Optimisation of graft function in liver transplantation: functional and metabolic aspects

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Optimisation of Graft Function in Liver Transplantation: functional and metabolic Aspects

Optimalisatie van de functie van de transplantatielever
na levertransplantatie:
functionele en metabole aspecten

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Chapter 1

1. INTRODUCTION

Patients with end-stage liver disease have a poor overall prognosis with a one-year mortality of more than 60% without liver transplantation (1-4). A wide variety of techniques has been developed to treat end-stage liver failure, but none has proven to be very effective. Except for the molecular adsorbent recirculating system (MARS) (5), none of the liver assist techniques like exchange transfusion, plasmapheresis, cross circulation, hemodialysis and hemoperfusion have been able to improve patient survival significantly (6-16). Extracorporeal liver perfusion using hepatocyte filled bioreactors has been reported to produce more promising results (17-21). However, the use of hepatoma cells or transgenic porcine hepatocytes is not without danger and a clinical trial in the Netherlands was stopped awaiting more detailed information regarding the transmission risks of transgenic viruses like the porcine endogenous retroviruses (PERVs) (18, 22, 23).

Therefore liver transplantation still represents the only treatment that offers survival benefit in children and adults with end-stage liver disease since the first successful human orthotopic liver transplantation was performed by Starzl in 1967 (24). In the early days, liver transplantation was a rescue therapy for decompensated liver disease and associated with high morbidity and mortality. Since then refinements in surgical technique, peroperative monitoring and postoperative immunosuppressive regimens resulted in an increased overall survival of patients undergoing liver transplantation. There are now many diseases for which orthotopic liver transplantation is performed and the main indications are given in table 1.

Table 1. Main indications for orthotopic liver transplantation (25, 26).

Viral hepatitis
Cholestatic liver diseases (incl. primary biliary sclerosis and sclerosing cholangitis)
Post alcoholic liver cirrhosis
Malignancy (hepatocellular carcinoma, solitary endocrine tumours, endothelioma)
Acute liver failure (Toxic, viral or auto-immune)
Cryptogenic liver cirrhosis
Inborn errors of hepatic metabolism
Congenital biliary disorders
Hepatic vein thrombosis (Budd-Chiari syndrome)

In specialised clinics the one-year patient survival is better than 90% and liver transplantation has become a semi-elective procedure (25, 27). However, new challenges

were brought by the emerging view that orthotopic whole liver transplantation may not be the best solution for all transplant patients (28), high expectations regarding functional outcome and patient survival and the scarcity of donor organs (29-31). This resulted in discussion about the use of auxiliary liver grafts in selected patients, the need for optimisation of clinical orthotopic liver transplantation and the use of a partial liver graft instead of whole liver grafts (32).

2. CURRENT PROBLEMS IN AUXILIARY LIVER TRANSPLANTATION

2.1 Auxiliary partial heterotopic liver transplantation (APHLT)

Orthotopic liver transplantation (OLT) was in the first two decades after the introduction a difficult operation with considerable morbidity, mortality and cost. Auxiliary liver transplantation was then introduced by Terpstra et al. as a less invasive procedure for patients with end-stage chronic liver disease who could not stand a normal orthotopic liver transplantation (33-35). Theoretically, auxiliary liver transplantation has several advantages over whole liver transplantation. Transplantation of an organ in the heterotopic position evades the surgical trauma of removal of the cirrhotic liver, which is a demanding procedure in patients with portal hypertension, abundant venous collaterals and severe clotting disorders (36, 37). Without an anhepatic phase, the hemodynamic condition of the patients is more stable (38, 39) and the native liver can provide some synthetic and clearing function during reperfusion of the donor liver (40). Also, a primary dysfunction of the donor liver does not necessarily lead to death or emergency re-transplantation as the remnant function of the native liver may enable elective re-transplantation. There is however a serious conceptual problem in selecting patients with chronic active hepatitis for this procedure. There is a substantial risk of development of hepatocellular carcinoma in the native cirrhotic liver, which is left in place (34). Therefore auxiliary heterotopic liver transplantation was abandoned in patients with a cirrhotic liver.

Complete resection of the native liver may also not be indicated in acute liver failure. In this situation auxiliary liver transplantation may serve as a bridge to recovery with the possibility to withdraw immunosuppressive therapy after regeneration of the native liver. Finally, auxiliary liver transplantation was proposed for patients with selected inborn errors of hepatic metabolism, in whom only one enzyme or cell membrane receptor is absent and have otherwise normal function of the liver (41). In those patients auxiliary liver

transplantation would provide metabolic correction and preserves the possibility of future gene therapy in the native liver. Due to complications associated with the heterotopic position of the graft (42), auxiliary liver transplantation never gained wide acceptance. This was further enhanced by the strongly improving results of orthotopic liver transplantation over the years. Despite its theoretical advantages, clinical auxiliary heterotopic liver transplantation was performed in selected cases only.

2.2 Auxiliary partial orthotopic liver transplantation (APOLT)

In 1990 a new concept of auxiliary liver transplantation was introduced, in which a partial liver graft was placed in the orthotopic position after removal of liver segments 2-4 (43). The patient, suffering from an HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) and probably hepatitis A, showed recovery of her own liver in six months and immunosuppression could be tapered off. Since then auxiliary partial orthotopic liver transplantation has been performed successfully for acute liver failure and inborn errors of metabolism (44-49). Although many of the complications associated with the heterotopic position were solved by the new technique, the need for sharing the portal blood flow between liver graft and native liver remains controversial.

3. CURRENT PROBLEMS IN ORTHOTOPIC LIVER TRANSPLANTATION

In chapter 3 the development of orthotopic liver transplantation is described in more detail. Since its introduction the one-year patient survival improved from approximately 30% to better than 80%. Although excellent results can be achieved, there are still some subjects that need to be elucidated.

3.1 Primary graft dysfunction

Despite the use of specially designed preservation solutions, the donor liver does not function well after orthotopic liver transplantation in 5-15% of the patients (50, 51). This primary graft dysfunction (PGDF) can resolve or deteriorate into primary non-functioning (PNF). The condition is associated with increased need for re-transplantation and mortality (52, 53). The initial clinical presentation of PGDF includes serum transaminases over 2500 U/l, severe coagulopathy and lack of bile production in the first days after transplantation. The pathogenesis of PGDF is attributed to cold storage of the liver, which activates sinusoidal macrophages (Kupffer cells) and induces preservation injury to endothelial cells

with progressive loss of viability (54-59). Subsequent reperfusion causes many reactions involving nitric oxide, reactive oxygen species, cytokines and eicosanoids (52, 60-62). Leukocyte margination and platelet adherence in the damaged sinusoids may lead to poor tissue perfusion and oxygen delivery (63-66). Ultimate result of this ischaemia / reperfusion damage is mitochondrial derangement with a loss of ATP synthase activity and reduction of ATP levels (67-75).

Recently, an alternative hypothesis for the cause of mitochondrial dysfunction was formulated. Direct inhibition of mitochondrial respiration by nitric oxide and oxygen radicals was described in several experimental studies (76-83). The main product of this reaction is peroxynitrite, which is held responsible for damage to numerous biologic targets. To optimise the quality of the graft and reduce the incidence of PGDF, the clinical role of nitric oxide and peroxynitrite needs to be elucidated in patients with PGDF.

3.2 Peroperative coagulopathy

A second problem that has to be solved is the large amount of blood loss during liver transplantation as recent publications indicate that peroperative blood loss still affects postoperative outcome (84-86). Surgery in general imposes a situation of hypercoagulability through a combination of activation of haemostasis due to tissue damage and release of hormones and cytokines, which affect haemostasis (87, 88). In response, fibrinolysis is activated by a rise in tissue plasminogen activator activity to maintain the pro- and anticoagulant equilibrium. Patients with preceding liver disease however often have profound disturbances in the haemostatic system, including a low platelet count, low levels of clotting factors and increased levels of fibrinogen degradation products due to disseminated intravascular coagulation and subsequent fibrinolysis (89-91). During the liver transplantation procedure, this accelerated fibrinolysis (hyperfibrinolysis) and coagulopathy are recognised complications and massive blood loss remains an important clinical problem. In 1989, bleeding complications was the most frequent cause of death during the procedure and in the first week after transplantation (92). Refinements of the surgical procedure and improved perioperative management have decreased blood loss over the years. Still, enhanced fibrinolysis plays an important role in the pathogenesis of peroperative bleeding in the anhepatic and reperfusion phase, due to compromised hepatic clearance and release of t-PA from the donor liver (40, 93, 94). Once large amounts of t-PA are released in the circulation, α2-antiplasmin plays a key-role in scavenging the formed free plasmin (95).

When in 1996 solvent/detergent virus-inactivated plasma was introduced in our hospital to minimise the risk of viral transmission, suddenly an increase in hyperfibrinolysis in patients undergoing OLT was observed with detrimental effects on peroperative transfusion requirements. A study was initiated to establish the role of this new plasma in the increased incidence of fibrinolysis.

