (median 300 IU/kg bodyweight), but 5 did not. The thrombin time (TT), activated partial thromboplastin time (aPTT) and heparin neutralisation test were determined at several intervals during the transplantation.

In the recipients of a heparinized donor a significant systemic prolongation of the TT ($p<0.004$) and aPTT ($p<0.0002$) was observed after reperfusion, that could be reversed with protamine sulfate. The recipients of an unheparinized graft did not reveal a significant prolongation of the TT ($p<0.2$). The first venous hepatic outflow was collected from 10 heparinized donors and contained heparin as reflected by a prolongation of the TT and its reversal by protamine. In 4 of the 5 samples from an unheparinized graft, no heparin was detectable and in 1 a heparin effect was detectable, suggesting a component of endogenous heparin release

The results suggest that exogenous heparin bound to the endothelium during the donor procedure may be released into the recipient upon reperfusion, and may contribute to the reperfusion coagulopathy.

Introduction

Coagulation problems have complicated clinical and experimental transplantations of the liver since the first was performed [1-5]. Increased fibrinolysis [6,7] and disseminated intravascular coagulation [8-10] both have been implicated. In addition an isolated prolongation of the thrombin time has been described, that could be (partially) neutralized by protamine sulfate, suggesting the presence of heparin [2,11,12]. The question then arises as to the source of the heparin, which is exerting the heparin effect. Some investigators have ascribed it to endogenous heparin or heparin-like substances released from donor livers [2,11,12], while others have suggested that the heparin in the preservation fluid becomes sequestered in the hepatic graft and enters the circulation after reperfusion [13,14]. Studies advocating the endogenous release of heparin are based on studies performed in dogs. These animals are especially susceptible to release of these substances during period of shock or acute phase reactions [15,16] and the results therefore do not necessarily apply to humans. Although most transplantation centres avoid systemic heparinization of the recipient, a "heparin effect" may still be seen after graft reperfusion in some patients, that can be easily treated with protamine sulfate [13]. As most donors receive a dose of heparin immediately before the liver is removed, in case of a simultaneous donation of the heart, the question arises whether this heparin may be retrieved in the recipient after reperfusion. The present study was designed to investigate whether systemic administration of heparin to the donor may be responsible for the appearance of heparin in the recipient after graft reperfusion.

Materials and methods

Twenty consecutive patients undergoing auxiliary heterotopic liver transplantation (HLT) were included in this study. No heparin was used during the transplantation, either in the preservation fluid, the perfusate, in the arterial lines or the syringes used to draw blood samples. The clinical characteristics of the patients are shown in table 1.

Table 1

Diagnosis in 20 patients undergoing HLT

Diagnosis	No	F	M	Age range
Fulminant hepatitis	4	3	1	18-35
Viral hepatitis	5	5	-	32-50
Autoimmune hepatitis	1	1	-	20
PBC/PSC*	8	5	3	26-57
miscellaneous	2	1	1	43,59

*Primary biliary cirrhosis/sclerosing cholangitis

Fifteen donors received heparin (median 300, range 83-385 IU/kg bodyweight) shortly before the donor hepatectomy and 5 donors received no heparin. The first 8 liver grafts (3 from a heparinized donor and 5 from an unheparinized donor) had been flushed during the implantation with 0.9% saline at a temperature of 20°C, whereas the remainder 12 had been flushed with 0.9% saline at a temperature of 4°C.

The surgical technique of HLT has been described in detail elsewhere [17]. Segments II and III of the left lobe of the liver and the gallbladder were removed in bench surgery and the graft was placed in the right subhepatic region. Three vascular anastomoses were made: the suprahepatic vena cava of the graft was placed end-to-side to the infrahepatic vena cava of the recipient; the portal vein was placed end-to-side to that of the recipient, and the hepatic artery, with an aortic patch, was placed end-to-side to the infrarenal aorta of the recipient. Finally, the common bile duct was anastomosed to a Roux-and-Y jejunal loop.

Blood samples were collected according to the following schedule: immediately after induction of anaesthesia, 60 and 90 minutes into surgery, 10 minutes before reperfusion and 5 to 10 minutes, 30, 60 and 90 minutes after reperfusion. In addition, a blood sample was taken from the graft liver vein, at the moment the graft was reperfused with the recipient's systemic blood (first venous hepatic outflow). The samples were collected from an unheparinized arterial line in plastic

tubes and were anticoagulated with one-tenth volume cold trisodium citrate 0.11 mmol/l. The samples were immediately placed on melting ice and centrifuged (30 min., 2800 g, 4°C) and assayed within 20 minutes.

Assays

The activated partial thromboplastin time (aPTT) was performed according to routine procedures. The reagent used was Actin^R (Baxter Dade AG, Dübingen, Switzerland). The thrombin time (TT) was measured by adding 200 µl bovine thrombin (±5 IU/ml) (Baxter Dade AG, Dübingen, Switzerland) to 200 µl prewarmed

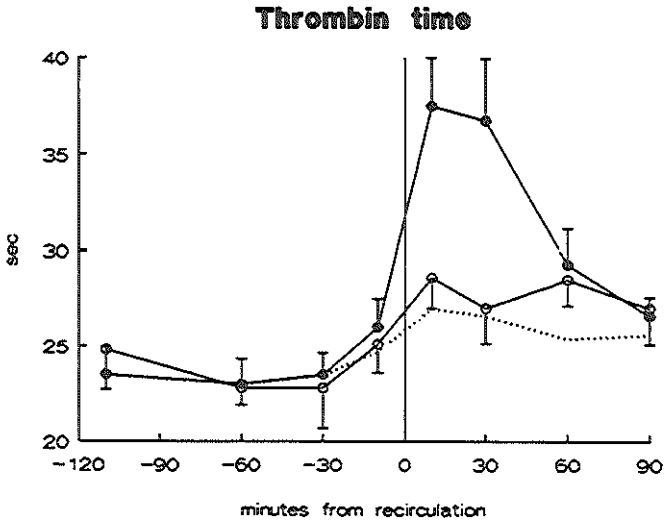

Fig. 1 Intraoperative course (mean±sem) of the thrombin time in heterotopic liver transplants receiving a graft from a heparinized (closed circles) compared those receiving a graft from an unheparinized donor (open circles). The broken line reflects the reversal of the thrombin time with protamine sulfate in recipients of a heparinized graft.

plasma. The heparin neutralisation test was performed by titration of the TT with protamine sulfate.

Both aPTT and TT were used as an indicator of biological activity of heparin. The heparin concentration was not measured, because of the very high bilirubin concentration in the samples, which is known to interfere with the assay.

When protamine sulfate substantially shortened a prolonged TT, it was assumed that heparin was present in the sample.

The prothrombin time (PT) was performed according to a standard procedure. The reagent used was Thromborel-S^{R}

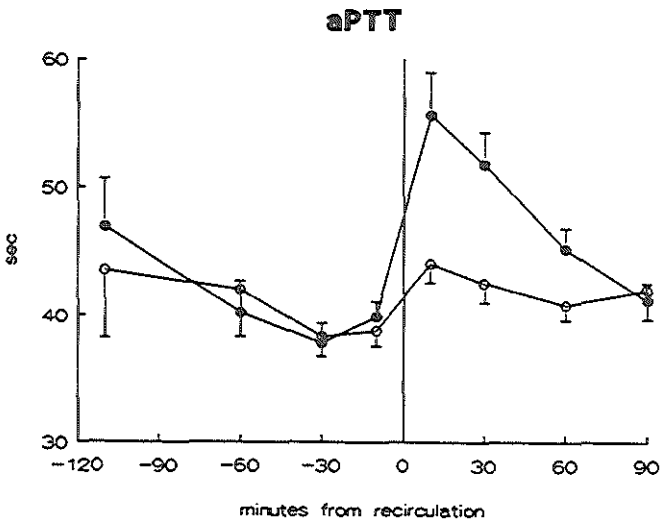

Fig. 2 Intraoperative course (mean±sem) of the aPTT in heterotopic liver transplants receiving a graft from a heparinized (closed circles) compared those receiving a graft from an unheparinized donor (open circles) *$p<0.05$

Statistical analysis

Statistical analysis was performed using non-parametric tests. P-values of < 0.05 were considered to be significant.

Results

The courses of the thrombin time of recipients of heparinized and unheparinized donors are depicted in figure 1. The thrombin time (normal range: 16-23 sec) increased significantly upon reperfusion in recipients of a heparinized graft ($p<0.004$) compared to those of an unheparinized graft ($p<0.2$). The thrombin time could be shortened by protamine sulfate in recipients of a heparinized donor, as is shown in figure 1, suggesting the presence of heparin ($p<0.01$). In contrast, protamine sulfate had no effect on the thrombin time in recipients of a graft from an unheparinized donor (not shown). Also the aPTT (normal range:22-45 sec) increased dramatically after reperfusion in recipients of a heparinized donor ($p<0.0002$) (figure 2).

The first venous hepatic outflow from the graft after reperfusion was available in 9 recipients of a heparinized donor and in all 5 recipients of an unheparinized donor. The first hepatic outflow from all grafts of heparinized donors contained heparin as measured by a prolonged aPTT and TT, and their shortening by protamine. Four samples of a graft from an unheparinized donor did not reveal a heparin effect in the first hepatic outflow. In one case a mild heparin effect was detectable.

The prothrombin time did not change appreciably during liver transplantation. No significant difference in the course of the prothrombin time could be observed between recipients of heparinized and unheparinized donors.

Discussion

The release of heparin has been mentioned as one of the causes of hypocoagulability after reperfusion of the graft after liver transplantation. The

question arises as to the source of the heparin which is exerting the heparin effect. It has been ascribed to endogenous heparin released from the donor liver or to exogenous heparin in the preservation fluid that is released into the recipient after reperfusion after sequestration into the graft during preservation. Although systemic heparinization of the recipient is no longer performed as a standard procedure, a "heparin effect" may still be observed after graft recirculation. The present study shows that systemic administration of heparin to the donor before removal of the liver may result in the appearance of heparin in the circulation of the recipient after graft recirculation. Both aPTT and thrombin time were significantly prolonged after graft reperfusion in recipients from a heparinized donor as compared to recipients of an unheparinized donor. This effect could in most cases be reversed by protamine sulfate, indicating heparin as its cause. The first venous outflow from grafts from heparinized donors contained heparin. This strongly suggests that heparin binds to the endothelium during the donor procedure and is released into the circulation upon reperfusion. It has earlier been demonstrated that heparin binds to the vascular endothelium after intravenous injection [18-20]. The process is reversible. Heparin molecules, released from endothelial cells retain their anticoagulant ability [19,21]. Apart from endothelial binding, binding (and catabolism) to extracelluar matrix has also been reported in the event of functionally damaged or even de-endothelialized vessel surfaces [22].

The fact that the first venous outflow in one recipient of an unheparinized graft revealed a heparin effect, may suggest an additional component of endogenous heparin release. The significant difference between recipients from heparinized and unheparinized grafts regarding the heparin effect, makes it unlikely that this effect can solely be attributed to endogenous heparin.

The effect imputed to heparin (released from the graft) is greater than might be expected in a normal situation, but heparin tolerance is greatly diminished in the recipient at the end of the operation and the capacity of the liver to eliminate exogenous heparin is often reduced or nearly lost [5,23]. As in HLT the remaining function of the host liver is retained and might account for some clearance of heparin, the effect of heparin released from the graft may be even more pronounced in orthotopic liver transplantation.

In conclusion: systemic administration of heparin to the donor, may result in the appearance of heparin into the circulation of the recipient after graft reperfusion and may contribute to the bleeding complications after reperfusion. Definite proof awaits studies using labelled heparin.

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Chapter 7

Recovery of hemostasis after orthotopic and heterotopic liver transplantation:

Is there a contribution of the host liver?

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Submitted

Abstract

Recovery of hemostasis after liver transplantation depends on the functional integrity of the allograft. Graft dysfunction may lead to a life-threatening coagulopathy. In heterotopic liver transplantation (HLT) the residual function of the host liver is retained and may contribute to the synthesis of hemostatic factors postoperatively.

We have measured the activated partial thromboplastin time, prothrombin time, fibrinogen, individual coagulation factors and antithrombin-III, after OLT (n=18) and HLT (n=22) to investigate whether there is a difference in postoperative hemostatic recovery and what is the contribution of the host liver after HLT.

There was no significant difference between OLT and HLT, either in the total group or in a selected group with an adequate graft function (OLT n=8, HLT n=12). However in the group with a poor graft function (absence of bile flow and peak transaminases>1500 U/l, OLT n=4, HLT n=6) the hemostasis profile was better in HLT than in OLT ($p<0.05$). In HLT retransplantation was performed after 15.2 days whereas in OLT retransplantation had to take place within 4.5 days ($p<0.03$).

We therefore conclude that in patients with an adequate allograft function, there is no difference in hemostatic recovery between OLT and HLT. In patients with an allograft dysfunction, however, the postoperative hemostatic levels were better in HLT as compared to OLT. These data suggest that the capacity of the diseased host liver to synthesize is small, but may be important in case of allograft dysfunction.