3.3 Preservation of the recipient vena cava inferior

The technique of orthotopic liver transplantation is well standardised and results have improved significantly over the years. Despite refinements, conventional orthotopic liver transplantation requires crossclamping of the inferior vena cava, reducing venous return and resulting in decreased cardiac output and systolic blood pressure and increased systemic vascular resistance (96-98). These changes are poorly tolerated in hemodynamic unstable recipients and may contribute to renal impairment. The introduction of veno-venous bypass by Shaw in 1984 (99) improved the results of adult liver transplantation as it corrected hemodynamic and renal disturbances and decreased intraoperative blood loss. However, the use of a veno-venous bypass is associated with complications including thromboembolism, air embolism, hypothermia and wound infection (100-103). Liver transplantation with preservation of the recipient vena cava ("piggyback" technique) has been proposed as an alternative procedure, allowing normal caval blood flow during the anhepatic phase. The use of this piggyback technique was first mentioned by Calne in 1968 (104) and fully described by Tzakis et al. in 1989 (105). In these first cases the hepatic veins were crossclamped and a veno-venous bypass still was used in adult recipients. The concept of side clamping of the retrohepatic vena cava, introduced by Belghiti in 1992 (106), allowed preservation of caval blood flow during the hepatectomy phase and overcame the need for veno-venous bypass. Since then, (modified) piggyback techniques have been compared to the conventional orthotopic liver transplantation technique by several authors (107-113), reporting reduced operative time, blood loss, transfusion need and hospitalisation. So far, in retrospective studies where patients were not matched for other variables affecting outcome, no survival benefit has been established. In a prospective, but not randomised study, outcome in both groups was excellent and no differences could be established (109). We compared therefore our results of a modified piggyback technique to a matched historical group of patients transplanted with the conventional technique.

3.4 Partial liver transplantation

With improving results of liver transplantation, more and more patients were referred to a transplant centre. This resulted in a steady increase of adults on the waiting list, but the death rate on the waiting list increased at an alarming rate, especially for children (29, 30, 114-116). In chapter 10 an overview is given of the different techniques that were developed to solve this problem. The introduction of partial orthotopic liver transplantation resulted in a decrease on the paediatric waiting list to almost zero over the years. Refinements of the operation opened the gate to right lobe living donor liver transplantation as the demand for organs increase every year and the numbers of donors remain constant in western countries. There is however serious concern of the well being of the liver donor in terms of morbidity and mortality.

4. AIMS OF THE THESIS

This thesis is aimed at optimisation of the existing liver transplantation procedures. For most candidates for liver transplantation the orthotopic transplantation technique will be appropriate. If however the auxiliary transplantation technique is preferred for some selected patients, the following questions need to be answered.

1. How is the portal and arterial blood flow altered after preservation and reperfusion of the partial liver graft in the presence of a non-cirrhotic native liver?
2. Does intervention in the portal blood flow during the transplantation procedure change portal and arterial flow patterns?
3. Which intervention in the portal blood flow improves long-term graft function?

To optimise graft and patient survival after conventional orthotopic liver transplantation, the following questions were addressed:

4. Is primary graft dysfunction caused by mitochondrial damage due to nitric oxide derived radicals during preservation and reperfusion?
5. Is intra-operative fibrinolysis and subsequent blood loss related to the type of blood plasma used during the procedure?
6. Is the vena cava preserving liver transplantation technique better than the conventional transplantation technique?
7. How do we expand the donor pool for paediatric and adult liver transplantation?

5. OUTLINE OF THE THESIS

Part one of this thesis contains the general introduction to partial and whole liver transplantation. **Chapter 2** addresses the concept of auxiliary partial liver transplantation. Auxiliary partial *heterotopic* liver transplantation was first introduced as a less invasive procedure for patients who could not tolerate a standard orthotopic liver transplantation. Later on the technique was proposed for patients with acute liver failure in whom regeneration of the native liver was expected, but due to its complications it never gained wide acceptance. Since 1990 a new concept of auxiliary partial liver transplantation was introduced where the graft is placed in the *orthotopic* position.

In **chapter 3** the development and current surgical technique of orthotopic liver transplantation are discussed. Initially the diseased liver was removed with the vena cava and replaced by a whole liver graft. With increasing waiting lists for donor organs, surgical techniques were developed to split the donor liver and transplant both partial liver grafts in the orthotopic position.

In **part two** three studies regarding experimental auxiliary partial liver transplantation are presented. In **chapter 4** we report the long-term correction of an inborn error of metabolism with an auxiliary partial liver graft placed in a *heterotopic* position. Since the distribution of portal blood flow between liver graft and native liver remains controversial in auxiliary liver transplantation, the success of metabolic correction was related to four different forms of portal inflow. With the introduction of auxiliary partial *orthotopic* liver transplantation several disadvantages of the placement of the graft in heterotopic liver transplantation were overcome.

In **chapter 5** we therefore assess the importance of portal flow diversion in an experimental model of auxiliary partial liver transplantation in the *orthotopic* position. The portal blood flow was measured with Doppler ultrasonography and changes in portal flow distribution were recorded after surgical intervention in the portal blood flow.
In **chapter 6** the metabolic correction following these interventions in portal flow were reported.

Part three contains three studies performed in patients receiving an orthotopic *whole* liver transplantation. In **chapter 7** we present the problem of graft dysfunction in the first 7 days after liver transplantation. We report our data on mitochondrial dysfunction in patients with

primary graft dysfunction and the relation with toxic reaction products of the nitric oxide radical.

In **chapter 8** we report an increased incidence of fibrinolysis, the most prominent coagulation disorder during orthotopic liver transplantation, after introduction of virus inactivated plasma. Consequences of fibrinolysis and treatment options are discussed.

In **chapter 9** we evaluate patient and graft survival between patients transplanted with standard liver replacement or a vena cava preserving technique.

Chapter 10 provides an overview of the development of different techniques for splitting of the donor liver in an attempt to alleviate the shortage of donor organs in paediatric and adult liver transplantation.

Chapter 11 summarises the previous studies, bringing the data in perspective and provides future perspectives.

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Auxiliary Partial Liver Transplantation

Chapter 2

Introduction

The concept of transplanting a liver graft side by side to the native liver originated from Welch in 1956 (1). The idea is theoretically attractive because the recipient liver is left in situ, avoiding the strenuous removal of the cirrhotic liver, which is usually densely attached to the diaphragm and abdominal wall due to inflammation and development of large collateral vessels. Dissection of the cirrhotic liver is still associated with considerable blood loss during liver transplantation, which was the main cause of death during the procedure and in the first week after transplantation in the early days (2). As surgical trauma was limited in auxiliary liver transplantation, the patients were expected to recover more rapidly than after an orthotopic whole liver transplantation. Another advantage of auxiliary liver transplantation is the absence of the anhepatic phase during the procedure, which enables some function of the native liver in clearance of t-PA and other haemostatic factors during anastomosis and reperfusion of the graft (3-5). Without hepatectomy, also the venous return to the right heart is preserved, avoiding the need for veno-venous bypass of the blood flow of the splanchnic vessels and lower extremities and creating a hemodynamic stable procedure.

Auxiliary liver transplantation was first introduced as a less invasive procedure for patients with end-stage chronic liver disease, who were at high risk of not surviving a conventional orthotopic liver transplantation (6-9). However, in patients with chronic active hepatitis, the risk of development of hepatocellular carcinoma in the native cirrhotic liver, which is left in place has proven to be substantial (7). Therefore auxiliary liver transplantation was abandoned in patients with a cirrhotic liver.

Patients with acute liver failure form another indication in which auxiliary liver transplantation is preferable over orthotopic whole liver transplantation (10-13). In these patients complete resection of the native liver is not indicated. Auxiliary liver transplantation may serve as a bridge to recovery with the possibility to redraw immunosuppressive therapy after regeneration of the native liver. Actual 1-year survival after auxiliary partial liver transplantation was 61% and of these patients, 65% could be taken off immunosuppressive medication (11).

Finally, auxiliary liver transplantation was proposed for patients with selected inborn errors of hepatic metabolism, in whom only one enzyme or cell membrane receptor is absent and who have otherwise normal function of the liver (table 1) (14-17). In those patients, auxiliary liver transplantation provides metabolic correction and preserves the possibility of future gene therapy in the native liver.

Table 1. Inborn errors of metabolism that possibly can be cured with auxiliary liver transplantation.

Alpha 1 -antitrypsine deficiency	Protoporphyria
Crigler-Najjar syndrome	Gaucher's disease
Urea cycle disorders	Wilson's disease
Tyrosinaemia	Galactosaemia
Wolman's disease	Propionic acidaemia
Byler's disease	Congenital Haemochromatosis
Glycogen storage diseases	Familial hypercholesterolaemia
Familial amyloidotic polyneuropathy	Haemophilia
Protein C deficiency	

The role of portal flow in auxiliary liver transplantation.

After an auxiliary partial heterotopic liver transplantation, some functional competition exists between the liver graft and the native liver (18, 19). Marchioro first described this functional competition in 1965 between two parts of a liver receiving its blood supply partly from the vena cava and the other part from the portal vein (20). The important role of adequate portal blood flow for survival of a liver graft was early established in both experimental and clinical setting (21-24). In the first reports, the nature of the portal blood seemed to be important. Various authors have indicated that pancreatic venous blood, with presence of insulin and glucagon is needed for graft regeneration. (25-29). Later on, other authors suggested that it is not the source of the blood, but rather its quantity that is important (30-35). The direction of adequate blood flow through the portal vein of liver graft and host liver is therefore of critical importance to auxiliary liver transplantation. In acute liver failure, portal blood flow may be a self-regulating mechanism, as in the early phase vascular resistance in the native liver is expected to be high due to the complete collapse of the diseased liver. This results in a blood flow preferentially towards the graft. In later periods, when the native liver is regenerating, a reversal effect may occur due to rejection episodes in the graft, leading to preferential blood flow towards the recovered native liver.

When however an auxiliary transplantation is performed in the presence of normal hemodynamic conditions of the recipient, like in inborn errors of hepatic metabolism, the distribution of the portal flow remains a major concern. To overcome the problem of insufficient portal blood flow, arterialisation of the portal vein was attempted with variable outcome (36-39).

Auxiliary Partial Heterotopic Liver Transplantation (APHLT)

The concept of auxiliary liver transplantation was first described by Goodrich and Welch in 1956 in a dog model (1). Absolon performed the first APHLT in men in 1964, one year after the first attempted orthotopic liver transplantation by Starzl (40). The graft comprised a whole liver graft and was placed in the upper right quadrant of the abdomen after resection of the right kidney. A heterotopic liver transplantation that truly prolonged life was achieved in 1972 (41). In 1980 the 29-month survival of an adult recipient was reported in Paris (7). In the following years many experimental studies were performed to optimise the procedure.

The placement of a large additional organ in the abdomen precluded in several cases wound closure after transplantation (41). Forced closure results in increased intra abdominal pressure with the risk of kinking or compression of the blood vessels of the graft and subsequent portal thrombosis. Removal of one of the kidneys or the spleen has been suggested, but has been abandoned today (22, 40). To solve the problem of lack of space in the abdominal cavity, different positions of the heterotopic graft have been proposed with various transplantation techniques. Besides the most frequently used right hypochondrium (Figure 1), grafts were placed in the thorax (10), left upper abdomen (42) and right lower abdomen (1). The technique that was developed by Bismuth in Paris placed the graft directly under the native liver (figure 2). Also the necessity of unimpaired venous drainage from the hepatic veins was demonstrated (43) and a new technique of side to side caval anastomosis was developed in the Netherlands (44) (figure 3). The best results were reported with the graft draining in the inferior vena cava as close as possible to the diaphragm, as the pressure in the vena cava fluctuates with respiration and increases proportionally with the distance from the right atrium (45).

Although satisfactory experimental results have been reported, clinical heterotopic liver transplantation was successful in selected patients only. From 1964 until 1980, 47 patients had undergone APHLT, but only two of these patients survived more than one year (46). While in this period OLT evolved to be the procedure of first choice, the potential advantages of APHLT still inspired researchers to study experimental auxiliary models in an attempt to overcome the main problems associated with the procedure.

In the laboratory for experimental surgery of the Erasmus University of Rotterdam, the problems associated with the APHLT procedure were extensively reviewed. The optimal transplantation technique (47, 48), efficacy (49, 50), intraoperative haemostasis (3, 4, 51-53), hemodynamic stability (54) and requirements for metabolic correction in case of an inborn error of hepatic metabolism were investigated (55, 56).

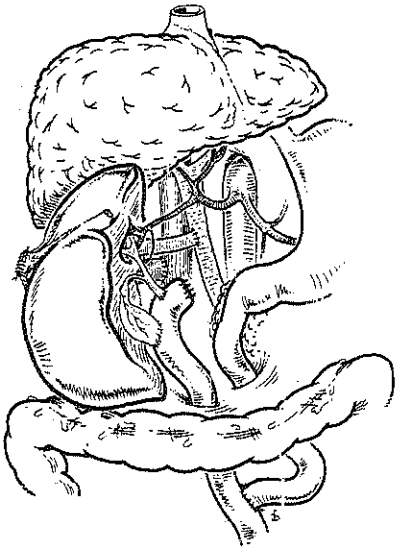

Figure 1. Auxiliary heterotopic liver transplantation with a whole graft, Bismuth 1988. From: Blumgart ed. "Surgery of the liver and biliary tract". London, Churchill-Livingstone 1988.

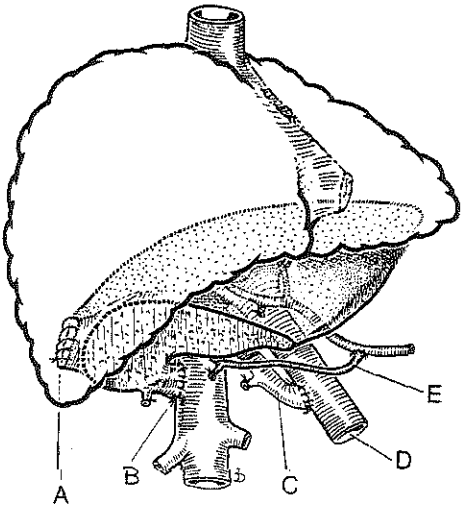

Figure 2. Auxiliary partial heterotopic liver transplantation, Bismuth 1984.
A) Suprahepatic vena cava inferior. B) Anastomosis with Infrahepatic vena cava. C) Graft left portal vein. D) Native common portal vein. E) Graft hepatic artery. From: Blumgart ed. "Surgery of the liver and biliary tract.". London, Churchill-Livingstone 1988.

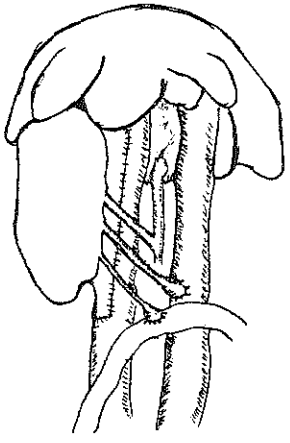

Figure 3. Side-to-side cavo-cavostomy, performed by Ligydakis to optimise hepatic outflow. From: J Surg Res 1985; 38: 246. Academic Press.

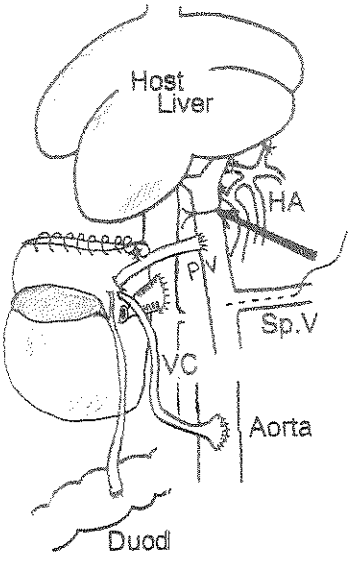

Figure 4. Auxiliary partial heterotopic liver transplantation developped in the Rotterdam laboratory for Surgical research.
HA: hepatic artery. PV: portal vein. SpV: splenic vein. VC: vena cava.

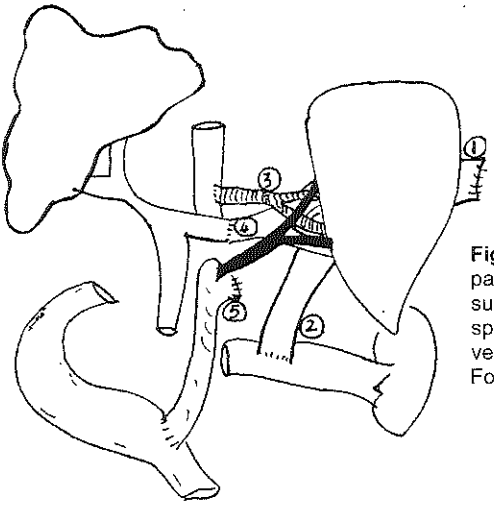

Figure 5. Auxiliary partial heterotopic technique for patients with portal vein thrombosis, Fournier 1990. 1) suprahepatic vena ava. 2) cavo-renal anastomosis. 3) spleno-hepatic arterial anastomosis. 4) spleno-portal venous anastomosis. 5) choledocho-jejunostomie. From: Fournier. Transplant Proc 1990; 22:1572.

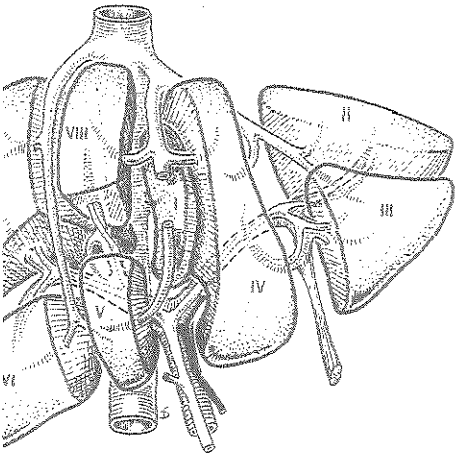

Figure 6. Liver segmental anatomy, proposed by Couninaud in 1957. Segm II +III = left liver lobe. II, III & IV= left hemi liver. V-VIII= right liver lobe. V-VIII & IV= right hemi liver. I=caudate lobe. from: Blumgart ed. "Surgery of the liver an bilary tract" London, Churchill-Livingstone 1994.

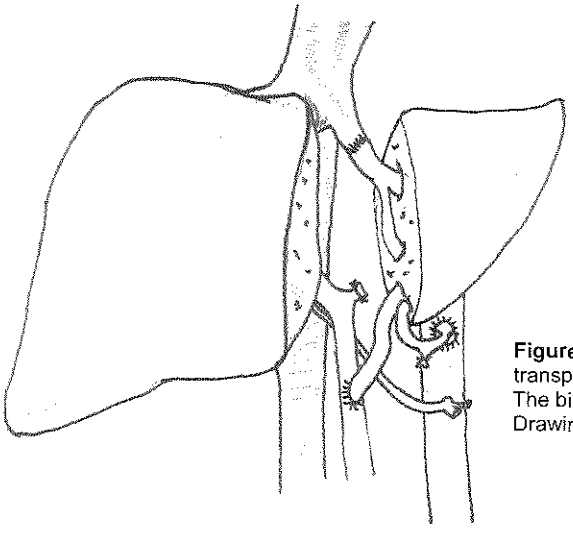

Figure 7. Auxiliary partial orthotopic liver transplantation according to Gubernatis (1990). The biliary tract is not drawn.
Drawing: de Jonge, 2001

A new concept of reduced-size heterotopic liver transplantation was developed with portal and arterial inflow, vital to graft survival and biliary tract integrity (57). The graft was placed in the right subhepatic space after removal of the left lateral and medial liver segments, leaving about 70% of the donor liver. The infrahepatic vena cava of the graft was ligated and venous drainage was achieved through anastomosis of the suprahepatic vena cava of the graft to the recipient's infrahepatic vena cava, proximal to the renal veins (figure 4).