Introduction

Orthotopic liver transplantation (OLT) has markedly improved the life expectancy of patients with acute and chronic liver failure [1]. However, in spite of refined surgical techniques and improved perioperative management, bleeding remains a critical problem [2,3]. In the report of the European Liver Transplant Registry, bleeding complications were the most frequent (34%) cause of death, both intraoperatively

and during the first postoperative week [4]. Several investigators have demonstrated that large perioperative blood loss is correlated to a high mortality after liver transplantation [5,6]. As the liver is the major site of synthesis and clearance of hemostasis proteins, hemostasis depends on the functional integrity of the transplanted liver upon revascularization. Graft dysfunction may lead to a life-threatening hypocoagulability in the immediate postoperative period, and the patient must urgently be retransplanted or will die [7,8].

Auxiliary heterotopic liver transplantation (HLT) is an alternative to OLT. In HLT the diseased host liver is left in situ and the liver graft is placed in a heterotopic position. In theory, the residual function of the host liver is retained and this could contribute to the postoperative recovery of hemostasis. Failure of the graft does not necessarily lead to bleeding complications, death or emergency retransplantation. In spite of these theoretical advantages, the clinical results f HLT were initially discouraging. The reasons for failure of earlier attempts at HLT included lack of space in the peritoneal cavity for the graft, atrophy of the graft due to a lack of portal blood inflow, and venous congestion of the graft. After extensive experimental studies using a graft reduced in size by partial hepatectomy, provided with both arterial and portal blood inflow, a clinical HLT program was started in our institution, and shows to date good clinical results in acute and chronic liver disease [9-11].

We have measured hemostatic parameters after 18 OLTs and 21 HLTs in order to address the following questions:

1. Is there is a difference in posttransplant hemostatic recovery between OLT and HLT?
2. Is there a contribution from the host liver in the hemostatic recovery after HLT?

Materials and methods

Patients

40 patients, receiving their first liver transplant, were enrolled in this study. One patient (OLT) died within 12 hours after surgery and was not included in the

Table 1

Diagnosis in 18 patients undergoing OLT and 22 undergoing HLT

Diagnosis	OLT	HLT	
Fulminant hepatitis	4	4	
Hepatitis B	4	5	
Autoimmune hepatitis	0	2	
PBC/PSC*	4	7	
Alcoholic	1	2	
Carcinoma	3	0	
miscellaneous	2	2	
Child-Pugh score** (median+range)	9(7-12)	10(8-15)	$p<0.05$

*Primary biliary cirrhosis/sclerosing cholangitis
**Child-Pugh score was calculated only in chronic liver disease.

analysis. The causes of the liver disease as well as the Child-Pugh score are listed in table 1 [12].

Initially, patients were separated into two distinct groups: OLT (n=18) and HLT (n=22). Subsequently, the OLT-group and HLT-group were each subdivided, based on their functional outcome; one subgroup (OLT n=8 and HLT n=12) consisted of patients, who exhibited an adequate postoperative graft function within at least 2 days after liver transplantation as defined by continuous bile flow, a peak ASAT and ALAT of less than 1500 U/l. The second subgroup consisted of patients (OLT n=4 and HLT n=6), who exhibited a (primary non-function or) poor graft function, as manifested by discontinuation/absence of bile flow and a peak ASAT and ALAT of above 1500 U/ml during the first 5 postoperative days. Patients with postoperative complications such as graft rejection, overwhelming infections or those that required surgical intervention because of technical causes, were excluded from these subgroups. The following comparisons were made with regard to postoperative hemostatic recovery;

1. Entire group of OLT versus HLT.
2. Subgroups of OLT versus HLT
   a. with an adequate graft function and
   b. with a poor graft function.

Perioperative management

Perioperative management and the immunosuppressive regimen were identical in both groups as described previously [9].

Postoperative substitution with fresh frozen plasma (FFP) was given depending on laboratory results and/or the presence of bleeding. In patients receiving substitution therapy with FFP, only trough levels were taken into account. Intravenous administration of heparin (at most 12,000 IU per 24 hours) was started 24 to 48 hours after surgery and was continued by subcutaneous injection (5000 IU twice daily).

Graft function was monitored by the daily measurement of liver enzymes, serum albumin and bile flow. Doppler ultrasound on days 1,2,3,7 and 21; and scintigraphy with technetium-99-labeled diethylimidodiacetic acid on days 3,7 and 21. Specimens of the graft were obtained by percutaneous needle biopsy at 7 and 21 days.

Materials

Blood samples for hemostatic analysis were taken preoperatively, twice daily (at 8 am and 8 pm) during the first 3 postoperative days and once daily (at 8 am) during the following days.

The blood was collected in 0.11 mmol/l trisodium citrate, placed immediately on melting ice and centrifuged (20 min, 2800g, 4°C) within 20 minutes. Plasma was snap-frozen and stored at -70°C.

Methods

The activated partial thromboplastin time (APTT) and the prothrombin time (PT) were performed according to routine procedures. Reagents used were Actin^{R} (Baxter Dade AG, Düdingen, Switzerland) for the aPTT and Thromborel-S^{R} (Behring Diagnostica, Amsterdam, The Netherlands) for the PT. Fibrinogen was measured according to Clauss [13]. Assays for coagulation factors II, V, VII, VIII, IX, X, XI and

XII were performed by a one-stage method using factor deficient plasma. Antithrombin-III activity was measured according to Abildgaard et al using Coatest^{R} antithrombin (Kabi Diagnostica, Amsterdam, The Netherlands) [14].

Statistical analysis

All values are given as mean and standard error of the mean. The Wilcoxon test for independent samples was used for statistical analysis. Any probability less than 0.05 was considered significant.

Results

The preoperative coagulation data (mean±sem) for the patients undergoing OLT and HLT are listed in table 2. Pretransplant coagulation results were worse in HLT as compared to OLT, but the difference reached no significance. The Child-Pugh score, though, was significantly higher in HLT than in OLT ($p<0.05$), indicating that patients undergoing HLT were in a more advanced stage of their liver disease than those treated with OLT.