The results of these experimental studies initiated a clinical program in Rotterdam starting in 1986. Two years later, the favourable outcome in six high-risk patients, rejected by another transplant centre, was reported (9). In an open comparative study between OLT and APHLT, Metselaar et al. showed similar results in the two groups (58). Blankensteijn reviewed the results of APHLT between 1980 and 1990 (59). In that decade 50 APHLTs were performed in 48 patients in 11 transplant centres. Overall one-year patient survival was 55%, but survival in patients transplanted since 1987 was 71%. In two recipients a new hepatocellular carcinoma developed in the native liver after transplantation, indicating the risk of application of APHLT for cirrhosis due to viral hepatitis (7).

Experimental studies performed in the last decade showed good results for metabolic support in acute liver failure (60-62) and addressed the subject of portal flow distribution between graft and native liver in absence of cirrhosis (63-65).

However, due to persisting complications associated with the heterotopic position of the graft, clinical auxiliary heterotopic liver transplantation was rarely performed, despite its theoretical advantages (66). In 1990 Fourtanier reported a new technique of APHLT that can be used in patients with portal vein thrombosis (67) (figure 5). Since then, some authors reported successes with auxiliary heterotopic liver transplantation in individual cases for acute liver failure (38, 68-72). Despite these reports, 1-year patient survival after heterotopic auxiliary transplantation was only 33% for acute liver failure in a review of the Eurotransplant area and primary non-function was more frequent than after orthotopic liver transplantation (11). Incidental reports of auxiliary heterotopic liver transplantation for Budd-Chiari syndrome (73), Alagille syndrome (74) and Protein C deficiency (75) were published in the last 3 years.

To overcome the problems of space, blood supply and drainage that hampered survival, in 1990 a new concept of auxiliary liver transplantation was introduced where a partial liver graft was placed in the orthotopic position after removal of liver segments II to IV.

Auxiliary partial orthotopic liver transplantation (APOLT)

The first report of the use of an auxiliary partial orthotopically placed liver graft comes from Bismuth in 1985 (76), but the patient died 13 days after the procedure. In 1989 the first successful clinical APOLT was performed in Hannover for acute liver failure due to the HELLP syndrome (hemolysis, elevated liver enzymes and low platelets) after pregnancy (77).

The donor liver was split by bench surgery to retrieve the left liver lobe (Couinaud segments 2-4 (figure 6, (78)). The vena cava inferior was dissected from the liver, except for a small part that served as a patch for anastomosis to the recipient left hepatic vein. The whole celiac axis including an aortic patch was preserved for the graft. In the patient a resection of segments 2 and 4 was performed, preserving portal and arterial blood flow to segment 4. The left hepatic vein was carefully dissected to obtain sufficient length for reanastomosing. Hereafter first an end-to-end anastomosis of the hepatic vein was performed, without clamping the vena cava inferior or veno-venous bypass. The celiac axis of the graft was anastomosed with a patch to the recipient aorta above the native celiac trunk. Portal vein reconstruction was performed by end-to-side anastomosis to the common portal vein at a sharp angle. The biliary tract was reconstructed with a Roux-en-Y jejunal loop (figure 7). After a complicated post-operative course with recurrent gastrointestinal tract bleeding, the patient could be discharged from hospital after 3 months and in the following year the immunosuppression could be tapered off.

In this situation no additional precautions were taken to ensure portal inflow in the graft as the vascular resistance in the native liver was expected to be much higher and gradual atrophy of the graft after native liver regeneration was aimed for. Since its introduction, auxiliary partial orthotopic liver transplantation has been mainly used for acute liver failure (70, 79-88). In general a 1-year survival comparable to that of orthotopic whole liver transplantation for acute liver failure can be achieved Azoulay, 2001 #1103] and 60% of the patients can be taken off immunosuppressive medication (11).

In 1990 APOLT proved also to provide total metabolic correction in a 14-month-old patient with ornithine transcarbamylase deficiency (OTCD), a urea cycle defect (89). In this case a segment 2/3 graft was placed after resection of the patient's left liver lobe. Portal flow reconstruction was achieved by end-to-end anastomosis of the graft left portal vein to the preserved vascular pedicle of the resected left lobe. Within 1 week after transplantation a normalisation of blood ammonia was seen and normal diet could be taken. Also in this particular case no precautions were taken to ensure portal inflow to the graft, as intra-

operative ultrasound indicated sufficient portal inflow. If, however the vascular resistance in the graft increases e.g. due to rejection episodes, the portal flow may be completely divided to the patient's own normal liver. This competition for portal blood between the two livers in auxiliary transplantation seems difficult to overcome when the patient's liver has only metabolic disease and is otherwise unimpaired.

Since this first report, APOLT procedures for other inborn errors of hepatic metabolism have been described in 14 patients with various metabolic disorders: Crigler-Najjar syndrome (90-93), OTCD (82, 94-98) and other metabolic diseases (99, 100).

In some of the transplanted patients the portal blood flow towards the native liver was artificially obstructed to ensure adequate blood flow towards the graft, so called "banding" (90, 91). In other cases the host portal vein had to be ligated after initial successful transplantation without intervention to guarantee adequate perfusion of the graft after a rejection episode (97, 98). For this indication, the complicated hemodynamic situation of the liver graft - side by side with the non-cirrhotic native liver - still is a critical issue at the time of surgery and of major importance to long-term graft survival. Only few experimental studies addressed the problem of portal blood distribution between the native liver and the auxiliary graft (29, 101, 102) and therefore this problem is further assessed in chapters 5 and 6.

The technical advances that enabled auxiliary orthotopic liver grafting had also other consequences to liver transplantation (89, 103). It opened the way for living donor hemi-liver transplantation in the situation of acute liver failure (82, 104, 105) and metabolic diseases (15, 82). There is however great reservation to put a healthy donor at risk and ethical issues of donation remain to be solved as long-term donor safety must be established. So far two donor deaths are reported with more than 1000 living donor procedures performed, but this is probably underreported. For the sake of donor safety in living donor liver transplantation, the left lobe is currently being used most often for the graft. However, in countries where living donors are the only source of liver grafts (as in Japan where brain death is non commonly accepted), restrictions on graft size are a serious obstacle for the expansion of indications for adult recipients. To overcome this problem, auxiliary partial orthotopic liver transplants was performed on the basis of the concept that the residual native liver would support the graft function until the graft had grown enough to function by itself (106). From 1995 until 1998, 20 recipients underwent living donor APOLT, which was indicated because a small for size graft (estimated graft/recipient's body weight ratio <0.8%) was harvested. Post-operative survival was 65% in these patients with signs of advanced liver failure, such

as massive ascites or hepatic coma. Mortality was not related to technical failure, but associated with sepsis.

This indicates that auxiliary partial liver transplantation is not only useful to bridge a patient in acute liver failure to recovery or provide metabolic correction in inherited metabolic liver diseases, but also can help to expand the indication of living donor liver transplants for adult recipients.

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Orthotopic liver transplantation:

whole and partial liver transplantation

Chapter 3

Orthotopic whole liver transplantation (OLT)

The concept of orthotopic replacement of the liver was first mentioned by Claude S. Welch (1), although the first reported experiments with canine orthotopic liver transplantation were done by Cannon (2) in 1956. None of the animals survived the operation and it took four years until the technique was performed successfully by teams in Denver and Boston (3, 4). Human orthotopic liver transplantation was first attempted on 1 March 1963, at the University of Colorado by Starzl and co-workers (5). This first transplantation and six others in Colorado, Boston and Paris were unsuccessful and clinical trials were halted for 3 years. In 1967 Starzl performed the first clinical transplantation that extended life, when a child lived 1.5 years after transplantation for hepatic malignancy before dying of metastasis (6).

Operation technique

Transplantation of the liver is a major operation, often offered to some of the sickest patients who are submitted to surgery. The technique of liver transplantation is divided into three stages: the hepatectomy phase, the anhepatic phase and the reperfusion phase. Hepatectomy is the most difficult part of the procedure because of the presence of portal hypertension and severe coagulopathy. Control of haemorrhage during the removal of the patient's owns liver can be difficult. Blood loss in the hepatectomy phase is often significant, with the need of large amount of packed red blood cell, fresh frozen plasma and thrombocyte substitution. After resection and haemostasis, particularly of the right adrenal area, the liver graft is anastomosed to the supra and infrahepatic vena cava inferior, portal vein and hepatic artery (figure 1). Biliary reconstruction may involve an end-to-end choledocho-choledochostomy or Roux-en-Y choledocho-jejunostomy with or, currently, without t-tube (7-9) (figure 2). After completion of the portal vein anastomosis the liver graft is often already reperfused to keep the warm ischaemic period as short as possible. Before reperfusion however, the organ is perfused with albumin or another fluid to wash out the preservation solution, which contains a high concentration of potassium.

In the reperfusion phase there are always signs of potassium and acid release with a 50% reduction in cardiac output, pulmonary hypertension and marked increase in systemic vascular resistance (10-13). Also profound haemostatic disturbances with onset of hyperfibrinolysis and depletion of clotting factors may be present in this stage of the operation (14-17). Adequate surgical haemostasis is promoted by interventions of the anaesthetist to achieve normal clot formation. After wound closure over closed sump drains, the patient is rewarmed in the intensive care unit where vital functions are supported.