Table 2

Preoperative coagulation findings (mean±sem) in patients undergoing OLT and HLT.

	reference	OLT	HLT
APTT sec	26-36	39.4 ± 10.1	41.3 ± 2.1
PT sec	15-19	27.5 ± 5.1	25.2 ± 1.8
Fibrinogen g/l	1.6-2.8	2.4 ± 0.36	2.0 ± 0.3
Factor V U/ml	0.50-1.50	0.53 ± 0.13	0.44 ± 0.07
Factor VII U/ml	0.60-1.40	0.43 ± 0.09	0.27 ± 0.04
AT-III U/ml	0.85-1.20	0.60 ± 0.10	0.39 ± 0.05

The difference between OLT and HLT was never significant

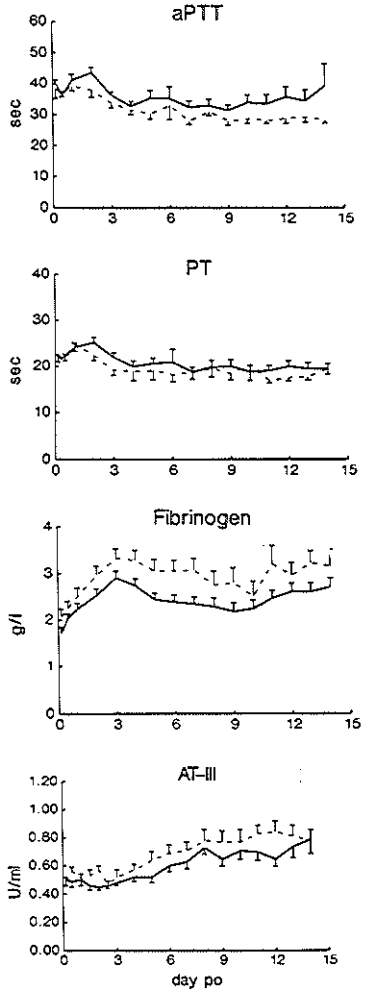

Fig. 1 Postoperative courses (mean±sem) of aPTT (normal range 22-45 sec), PT (normal range 1-19 sec), fibrinogen (normal range 1.6-2.8 g/l) and antithrombin-III (normal range 0.85-1.20 U/ml) of 18 patients who underwent OLT and 22 patients who underwent HLT.

Recovery of hemostasis in the total group of OLT and HLT

The postoperative courses (mean±sem) of several representative coagulation parameters, the aPTT, PT, fibrinogen and antithrombin-III, of patients treated with OLT and HLT are shown in figure 1. The courses of coagulation factor II, V, VII, IX, X, XI and XII are shown in table 3. A rapid recovery of all measured parameters is seen; aPTT and fibrinogen became normal within the first postoperative day. The PT improved more slowly and did not reach the normal range before day 3 for OLT and day 4 for HLT. The individual coagulation factors were within the normal range

Table 3

Postoperative courses of the individual coagulation factors (mean±SEM) in OLT and HLT*.

Factors	Type	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15
Factor II	OLT	0.47±0.03	0.50±0.04	0.62±0.04	0.64±0.04	0.62±0.10	0.60±0.06
	HLT	0.45±0.03	0.50±0.03	0.60±0.03	0.61±0.04	0.68±0.05	0.72±0.08
Factor V	OLT	0.38±0.05	0.46±0.06	0.84±0.06	0.92±0.05	0.85±0.06	0.92±0.06
	HLT	0.29±0.03	0.43±0.05	0.76±0.06	0.76±0.05	0.90±0.05	0.92±0.10
Factor VII	OLT	0.44±0.05	0.34±0.06	0.45±0.06	0.53±0.07	0.59±0.12	0.60±0.05
	HLT	0.35±0.03	0.27±0.03	0.36±0.03	0.46±0.06	0.69±0.08	0.61±0.10
Factor IX	OLT	0.49±0.03	0.66±0.05	0.95±0.09	1.13±0.09	1.07±0.14	1.14±0.24
	HLT	0.48±0.04	0.60±0.04	0.77±0.05	0.99±0.07	1.25±0.12	1.16±0.16
Factor X	OLT	0.40±0.03	0.42±0.04	0.64±0.05	0.75±0.05	0.70±0.12	0.70±0.10
	HLT	0.42±0.07	0.45±0.03	0.56±0.03	0.67±0.05	0.75±0.06	0.78±0.07
Factor XI	OLT	0.51±0.04	0.52±0.05	0.73±0.07	0.80±0.06	0.64±0.13	0.66±0.34
	HLT	0.58±0.05	0.49±0.04	0.63±0.04	0.70±0.06	0.74±0.07	0.69±0.08
Factor XII	OLT	0.60±0.04	0.73±0.06	0.73±0.07	0.73±0.06	0.93±0.16	0.81±0.09
	HLT	0.74±0.08	0.65±0.06	0.61±0.07	0.61±0.08	0.92±0.13	1.06±0.18

* The difference between OLT and HLT is never significant.

Normal range : 0.60-1.40 U/ml (factor II, VII, IX, X, XI, XII)
: 0.50-1.50 U/ml (factor V)

by day 3, both for OLT and HLT, with the exception of coagulation factor VII, which remained just subnormal and reached the normal range by day 7. Antithrombin-III needed two weeks to reach normal values. Overall, patients treated with HLT recovered somewhat slower than patients who underwent OLT, but no significant difference was observed between OLT and HLT.

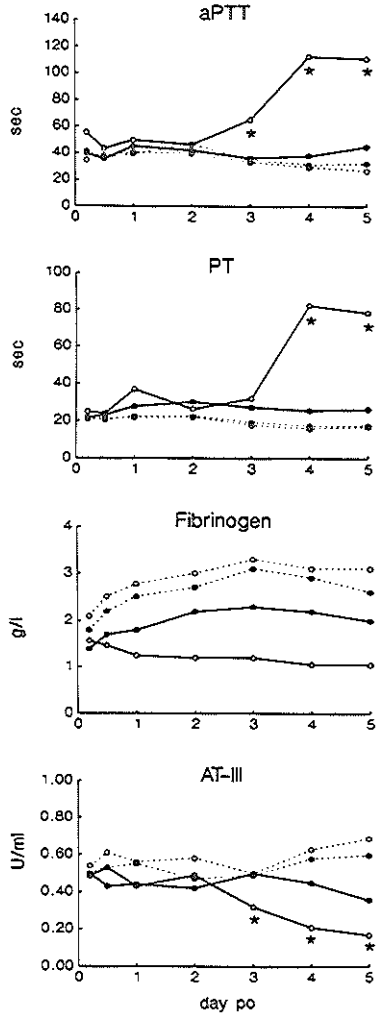

Fig. 2 Postoperative courses (mean) of aPTT, PT, fibrinogen, and antithrombin-III in OLT (open circles) and HLTs (closed circles) with an adequate (broken lines) and a poor (solid lines) allograft function, respectively. *$p<0.05$ in OLT compared to HLT with a poor graft function.

Recovery of hemostasis in OLT and HLT with an adequate and poor graft function

Figure 2 depicts the postoperative courses of the above mentioned parameters in OLTs and HLTs with an adequate and a poor graft function. In the group with an adequate graft function, there was no difference between OLT and HLT for the aPTT, PT, fibrinogen, and antithrombin-III. However, in patients with a poor graft function, levels of these parameters were appreciably worse in OLT than in HLT. The difference between OLT and HLT was significant for the aPTT and PT at day 4 and 5 ($p<0.05$). A significant difference between OLT and HLT was reached at day 3 for antithrombin-III. Although fibrinogen levels were lower in OLT than in HLT, the difference here was not significant.