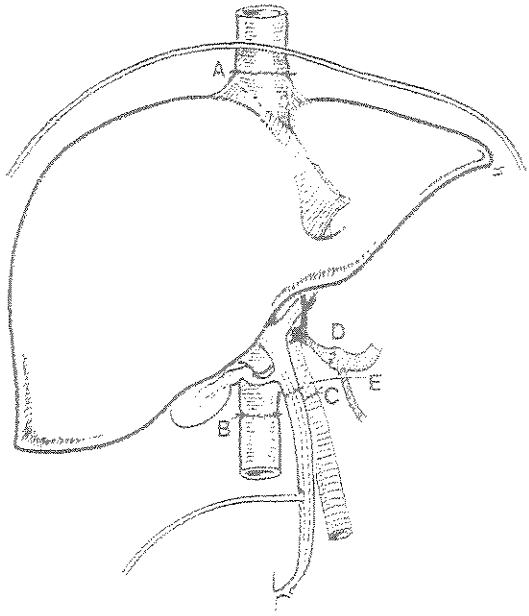

Figure 1. Orthotopic whole liver transplantation. The supra (A) and infrahepatic (B) vena cava inferior, portal vein (C), hepatic artery (D) and common bile duct (E) are anastomosed. From: Forsythe ed. "Transplantation Surgery". London, WB Saunders, 1995

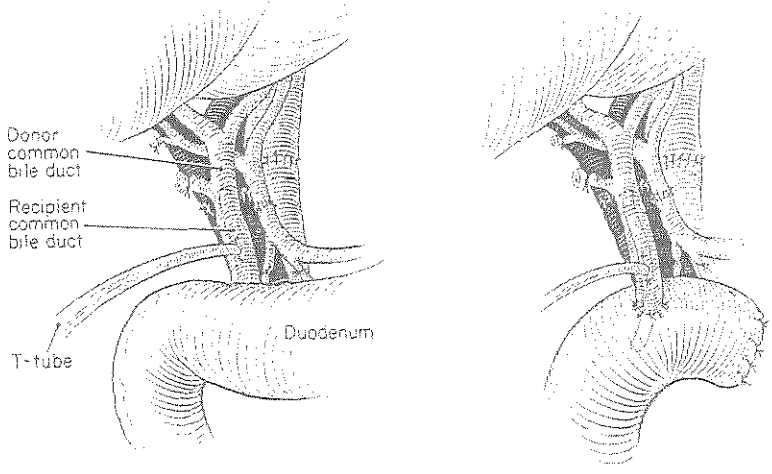

Figure 2. Biliary tract reconstruction with end-to-end anastomosis or choledocho-jejunostomy and the use of a T-drain. From: Busuttil et al. Ann Int Med 1986; 104:377-389.

Results

In the initial phase, one-year patient survival was poor and restricted to approximately 30% (8, 18). In the era 1963-1979 immunosuppression was achieved by steroids, azathioprine and sometimes anti-thymocyte globulines (ATG). Technical post-operative problems included ischaemic injury to the liver graft with poor initial function, anastomotic problems, graft vascular injuries, and bile duct problems. Extrahepatic problems were mainly caused by too much immunosuppression with bacterial, viral and fungal infections or too little immunosuppression with rejection on day 4 or 5 after transplantation. Late problems included drug-induced nephrotoxic and cardiotoxic side effects, chronic rejection and recurrence of the liver disease.

In 1980 there was a major step forward with the introduction of Cyclosporine A (Novartis) as the main immunosuppressive drug (19). The predictability and reliability with which liver transplantation could be carried out improved steadily with the first trials of cyclosporine / steroid therapy and since 1980 the majority of liver recipients has survived the initial post-operative phase. The most specific survival information was obtained by the groups of Starzl (Pittsburgh) and Calne (Cambridge / King's College hospital). One-year survival rates increased to 60-75% (20-22). Part of this improved survival was attributed to the inclusion of paediatric patients, who had about a 10% survival advantage over adult recipients, in the liver transplantation programmes. Several other factors played an important role in the improvement of survival rates.

One important technical factor improving patient survival was the introduction of a veno-venous bypass by the Pittsburgh group in 1984 for decompression of the blood flow of the splanchnic vessels and lower extremities (23). Most of the surgical difficulties were centred on the anhepatic phase. Approximately 50% of the patients who died within the first year following liver transplantation were lost in the first post-operative month. In addition, operative mortality of the first patients treated with cyclosporine still was 9.5% and blood loss was the main cause of death in these patients (18).

Crossclamping of the portal vein and abdominal vena cava inferior causes several problems. It leads to impaired venous return to the right heart, portal hypertension and a sudden return of the blood from obstructed venous beds into the circulation after reperfusion. To overcome these problems, a passive veno-venous bypass was tried but abandoned after embolic complications (5, 24). A clinical trial with systemic heparinisation initiated in 1982 led to increased blood loss and reduced patient survival, but the need for some sort of bypass remained evident. An experimental method of bypass without the need

for system anticoagulation by heparin coated tubing was successfully developed by the Pittsburgh group (25). This enabled early implementation of the bypass (figure 3), greatly decreasing the extent of preliminary dissection of the liver. The hepatectomy could then be performed with hemodynamic stability and the raw resection surfaces that were created during dissection could be systematically closed. All together the technique offered a 10% survival benefit in the first 30 days after transplantation to all patient categories (26).

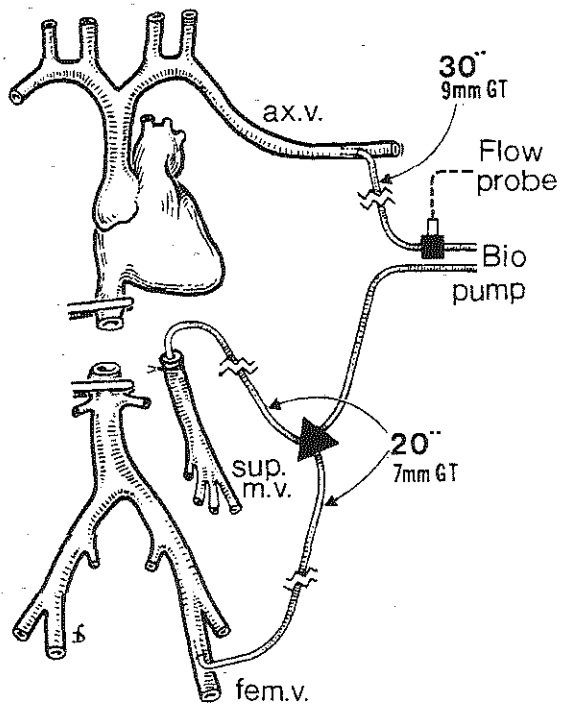

Figure 3. Diagram of the veno-venous bypass. The venous return of the splanchnic and lower extremity vascular beds is transferred to the axillary vein.
From: Starzl in "Surgery of the liver and biliary tract" Blumgart ed. London, Churchill-Livingstone 1988

Another improvement in graft and patient survival was obtained by the development of a special preservation solution to keep the harvested donor liver in good condition during cold storage. At the University of Wisconsin, Belzer and co-workers developed in 1989 a preservation solution ("UW-solution"), which contained all prerequisites for cell survival and energy preservation (27, 28). The introduction of the UW-solution enabled safe storage of the donor liver up to 16 hours and changed the liver transplantation from an emergency into a semi-elective operation (29). Additionally other preservation solutions were designed with equal results (30, 31).

Further improvements in operation technique and perioperative care included the aggressive intra-operative monitoring of the coagulation cascade by thromboelastography (TEG), introduced by Kang in 1985 (32). This enabled detection of hyperfibrinolysis, a serious coagulation disorder where the blood clot is dissolved by tissue type plasminogen. The standard use of anti-fibrinolytic medication during the procedure further reduced blood loss (33-38).

The introduction of a transplantation technique, which preserves the vena cava during the transplant procedure further reduced transfusion requirements and shortened the warm ischaemic period. This "piggyback" technique was first mentioned by Calne in 1968 (39), but fully described by Tzakis in 1989 (40). The technique originally uses the hepatic veins of the recipient for cavo-caval end-to-end anastomosis, obviating the need for cross clamping of the vena cava inferior. Initially the veno-venous bypass was used during the procedure, but in 1992 Belghitti introduced a modification with side-to-side cavo-cavostomy (41), which enabled transplantation without bypass (42).

Parallel advances in immunosuppression were made by the introduction of tacrolimus (FK-506, Fujisawa) (43), which is comparable with Cyclosporine (CsA) in efficacy, but sometimes can be used in CsA resistant rejection or CsA induced nephrotoxicity. Rapamycine (Sirolimus, Wyeth), an inhibitor of the mammalian target of rapamycine (mTOR) is another new immunosuppressive agent with promising short-term results (44, 45). Recently the recombinant human monoclonal CD-25 receptor antagonists Daclizumab (Roche) (46) and humanised Basiliximab (Novartis) (47) were engineered to reduce acute rejection in the first year after transplantation. At this moment steroid withdrawal and even steroid-free immunosuppression regimens are under study.

These factors together made that orthotopic whole liver transplantation is nowadays a safe option for end-stage chronic and acute liver failure. Currently many centres report one-year survival rates of 90% and about 4500 liver transplantations are performed annually in the USA with 16000 patients on the waiting list (48) and about 4000 liver transplantations are performed in Europe with 9000 patients waiting on the list (49, 50).

Orthotopic liver transplantation in Rotterdam

In the Netherlands, orthotopic liver transplantation was first performed at the University hospital in Groningen in 1979. Until 1982 transplantations were only performed in adults because survival results did not justify paediatric transplantation. The first experience with

liver transplantation in the Rotterdam University hospital was gained in 1986. Since then more than 300 orthotopic liver transplantation have been performed.