The mean time between transplantation and retransplantation (or death) in the group with a failing liver graft was 4.5 days for OLT and 15.2 days for HLTs ($p<0.03$).

The postoperative usage of blood products was not significantly different between OLT and HLT; in the group with an adequate graft function blood replacement was only given during the first 2 postoperative days (range 1-4). The median (range) usage of FFP was 0 (0-13) U in the OLT group and 6 (0-21) U in the HLT group ($p<0.16$). The postoperative usage of FFP in recipients with a failing graft was 8.2 (6-12) U/day in the OLT group and 7 (4-13) U/day in the HLT group ($p<0.5$). (Values are given as U/day, because of the wide difference in survival of the individual liver transplants)

Discussion

One of the main differences between OLT and HLT is that the residual function of the host liver is retained. We have demonstrated that the presence of a host liver prevents the development of a hyperfibrinolytic state during HLT [15,16]. Postoperatively, the host liver may contribute to the synthesis of hemostatic factors. This may result in a speedier hemostatic recovery and in case of allograft dysfunction a life-threatening hypocoagulability may be prevented. The present

study investigates whether there is a difference between OLT and HLT regarding the recovery of hemostasis and whether there is a contribution from the host liver after HLT.

A rapid normalisation was seen after liver transplantation for the aPTT, PT and the individual coagulation factors. There was no significant difference between OLT and HLT, either in the total group or in a selected group with an adequate graft function. However, in the group with a inadequate graft function, hemostatic levels were appreciably better in HLT than in OLT. Moreover, in HLT retransplantation was performed after 15.2 days whereas in OLT retransplantation had to take place within 4.5 days. These data suggest that the remnant function of the host liver in HLT contributes to the synthesis of coagulation factors and protects the recipients from the effects of a failing graft. An emergency retransplantation was prevented, allowing the procedure to be performed (semi-)elective.

There are several explanations for the lack of difference in the recovery of hemostasis between OLTs and HLTs with an adequate graft function. Firstly, the function of the diseased host liver may be compromized by the surgical procedure and pales into insignificance besides a good functioning liver graft. It is well known from the literature that surgery and particularly general anesthesia, which reduces the hepatic blood flow by 30-50% during induction, exacerbates pre-existent liver failure [17,18]. Secondly, the remnant function of the host liver may be suppressed in the presence of a good functioning graft, in terms of "functional competition", but stimulated to maximum synthesis in the case of an allograft failure. We have earlier demonstrated "functional competition" in HLTs with an excellent graft function, which resulted in a rapid regeneration of the liver graft with atrophy of the host liver [19].

Notwithstanding all that, the better hemostatic levels in HLTs compared to OLTs with allograft dysfunction indicate that there is synthesis of coagulation factors by the host liver, which may contribute to a reduction of perioperative bleeding problems and mortality.

In conclusion, there is no difference in the postoperative hemostatic recovery between OLT and HLT with an adequate allograft function. In patients with an allograft dysfunction, however, postoperative hemostatic parameters were better in

HLT than in OLT. Moreover, an emergency retransplantation could be prevented. These data suggest that the postoperative capacity of the host liver to synthesis is small, but may be very important in case of allograft dysfunction.

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Chapter 8

General discussion and conclusions

General discussion and conclusions

8.1 *Introduction*

The liver plays a central role in the maintance of hemostasis. It is the major site of synthesis and clearance of most coagulation and fibrinolytic factors as well as their inhibitors.)is the major site of synthesis and clearance of most coagulation and fibrinolytic proteins as well as their inhibitors [1]. Consequently, either diseases of the liver or procedures on the liver are associated with hemostatic abnormalities, let alone procedures on the diseased liver [2,3]. It has been suggested that disseminated intravascular coagulation (DIC) contributes to the coagulopathy of cirrhosis, but assessing its contribution is difficult since many coagulation changes can be attributed to the impairment of liver function itself [4-6]. DIC implies the activation of both thrombin (coagulation) and plasmin (fibrinolysis) enzyme systems. Over the last few years specific biochemical markers of activated coagulation (thrombin-antithrombin III complex [6] and soluble fibrin [7]) and fibrinolysis (D-Dimer) have been developed. These test have been applied in patients with liver cirrhosis of various severities (chapter 3). It was found thrombin formation was elevated in liver cirrhosis, irrespective of the severity of the disease. The concentration of antithrombin-III was however of major importance for the subsequent development of DIC

Liver transplantation is the ultimate step in the treatment of liver disease. The operation is a major undertaking and is associated with a considerable blood loss [8]. Part of the blood loss is related to the magnitude of the surgical undertaking and part to hemostatic abnormalities. Most patients come to surgery with a coagulopathy, related to end-stage liver disease. This coagulopathy is further compounded during the operation. Specific hemostatic changes occur, especially during the anhepatic and reperfusion period [9]. These have been ascribed to increased fibrinolysis, disseminated intravascular coagulation and possibly the release of heparin(-like) substances. Lack of clearance of procoagulant or profibrinolytic factors during the anhepatic period as well as their release from the ischemically damaged graft upon reperfusion, presumably play a role. Despite extensive research in the field, the pathogenesis of these changes is still not

completely understood. As a consequence the management of hemostasis during the operation is not optimal, causing an increased morbidity and mortality in liver transplant patients.

An alternative to OLT is auxiliary heterotopic liver transplantation (HLT), whereby the diseased host liver is left in situ and the graft is placed in a heterotopic position [10]. The traumatic removal of the host liver, with the associated blood loss is avoided and there is no anhepatic period. The host liver can still, to a greater or lesser extent, participate in the metabolism of hemostasis proteins and thus hemostatic deterioration may be less severe. HLT provides us with a useful model to differentiate between the effects of graft reperfusion and the anhepatic period on hemostasis and give more insight into the mechanisms underlying specific hemostatic changes during OLT.

Fibrinolysis - Increased fibrinolysis has been recognized as a major cause of bleeding during OLT, related to increased levels of tissue-type plasminogen activator (t-PA) activity [10,11]. The underlying mechanism is still not clear. Lack of hepatic clearance and/or increased endothelial release of t-PA during the anhepatic period and release from the ischemically damaged graft upon reperfusion has been described. We undertook a comparative study on t-PA activity during OLT and HLT (chapter 2). As any surgical procedure is accompanied with an increased fibrinolytic activity, we also included a reference group of patients undergoing partial hepatic resections (PHR). t-PA activity was found to be increased during the anhepatic period of OLT but not during HLT or PHR. There were no signs of t-PA release from the hepatic graft. This suggested that the lack of clearance of t-PA during the anhepatic period is currently the major cause in the evolution of increased fibrinolysis during OLT.

However, orthotopic transplantation of a **long-term** preserved graft (at most 72 hours) did induce a significant increase in t-PA activity after graft reperfusion in pigs. The increase in t-PA was proportional to the preservation damage (chapter 5). Also under these extreme conditions, HLT protected the recipient from the effects on hemostasis. These results underscore the importance of a graft preservation, as optimal as possible. Most probably t-PA release from the liver graft may have

played an important role in the earlier experiments and clinical studies, but the improvement in graft procurement and preservation have made this factor negligible compared to the effect of lack of hepatic clearance.

Disseminated intravascular coagulation - Specific hemostatic changes, akin to those in DIC, have been encountered during OLT [12-14]. Although improvements in graft preservation techniques, surgical and anesthetic procedures and a more stringent substitution scheme have led to a reduction of the coagulation changes, it is still controversial whether DIC contributes to the bleeding problems or not. Theoretically, there are ample pathogenetic mechanisms for DIC; lack of clearance of procoagulant factors during the anhepatic period as well as release of thromboplastic material or local clotting activation in the liver graft upon reperfusion have been suggested to be involved. In HLT, there is no anhepatic period and DIC, if present, will not occur until after reperfusion. It was found that thrombin formation, as reflected by a rise in TAT levels, dramatically increased at reperfusion of the graft both OLT and HLT, but there were no signs of the subsequent development of DIC, as the conventional DIC parameters remained more or less unchanged (chapter 4). Any thrombin formed, is most probably rapidly inhibited by antithrombin-III. The concentration of antithrombin-III could be of major importance for the subsequent development of DIC.

Heparin(-like) substances - The release of heparin(-like) substances from the graft has been reported in several studies [15-17]. The heparin could be of endogenous or exogenous origin. Currently the use of heparin in preservation and flush fluids is avoided, but still a "heparin" effect may be observed. It was found that the heparin effect, as measured from the aPTT and thrombin time, was significantly more pronounced in recipients from a heparinized donor as compared to recipients from an unheparinized donor (chapter 6). This suggests that heparin, administered to the donor shortly before hepatectomy, may be retrieved in the recipient after graft reperfusion and may be considered as a risk factor for bleeding during liver transplantation.

Hemostatic recovery after liver transplantation depends on the functional integrity of

the transplanted liver upon revascularization. Graft dysfunction may lead to a life-threatening hypocoagulability in the immediate postoperative period. It was demonstrated that, in the event of an adequate graft function, hemostatic parameters recovered within several days after liver transplantation. There was no difference in postoperative recovery between OLT and HLT, as might be expected when the host liver contributes to postoperative synthesis of coagulation factors. However, in case of an allograft dysfunction, hemostatic levels were significantly better in HLT than in OLT. An emergency retransplantation could be prevented, allowing the procedure to be performed semi-elective. These results implicated that contribution of the host liver to postoperative hemostatic levels is small, but may be life-saving in case of allograft failure.

8.2 Major conclusions:

1. Liver cirrhosis is associated with increased thrombin formation, irrespective of the severity of the disease. The subsequent development of (low-grade) DIC occurs only if antithrombin-III levels fall below 0.30 IU/ml. TAT are of no diagnostic use for DIC in liver cirrhosis, but soluble fibrin levels may be.

2. With the current preservation and procurement techniques, lack of hepatic clearance of t-PA during the anhepatic period is the most important factor in the evolution of increased fibrinolysis in OLT.

3. DIC does not contribute to a large extent to the bleeding problems in OLT. There is no significant difference of DIC parameters between OLT and HLT.

4. Heparin, administered to the donor shortly before the hepatectomy, may be reappear in the recipient after graft reperfusion and can be considered as a possible reason for bleeding during liver transplantation.

5. Transplantation of long-term preserved porcine grafts is accompanied by an increase in t-PA after reperfusion in OLTs, that is proportional to the preservation damage. HLT protects the recipient from these effects.

6. There is no difference between OLT and HLT regarding postoperative recovery of hemostasis parameters in the event of a adequately functioning graft. However, in the event of allograft failure, hemostatic levels are significantly better in HLT, suggesting a contribution of the host liver. An emergency retransplantation is prevented in these cases..

8.3 Suggestions for perioperative hemostatic management

Despite the great attention paid to bleeding in OLT, no consensus has developed concerning the optimal degree of hemostatic management.
During liver transplantation specific hemostatic changes may occur, that include hyperfibrinolysis, disseminated intravascular coagulation, the release of heparin. OLT can be divided into three operative phases and each phase is characterized by one or more of these specific abnormalities (table 1). Based on data from literature completed with own results, the following recommendations for perioperative hemostatic management during liver transplantation are made; generally, adequate coagulation monitoring may have to be minimal routine testing (prothrombin time, activated partial thromboplastin time and platelet count) (supplemented with) and thrombelasthography, with more intense monitoring only as dictated by the clinical situation.
If bleeding occurs, the following causes are most likely, depending on which stage of the operation it occurs;
Preanhepatic period - Most patients come to liver transplantation with a coagulopathy. There is still no consensus on the benefit of preoperative correction of coagulopathy in reduction of perioperative blood use, morbidity and mortality. Routine correction of hemostasis most probably is not eligible. The reached effect is quickly nullified, (amongst others) via loss to ascites or via blood loss. Likewise, transfused platelets will be pooled in the spleen.

Table 1

Most likely causes for bleeding during OLT (besides "surgical"bleeding).

Preanhepatic period	Preoperative coagulopathy Bleeding from collaterals (vasopressin)
Anhepatic period	Hyperfibrinolysis Heparin derived from venovenous bypass (DIC)
Postanhepatic period	DIC Heparin

therefore be reserved for cases with a preoperative bleeding tendency due of lowlevels of coagulation factors (e.g. under the hemostatic levels), or platelets, as manifested by secondary haemorrhage of the vena puncture and skin incisions.
As vasopressin induces an increase in t-PA activity, its use during liver transplantation has to be weighed carefully, since it carries a considerable risk of bleeding (capacity to clear t-PA is greatly reduced)

Anhepatic period - Especially during the anhepatic period, one should be apprehensive of the development of hyperfibrinolysis, due to lack of clearance of t-PA. It is eligible to keep the anhepatic period as short as possible.
(During the anhepatic period, the risk of the development of hyperfibrinolysis is the greatest.) Monitoring of hemostasis during this period should be focused on fibrinolysis. This can be accomplished either by the thrombelasthography or by specific hemostatic tests. (The latter have as disadvantage that it takes time before the results are available.)
If present, hyperfibrinolysis can be treated with EACA, tranexaminic acid or aprotinin. It is advisable to test its effect first in vitro (thrombelasthograph).
During HLT the risk of the development of hyperfibrinolysis is low.