Partial orthotopic liver transplantation

Worldwide shortage of donor livers was the main obstacle for further expansion of liver transplant programs during the last decade. During this period the waiting lists for adults showed a steady increase all over the world, but the death rate on the waiting list for children increased at an alarming rate. To solve this problem several alternative techniques of partial liver transplantation were developed.

The earliest form consisted of a reduction of a whole liver graft to the left lateral segment or the left liver lobe, small enough to transplant to a young child. The remaining part of the liver was discarded. This method solved part of the great need of liver grafts for children but as the remaining part of the liver was lost, the method was in fact detrimental for adults on the waiting list.

Further surgical development resulted in splitting of the liver *ex-vivo* into two transplantable partial grafts, the left part to a child and the right lobe to an adult. The procedure was successfully introduced but the complicated logistics resulted in prolonged cold ischaemia times of the grafts. In order to keep the cold ischaemia time as short as possible Rogiers developed the *in-situ* split liver technique in which the liver was split in the post-mortem donor (51). Refinement of this operation led to results, superior to the *ex-vivo* method. It opened the gate to living donor liver transplantation. The first successful living donor liver transplantation was performed in Australia from a mother to a child who received the mother's left liver segment (52). The introduction of living donor transplantation resulted in a decrease over the years on the paediatric waiting list to almost zero. As the demand for organs increases every year and the numbers of donors are constant in western countries, the right lobe living donor liver transplantation for adults was introduced. There is however concern of the well being in terms of morbidity and mortality of the living donor. Until now there are in the literature two donors who died after living liver donation but the true figure, after more than 1000 living donor liver transplantations is expected to be higher judged by experts (53, 54).

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Part II

Experimental auxiliary partial liver transplantation: optimisation of portal flow

Directing the portal flow is essential for graft survival in auxiliary partial heterotopic liver transplantation in the dog

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Chapter 4

Abstract

Auxiliary liver transplantation is an attractive alternative for orthotopic liver transplantation in patients with certain inborn errors of metabolism of the liver, where complete resection of the liver is unnecessary or even contraindicated. As in these diseases portal hypertension is mostly absent, finding a balance in portal blood distribution between native liver and graft is complicated. Our objective was to investigate requirements for long-term (180 days) graft survival in auxiliary partial heterotopic liver transplantation (APHLT) in a dog model. A metabolic defect was corrected in 26 Dalmatian dogs with a 60% Beagle heterotopic auxiliary liver graft. Grafts were preserved in UW solution (Viaspan, DuPont). Four groups of different portal inflow were studied: In the *ligation* group the portal vein to the host liver was ligated. In the *split-flow* group graft and host liver received separate portal inflow. In the *banding* group the distribution of the portal flow was regulated with an adjustable strapband and in the *free-flow* group the portal blood was allowed to randomly flow to host or graft liver. Post-operative the dogs received Cyclosporine in an oral dosage of 10 mg/kg/day. Metabolic correction increased in all groups after transplantation from 0.19 ± 0.02 to 0.70 ± 0.05 ($p<0.0001$), but remained significantly better in the ligation and split-flow groups (graft survival 135 ± 27 and 144 ± 31 days). In the banding group metabolic correction decreased significantly after 70 days and although the grafts kept some function for 155 ± 14 days, in 4/6 dogs portal thrombosis was found. In the free-flow group, competition for the portal blood lead to reduced correction within 12 days and total loss of function in 96 ± 14 days. Graft function was also assessed with ^{99m}Technetium dimethyl-iminodiacetic acid uptake. A good linear association between HIDA uptake and metabolic correction was observed ($r=0.74$; $p<0.0005$). Grafts that contributed more than 15% to the total uptake of HIDA showed biochemical correction. This indicates a critical graft mass of about 15-20% of the hepatocyte volume to correct this metabolic defect. Auxiliary partial heterotopic liver transplantation can be a valuable alternative treatment for inborn errors of hepatic metabolism, if the native liver and the graft receive separate portal blood inflow.

Introduction

Orthotopic liver transplantation (OLT) has become the standard therapy for end-stage cirrhotic liver disease. Still, children with some life threatening *inborn errors of hepatic metabolism*, in which an enzyme or cell membrane receptor is absent, have an otherwise normal function of the liver and complete resection of the liver in OLT is only performed for technical reasons (1,2). Auxiliary partial heterotopic liver transplantation (APHLT) can be an

attractive option as it is a less demanding surgical procedure than OLT and primary graft non-function does not lead to an emergency re-transplantation. With partial liver transplantation, living-related procedures come into focus, allowing optimal timing and good graft quality (3-5). In addition auxiliary transplantation preserves the possibility of future gene therapy (6). In spite of these benefits, auxiliary heterotopic liver transplantation never gained wide acceptance due to its initially poor results (7) and the success of OLT. In previous animal experiments the surgical technique of APHLT and its efficacy to provide temporary support in (sub)acute liver failure have been assessed (8,9). In that situation the portal blood flow is preferentially toward the graft due to the increased vascular resistance in the native liver. In the correction of an inborn error of metabolism however, portal hypertension is absent and great attention must be paid to the prevention of portal vein thrombosis due to insufficient portal inflow or insufficient venous outflow. In this study we use the metabolic defect in the Dalmatian dog as a stand in for a life threatening inborn error and we evaluate long-term correction after transplantation of a 60% Beagle liver graft in four groups with different portal vascularisation.

Methods

Transplantations were performed from 26 Beagles to 26 Dalmatian dogs. The Dalmatian dog lacks a normal transport system for uric acid into the hepatocyte, which impedes the conversion of uric acid into allantoin and results in significantly depressed allantoin formation (10). Of this formed allantoin, 94% is excreted in urine by renal clearance (11) and the ratio of allantoin / creatinin in urine proved to be nearly constant, allowing correction for daily variations in renal function. Pre-transplantation excretion ratio of allantoin was 1.25 ± 0.07 in Beagles and 0.19 ± 0.02 in Dalmatian dogs (95% confidence interval: 0.16-0.22). In donors total hepatectomy was performed and after in situ perfusion with 1 litre of cold University of Wisconsin solution (Viaspan, DuPont), the right lateral and right medial lobe were prepared for transplantation by bench surgery. In the recipient the pressure in the common portal vein and vena cava was registered simultaneously before transplantation. The graft was placed in the right subhepatic region and three vascular anastomoses were made: the suprahepatic vena cava of the graft was placed end-to-side with the infrahepatic inferior vena cava of the recipient; the hepatic artery was anastomosed to the infrarenal aorta. The portal vein was anastomosed end-to-side to the host portal vein and animals were divided in three study groups: in the *ligation group* (n=8) the host portal vein was ligated, the *free flow group* (n=6) had random flow to both livers. In the *banding group* (n=8) an adjustable strapband round

the host portal vein was carefully tightened to decrease the pressure proximal to the banding site to 50 % of the pre-banding portal pressure.

In the *split flow group* (n=4) the graft portal vein was anastomosed to the host superior mesenteric vein, which was ligated at its insertion in the portal vein. In this way the flow of the mesenteric vein was directed to the graft and the flow of the splenic vein to the host liver. Bile drainage was achieved by choledocho-duodenostomy. After reperfusion pressure recordings in the graft portal vein and vena cava were performed. Recipients received Cyclosporine A (Novartis, Uden, The Netherlands) in an oral dosage of 15 mg/kg/day. Changes in uric acid and allantoin levels were measured weekly in plasma and 24 hours collected urine samples. Graft function was determined by urinary excretion of allantoin (12). Intravenous angiography was performed 14 days after transplantation. At 3, 7, 16 and 24 weeks after transplantation the liver function of both graft and host liver were quantitated by determination of ^{99M}Tc labelled dimethyl-iminodiacetic acid (^{99M}Tc-HIDA) uptake. Autopsy and registration of the wet liver weight were done in all animals. Surviving animals were sacrificed 180 days after transplantation. All data are presented as mean ± SEM. Differences between groups were detected by analysis of variance (ANOVA) with the Bonferroni *post hoc* test, after testing normality with Shapiro-Wilk statistics. For correlation analysis the Spearman rank correlation was calculated. Graft survival was analysed by the method of Kaplan-Meier. Loss of graft function was scored when post-transplantation allantoin excretion ratio in urine reached the 95% confidence interval of the pre-transplantation level (mean ± 2 SEM). Differences were considered significant for $p<0.05$.

Results

The harvested grafts were weighed before implantation (231±12 g). After reperfusion the portal pressure was significantly increased in the ligation group (11.8 ±0.8 mm Hg; $p<0.05$ vs. free-flow and banding). In the free-flow group portal pressure measured 4.2 ± 0.7 mm Hg, 6.4 ± 0.3 mm Hg in the banding group and 7.8 ± 3.0 mm Hg in the split-flow group (Figure 1). The insertion of the graft resulted in a slightly increased central venous pressure; vena cava pressure increased from 3.4 ± 0.3 mm Hg to 4.4 ± 0.4 mm Hg (n.s.). None of the dogs died during surgery, but late mortality was high. In the ligation group 4/8 dogs died from an accident, gastric perforation, arterial thrombosis of the graft and sepsis. In the free-flow group 4/6 dogs died from severe necrotic grafts with secondary infection (2), bile peritonitis and haemorrhagic cystitis. In the banding group 2 dogs died from infected haematoma and unknown cause. In the split-flow group 1 dog died from severe rejection at

day 98. Graft failure due to hepatic vein thrombosis was not seen. In all 19/26 dogs in which an arteriogram could be performed, it showed patency of the graft hepatic artery.