Postanhepatic period - Reperfusion of the graft triggers a great amount of thrombin formation. Therefore during this period there is a appreciable risk of the development of DIC. The concentration of antithrombin-III could be a major contributing factor. If DIC is suspected, the use of heparin should still be avoided, as the use of even small amounts of heparin leads to bleeding. The use of antithrombin-III concentrates is still subject of research. As it has been demonstrated that coagulation changes after reperfusion are directly related to the quality of the graft, it is useful to limit the preservation damage as much as possible.
If there is a persistent bleeding tendency, keep also in mind that during reperfusion, heparin can be released in the circulation. This effect is easily corrected by administration of protamine. Again it is advisable to test the effect first in vitro.

Postoperative period - If the liver grafts picks up hemostatic parameters normalize within 2-3 days. If the clinical picture indicates an adequate graft function, a meticulous monitoring of hemostasis is not necessary and one can confine to

routine clotting assays (e.g. PT or NT and APTT) once daily

8.4 References

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Summary

This thesis comprises of clinical and experimental studies on hemostatic disorders in liver disease and liver transplantation. **Chapter 1** is a survey of literature on hemostasis in liver disease and liver transplantation. Based on this survey, the aims of our studies are formulated.

In **Chapter 2** we tried to determine whether disseminated intravascular coagulation (DIC) contributes to the hemostatic abnormalities encountered in liver cirrhosis. We assayed *thrombin-antithrombin-III complexes* (TAT) (indicative of thrombin formation) and *soluble fibrin* concentrations (indicative of fibrin formation) as well as *D-Dimer* levels (indicative of fibrin degradation) in patients with liver cirrhosis. It was found that thrombin formation is increased in liver cirrhosis irrespective of the severity of the disease, as reflected by elevated TAT levels. However, the subsequent progression to DIC (reflected by elevated soluble fibrin levels) is dependent on the antithrombin-III concentration. Evidence for DIC was only found in severe liver cirrhosis at antithrombin-III levels less than 0.30 U/ml.

In **Chapter 3** the fibrinolytic system was evaluated in orthotopic and heterotopic liver transplantation (OLT and HLT respectively) and in partial hepatic resections (PHR) in order to attain a better insight into the mechanism of increased fibrinolytic activity in OLT. Both lack of hepatic clearance of *tissue-type plasminogen activator* (t-PA) in the anhepatic period and increased endothelial release from the graft upon reperfusion have been proposed as the major causes. HLT avoids the resection of the host liver and is a useful model to help differentiate between these two possibilities. A marked increment in fibrinolytic activity was observed during the anhepatic period of OLT, as reflected by a rise in t-PA activity and plasma degradation products of fibrin and fibrinogen, which rapidly normalized after reperfusion. In contrast t-PA activity levels remained normal in HLT and PHR. The first venous hepatic outflow after reperfusion did not contain elevated t-PA activity levels. We concluded that the lack of hepatic clearance of t-PA during the anhepatic period is the major cause of increased t-PA activity during OLT.

In **Chapter 4** we have evaluated TAT-complex, fibrin degradation products and routine clotting times in OLT and HLT in order to answer the question whether (and when) DIC occurs in liver transplantation. Theoretically the lack of hepatic clearance

of procoagulant factors during the anhepatic period and the release of thromboplastic material from the graft might trigger DIC. During heterotopic liver transplantation the host liver is left in situ and procoagulant factors may still be cleared; DIC, if present, may not occur until after reperfusion. It was found that graft reperfusion triggers excessive thrombin formation both in OLT and HLT, as reflected by a sharp increase in TAT-complex levels, but there were no other signs of subsequent DIC. Any thrombin formed is probably rapidly inhibited by antithrombin-III and DIC only occurs at very low antithrombin-III levels (see Chapter 2)

In **Chapter 5** we compared the hemostatic changes in porcine OLT and HLT after various periods of graft storage (2,24,48,and 72 hours). Graft reperfusion was followed by a rise in t-PA in both OLT and HLT, probably due to an abundant release of t-PA from the damaged graft. In HLT, t-PA quickly returned to normal, whereas a continuous increase was found in OLT. This further rise may be caused by cytokines released from the damaged graft, that activate the recipient's endothelium to t-PA release. T-PA and cytokines may be cleared by the host liver in HLT. Coagulation parameters deteriorated only after reperfusion, and more severely in OLT than in HLT. It was concluded that the remaining (clearance) function of the host liver in HLT also prevents the development of hyperfibrinolysis and hypocoagulability induced by severe graft storage damage.

Chapter 6 investigates the origin of heparin, which may be observed in the circulation of the recipient after graft reperfusion. A comparison was made between recipients of donors that received heparin (in accordance of the terms of multiorgan donation) and those that did not, with regard to the thrombin time, activated partial thromboplastin time and the heparin neutralisation test. The results imply that heparin may be bound to the endothelium of the liver during the donor procedure and reappear in the recipient after reperfusion of the liver graft and thus may contribute to the reperfusion coagulopathy of liver transplantation.

In **Chapter 7** we investigate whether there is a difference in postoperative recovery of hemostasis between OLT and HLT and what is the contribution of the host liver after HLT.

There was no significant difference between OLT and HLT, either in the total group

or in a selected group with an adequate graft function. However in the group with a poor graft function (absence of bile flow and peak transaminases>1500 U/l) the hemostasis profile was better in HLT than in OLT ($p<0.05$). In HLT retransplantation was performed after 15.2 days whereas in OLT retransplantation had to take place within 4.5 days ($p<0.03$).

These data suggest that the capacity of the diseased host liver to synthesize is small, but may be important in case of allograft dysfunction.

Samenvatting

Dit proefschrift omvat een aantal klinische en experimentele studies naar hemostase afwijkingen bij leverziekten en levertransplantaties.

Hoofdstuk 1 geeft een overzicht van de literatuur over hemostase afwijkingen bij leverziekten en levertransplantaties. Aan de hand van dit overzicht zijn de vraagstellingen van onze studies geformuleerd.

In **Hoofdstuk 2** trachten we inzicht te krijgen of diffuse intravasale stolling (DIS) bijdraagt aan de hemostase afwijkingen van leverziekten. Daartoe zijn in patiënten met levercirrose de volgende parameters bepaald; *thrombine-antithrombine-III complex (TAT)* (een indirecte parameter voor thrombinevorming), *soluble fibrin* (een parameter voor fibrinevorming) alsmede *D-Dimeren* (een parameter voor fibrine afbraak). Thrombinevorming bleek over het algemeen verhoogd te zijn in patiënten met levercirrose, onafhankelijk van de ernst van de ziekte. Echter de verdere progressie naar een diffuse intravasale stolling was afhankelijk van de concentratie antithrombine-III. Diffuse intravasale stolling werd alleen gezien bij ernstige levercirrose, met een antithrombine-III gehalte lager dan 0.30 U/ml.