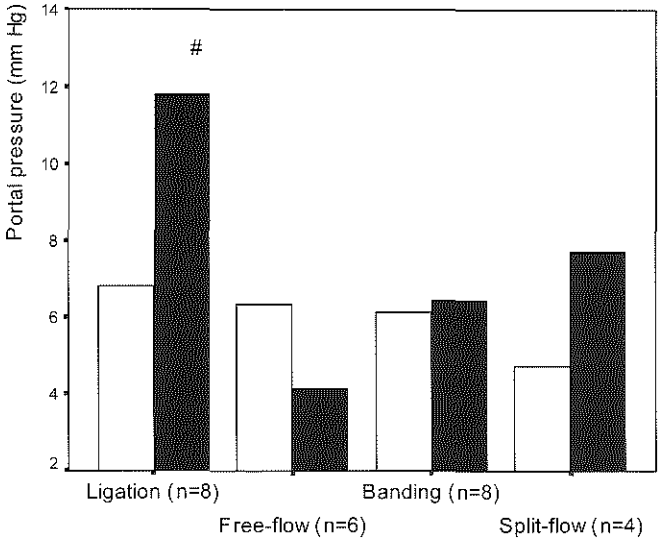

Figure 1. Pressure in the graft portal vein before and after transplantation.
□ pre-transplantation; ■ post-reperfusion. # $p<0.05$ vs. free-flow and banding group. Increased portal pressure after ligation and splitted portal flow. In the banding group the pressure in the host portal vein measured 50% of that in the graft portal vein. In the free-flow group the graft functioned as a porto-caval shunt with low perfusion pressure in the portal vein.

In figure 2 the allantoin excretion ratio in urine is shown during follow-up. After transplantation excretion of allantoin increased from 0.19 ± 0.02 to 0.70 ± 0.05 in all groups ($p<0.0001$). In the ligation and split-flow group metabolic correction remained stable at about 300% of the pre-transplantation level. Mean graft function lasted 135 ± 27 and 144 ± 31 days respectively. In the banding group the metabolic correction decreased significantly after about 10 weeks from 0.68 ± 0.05 to 0.33 ± 0.06 at week 16 ($p=0.01$). At autopsy late portal thrombosis was seen in 4/6 dogs surviving 180 days. Metabolic correction in the 2 dogs with patent portal veins was 0.49 compared to 0.19 in the dogs with portal thrombosis ($p<0.02$), but as these animals all survived with their poor functioning grafts, mean graft survival still was 155 ± 14 days. In the free-flow group the metabolic correction was significantly worse from that in the other groups by day 21 (0.39 vs. 0.81, 0.72 and 0.69; $p<0.02$) and returned to pre-transplantation levels after 3 months; mean graft survival was 96 ± 14 days.

In table 1 the wet liver weight of all dogs at autopsy is shown. In the ligation group atrophy of the host liver was observed in 1 dog. The grafts in the ligation and split-flow group

showed hypertrophy compared with the pre-transplantation graft weight, while grafts in the free-flow group showed atrophy. Grafts in the banding group remained their original weight.

Table 1. Wet liver weights at autopsy.

	Host liver (g)	Graft (g)	Total (g)
1. Ligation group	527±83	530±81 †	1057±94
2. Free-flow	1102±122 *	168±44	1269±1 39
3. Banding	599±100	285±78	884±76
4. Split-flow	723±168	357±39	1080±1 91

*P<0.05 versus ligation and banding. †P<0.05 versus free flow.

Histology examination showed oedema in the grafts of dogs that died within three days after transplantation (N=3). An equal distribution of mild to moderate chronic rejection with mixed cellular infiltrate was observed in the ligation, banding and split-flow group. In the free-flow group however, extended (>75%) hepatocellular necrosis and destroyed architecture were seen. The wet liver weight of the graft at autopsy correlated significantly with its contribution to the total HIDA uptake, as determined in the last scintigraphy before autopsy (N=21, r=0.8-4; p<0.0005). In figure 3 excretion of allantoin in urine at the time of ^{99M}Tc-HIDA scanning is plotted against the relative graft contribution to the total HIDA uptake. 72 Scintigraphies could be performed in 21 dogs (5 dogs died before the first scan was made). There was a significant correlation between proportional correction of the inborn error and the graft contribution to the total HIDA uptake (r=0.74; p<0.0005). Grafts that contributed more than 15% to the total HIDA uptake, had graft function during 49/50 (98%) scans, where grafts that contributed less than 15% had graft function during 6/22 scans (27%); p<0.0001, Chi-square test.

Discussion

Auxiliary liver transplantation seems more and more an attractive option for orthotopic liver transplantation in the correction of metabolic diseases (13), but the technical difficulty in this specific situation, were the vascular resistance in the host liver is not increased as in acute liver failure, is to distribute the portal flow over the host liver and graft. Even the concept of auxiliary partial orthotopic liver transplantation (APOLT) does not overcome this problem

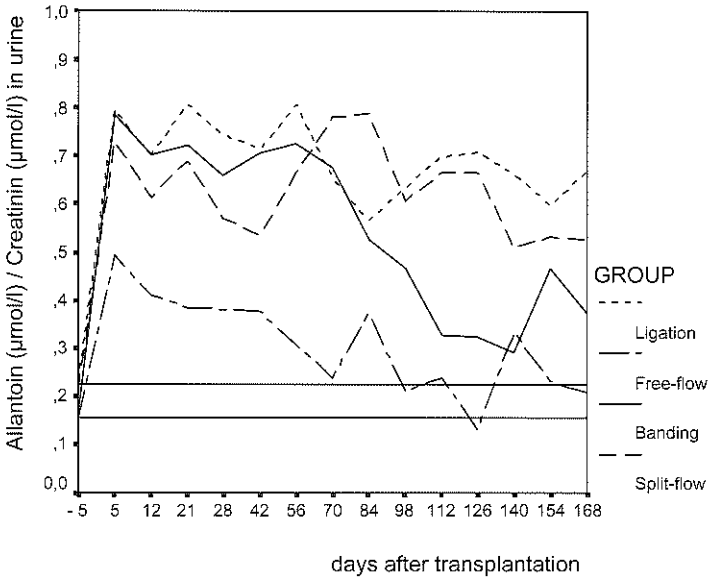

Figure 2. Excretion of allantoin -corrected for creatinin- in urine.
Metabolic correction remained stable in the ligation and split-flow group. In the banding group a decrease in graft function was seen, where in the free-flow group correction was less marked and returned to pre-transplantation levels. —— : 95% C.I. of the pre-transplantation allantoin excretion ratio.

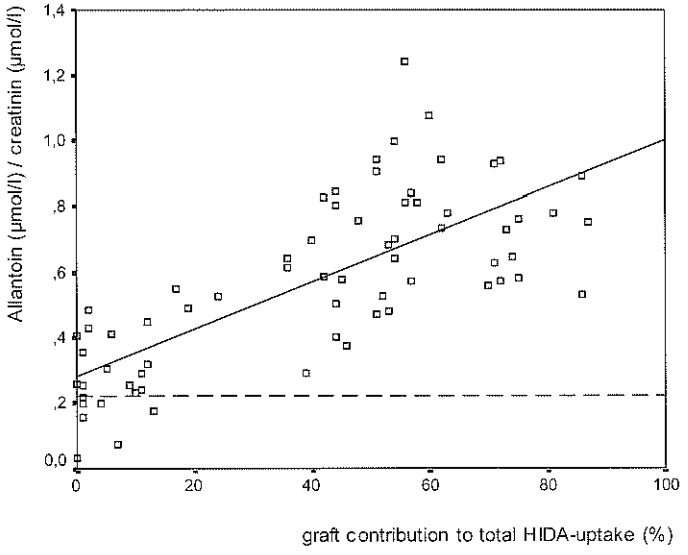

Figure 3. Correlation of HIDA-uptake and allantoin excretion in urine.
72 Scintigraphies were performed in 21 dogs. Graft contribution to the total HIDA-uptake during scintigraphy correlated significantly with the urinary allantoin level; r=0.74; p<0.0005.
- - - - : 95% pre-transplantation C.I. of the allantoin excretion ratio.

and also in this situation chronic rejection led to decreased portal inflow in the graft (14). In this experimental study both ligation of the host portal vein and separate blood flow for host liver and graft assured adequate portal flow to the graft and good graft function. However, ligation of the host portal vein may interfere with the concept of its life supporting function in case of nonfunction of the graft and caused host liver atrophy in 1 dog. In the banding group initial good metabolic function decreased in time with increasing vascular resistance due to chronic rejection. Unlike the ligation and split-flow group, were chronic rejection did not lead to loss of function, the portal flow in this group probably redistributed towards the host liver, resulting in decreased portal inflow in the graft and late thrombosis in 4/6 dogs. In the free-flow group acute competition for the portal blood flow led to rapid loss of function and atrophy of the graft as is described before (15,16). In our study we did not find evidence for functional competition between host liver and graft in the split-flow group due to a hepatotrophic factor, as was described in literature (17,18). The host liver, receiving the pancreaticoduodenal derived venous blood, did neither show hypertrophy, nor atrophy. The weight of the graft, receiving the superior mesenteric derived venous blood, was not different from that of the grafts in the ligation group, which received all portal blood. It therefore seems that the presence of portal flow, rather than its origin is essential for graft survival.