In **Hoofdstuk 3** evalueren we het fibrinolytisch systeem in orthotope en heterotope levertransplantaties (OLT en HLT respectievelijk) en in partiële leverresecties (PHR), om een beter inzicht te krijgen in het onderliggend mechanisme van de verhoogde fibrinolyse tijdens OLT. Zowel het gebrek aan klaring van *tissue-type plasminogeen activator* (t-PA) door de lever tijdens de anhepatische fase als het vrijkomen van t-PA uit het endotheel van de graft tijdens reperfusie zijn als oorzaak gesuggereerd. Omdat bij een HLT de eigen lever in situ blijft en derhalve de anhepatische fase achterwege blijft, vormt het een bruikbaar model om te differentiëren tussen beide bovengenoemde oorzaken. We vonden een sterke stijging van fibrinolytische activiteit tijdens de anhepatische fase van OLT, gereflecteerd door stijging van t-PA en afbraakprodukten van fibrine en fibrinogeen, welke snel normaliseerde na de reperfusie. Tijdens HLT en PHR bleef de fibrinolytische activiteit laag. Ook het eerste (portale) reperfusiebloed bevatte geen verhoogde t-PA activiteit. We concluderen dan ook dat de afwezigheid van t-PA klaring door de lever tijdens de anhepatische fase de belangrijkste factor is in het ontstaan van toegenomen fibrinolyse tijdens OLT.

Hoofdstuk 4 beschrijft de resultaten van een studie waarin geprobeerd wordt inzicht te krijgen of (en wanneer) diffuse intravasale stolling (DIS) optreedt tijdens levertransplantatie. Theoretisch kunnen de afwezigheid van hepatische klaring van procoagulante factoren en het vrijkomen van thromboplastisch materiaal uit de graft een DIS triggeren. Omdat tijdens HLT de eigen lever in situ blijft en dus procoagulante factoren geklaard kunnen worden, zal een DIS, indien aanwezig, pas na de reperfusie optreden. Uit de resultaten van de studie blijkt dat reperfusie van de graft een enorme thrombinegeneratie tot gevolg heeft, zonder dat er aanwijzingen zijn voor een verdere progressie naar DIS. Waarschijnlijk wordt het gevormde thrombine geremd door antithrombine-III en treedt een DIS pas op bij zeer lage antithrombine-III concentraties (zie hoofdstuk 2).

In **Hoofdstuk 5** vergelijken we in een varkensmodel de hemostase veranderingen tijdens OLT en HLT na verschillende periodes van graftpreservatie (2, 24, 48 en 72 uur). De t-PA activiteit onmiddellijk na de reperfusie steeg, zowel in OLT als in HLT, waarschijnlijk ten gevolge van een enorme t-PA afgifte door de ischemisch beschadigde graft. Hoewel de t-PA activiteit verder steeg in OLT, daalde deze weer in HLT. De verdere stijging in t-PA activiteit in OLT wordt mogelijk veroorzaakt door het vrijkomen van cytokines uit de graft, welke het endotheel aanzetten tot t-PA afgifte. De eigen lever bij HLT kan de cytokines en het t-PA klaren, zodat t-PA activiteit afneemt in HLT. De stollingsparameters verslechteren pas na reperfusie en veel ernstiger tijdens OLT dan HLT. We concludeerden dat de eigen lever in HLT de ontvanger ook beschermt tegen de effecten van *ernstige* preservatieschade op het hemostase systeem.

Hoofdstuk 6 beschrijft de resultaten van een studie, waarin de oorzaak wordt gezocht van het heparine-effect, dat na reperfusie van de graft kan worden waargenomen in de ontvanger. Daartoe zijn de thrombine tijd, de geactiveerde partiële thromboplastine tijd en de heparine neutralisatietest van ontvangers van een lever wiens donor heparine toegediend kreeg (in het kader van multiorgaan donatie) vergeleken met ontvangers wiens donor geen heparine kreeg. Uit de resultaten blijkt dat (exogeen) heparine, toegediend tijdens de donorprocedure, zich bindt aan het leverendotheel en tijdens de reperfusie van de graft weer loslaat en in de circulatie van de ontvanger komt. Deze heparine kan bijdragen aan de

reperfusie coagulopathie van lever transplantaties.

In **Hoofdstuk 7** onderzoeken we (1) of er een verschil is in postoperatief herstel van hemostaseafwijkingen tussen OLT en HLT en (2) wat de bijdrage van de eigen lever hierbij is. We vonden geen verschil in postoperatief herstel van hemostase noch in de totale groep noch in de groep met een goede graftfunctie. Echter, in de groep met een slechte graft functie waren hemostase-parameters beter in HLT dan in OLT. Retransplantatie vond in de HLT groep na gemiddeld 15.2 dagen plaats, terwijl bij OLT retransplantatie al na 4.5 dagen moest plaatsvinden. De resultaten suggereren dat de postoperatieve synthesecapaciteit van de eigen lever weliswaar klein is, maar juist belangrijke bijdrage kan leveren in geval van een slechte graft functie.

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Curriculum Vitae

De schrijfster van dit proefschrift werd geboren op 10 juli 1963 te Dordrecht. In 1981 behaalde zij het diploma Gymnasium ß aan het Johan de Witt Gymnasium te Dordrecht. In hetzelfde jaar werd de studie geneeskunde aangevangen aan de Medische Faculteit van de Erasmus Universiteit te Rotterdam. Tijdens de studie was zij gedurende 1 jaar als student-assistent werkzaam op de afdeling celbiologie (Hoofd: Prof. Dr. O. Vos).
Tevens werden 3 keuzecoassistentschappen in het buitenland gelopen, te weten: Inwendige Geneeskunde, Telhashomer Hospital, Israël; Gynaecologie & Verloskunde, Tijgerberg Hospital, Zuid-Afrika en Inwendige geneeskunde, Cikini Hospital, Indonesië.
Na het behalen van het artsexamen (cum laude) in 1988 was zij werkzaam als wetenschappelijk onderzoeker op de afdeling Inwendige Geneeskunde II (hoofd: Prof. J.H.P. Wilson) van het academisch Ziekenhuis Rotterdam-Dijkzigt, alwaar aangevangen werd met dit proefschrift. Vanaf februari 1990 is zij tevens werkzaam op de afdeling Inwendige Geneeskunde I (hoofd: Prof. Dr. M.A.D.H. Schalekamp), aanvankelijk als AGNIO, vanaf januari 1991 tot op heden in opleiding tot internist.

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- **Sanofi Winthrop**
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- **Kabi Pharmacia**
- **Organon Technica**
- **Behring Diagnostica**
- **Behring Biologicals**
- **Servier Nederland b.v.**
- **Duphar Nederland b.v.**
- **Kordia b.v.**