Also essential for success in auxiliary liver grafting is to determine the liver mass that has to be transplanted for correction of the inborn error. In some metabolic diseases probably not more than 1-2% of the total liver volume is required (19). In this study there was no absolute correlation (r=0.74) between HIDA-uptake and allantoin synthesis. Grafts that contributed more than 15% to the total uptake of HIDA had good metabolic function, probably indicating a critical liver mass of 15-20% of the functional liver weight for this specific inborn error. In conclusion, auxiliary liver transplantation remains of interest (20-23) and auxiliary partial heterotopic liver transplantation may be a valuable alternative for orthotopic liver transplantation for the correction of inborn errors of metabolism. Ligation of the host portal vein assures good graft function, but may interfere with the life supporting function of the host liver in a primary graft non-function. At this time we have no technical solution to find the delicate balance between portal inflow in the host liver and graft as is proven by the dysfunction in the free-flow group and the banding group.

This study confirms the good results (16,24) with separate portal blood flow for host liver and graft and therefore we advocate for the split-flow technique.

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Importance of portal flow diversion in experimental auxiliary partial orthotopic liver transplantation

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Chapter 5

Abstract

Background: Auxiliary partial orthotopic liver transplantation (APOLT) has successfully been performed in patients with non-cirrhotic metabolic diseases. It remains however unclear if intervention in the portal venous inflow is necessary to ensure adequate portal blood flow to graft and host liver. In this experimental study we evaluate the hepatic flow during APOLT.

Methods: Left lateral/medial segmental grafts were transplanted from Beagle to Dalmatian dogs. Grafts were preserved in UW solution (Viaspan, DuPont) until further use. Resection surfaces were sealed with Tissu-coll (Baxter). Vascular structures were anastomosed end-to-end. The effect of diversion of the portal flow was studied in three groups: in the ligation group (n=3) the host portal vein was tied off, the free flow group (n=6) had random flow to both livers. In the banding group (n=11) the host portal vein was banded with an adjustable strapband to restore the pre-transplantation flow distribution. Before resection of the left segments in the donor, after reperfusion of the graft and after intervention, the portal and arterial blood flow was measured with ultrasonography (T206, Transonic systems, USA).

Results: After reperfusion the blood flow through the common portal vein (CPV) decreased from 49 to 36 ml/kg/min ($p< 0.03$) in all animals. Flow through the left portal vein (LPV) decreased from 26 to 5 ml/kg/min ($p<0.0001$). Banding restored the flow in the LPV to 12 ml/kg/min, while the flow in the free-flow group remained 4 ml/kg/min. In the ligation group the total portal flow was forced towards the graft leading to the highest perfusion: 24 ml/kg/min ($p<0.005$). Adverse effect of this ligation was the development of portal hypertension.

Conclusions: This experimental study confirms that diversion of the portal flow is necessary for adequate graft perfusion in APOLT. Banding can restore the pre-transplantation flow distribution, without compromising the flow in the common portal vein.

Abbreviations

ALT	auxiliary liver transplantation		
APOLT	auxiliary partial orthotopic liver transplantation		
CHA	common hepatic artery	LPV	left hepatic vein
CPV	common portal vein	OLT	orthotopic liver transplantation
HBFI	hepatic blood flow index	PI	pulsatility index
LHA	left hepatic artery	RI	resistance index

Introduction

Auxiliary liver transplantation (ALT) is an attractive alternative for standard orthotopic liver transplantation (OLT) in selected patients with diseases that result from an inborn error in metabolic or synthetic processes mainly involving the liver and in patients with acute liver failure. In the latter group, ALT aims at recovery of the native liver and withdrawal of the immunosuppressive therapy. In some inborn errors however there is no structural liver damage and complete resection of the liver and whole liver replacement is unnecessary or even contraindicated. In this situation ALT has potential advantages over OLT. Should primary nonfunction occur, this does not necessarily lead to an emergency re-transplantation. Furthermore, with partial liver transplantation, living-related procedures are in focus (*1-3*). Also in the preserved native liver future gene therapy is still possible (*4*).

Although auxiliary partial heterotopic liver transplantation (APHLT) is a less demanding procedure than OLT, it has never gained wide acceptance because of initial poor results (*5*) and the success of OLT. Recently auxiliary partial orthotopic liver transplantation (APOLT) has successfully been performed in patients with non-cirrhotic metabolic diseases (*2, 3, 6-11*).

A technical problem rising in this situation is the complicated hemodynamical status of the liver graft side by side with the non-cirrhotic native liver, without the increased portal pressure due to increased vascular resistance (oedema), as in acute liver failure. The hemodynamics of liver transplantation have been studied extensively (*12-14*), but only few studies on the effect of APOLT and hepatic flow patterns have been published (*15-17*). It therefore still remains unclear if intervention in the portal venous inflow of the native liver is necessary to ensure adequate portal blood flow to both graft and host liver. In this experimental study we evaluate the intraoperative systemic hemodynamics and hepatic blood flow during APOLT in three groups of portal intervention.

Materials and Methods

Auxiliary partial orthotopic transplantations were performed from Beagles to Dalmatian dogs (n=20). The Dalmatian dog lacks a normal transport system for uric acid into the hepatocyte, impeding the conversion of uric acid into allantoin. This results in significantly depressed allantoin formation and excretion and has been used previously to evaluate the concept of

correcting an inborn error with heterotopic liver transplantation (*18*). For auxiliary segmental grafts, the left lateral and medial segments of the donor were split in situ to mimic a living related liver transplantation. The graft was isolated, immediately perfused with 1 litre cold University of Wisconsin solution (Viaspan, DuPont) and stored on melting ice. In the acceptor measurements of blood flow in the left and common portal vein and the left and common hepatic artery were recorded prior to resection of the left lateral and medial segments by perivascular transit-time ultrasonography (T206, Transonic Systems Inc, Ithaca NY, USA). After resection the segmental graft was placed in the orthotopic position. The donor left hepatic vein, left portal vein and left hepatic artery were anastomosed end to end to the preserved vascular pedicles of the acceptor with loupe magnification (figure 1).

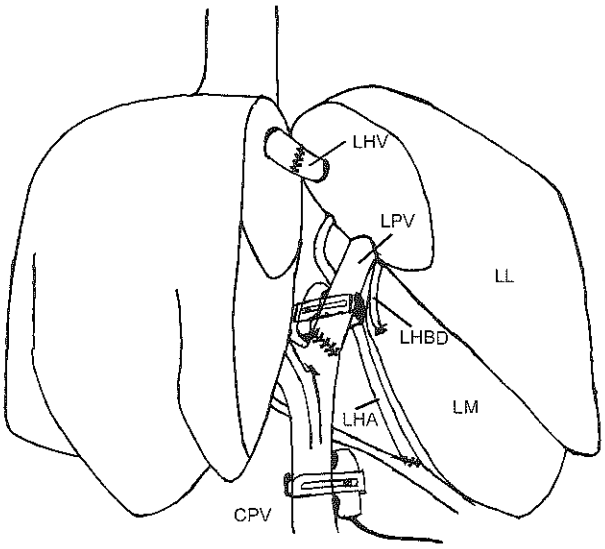

Figure 1. Adapted from D. Cherqui et al. HPB Surgery 1990; 2:193. Gordon & Breach Publishers, with permission.
Implantation of the left lateral (LL) and medial segments (LM) with end-to-end anastomosis of the left hepatic vein (LHV), left portal vein (LPV) and left hepatic artery (LHA). Flow probes in situ for measurement of the portal flow in the left and common portal vein (CPV). The left hepatic bile duct (LHBD) was anastomosed to a Roux-Y loop.

The left hepatic bile duct was anastomosed to a Roux-Y loop. After declamping and stabilisation of the circulation measurements of the hepatic blood flow were repeated. The effect of diversion of the portal flow on the hepatic hemodynamics was studied in three groups (figure 2): in the ligation group (n=3) the host portal vein was tied off, directing the total portal flow towards the graft. The free flow group (n=6) had random flow to both livers.

In the banding group (n=11), the host portal vein was banded with a adjustable strapband to restore the pre-transplantation flow distribution in terms of percentage between native liver and graft segments.

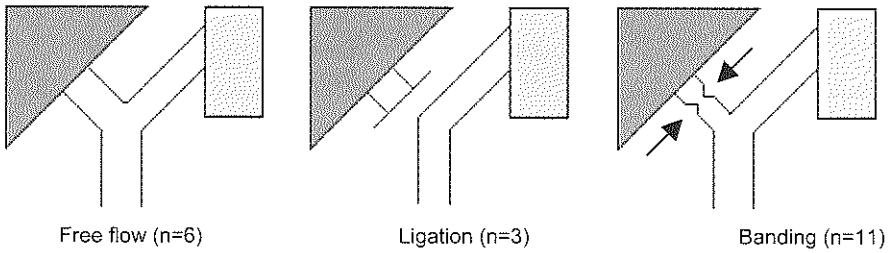

Figure 2. The three study groups.

A last series of measurements was performed 60 minutes later. Curve analysis was performed on the recorded signal with a personal computer (Burr-Brown AD-converter card) with multichannel registration software (MKR, AZR Dijkzigt, the Netherlands). Portal pressure was not routinely measured, as it was difficult to control the resulting bleeding.

Therefore, changes in the vascular resistance between the left lateral liver lobes before transplantation and the liver graft after reperfusion were investigated by calculating the resistance index (RI), pulsatility index (PI) and hepatic blood flow index (HBFI) (*19*).

RI = (peak systolic velocity - minimum diastolic velocity) / peak systolic velocity, PI = (peak systolic velocity - minimum diastolic velocity) / mean systolic velocity and HBFI = (portal vein blood flow - hepatic artery blood flow) / total hepatic blood flow. During transplantation, arterial blood pressure, central venous pressure and cardiac output were recorded at 30 minutes intervals. Liver enzymes in arterial blood samples were determined and compared to assess the impact of the different interventions in the portal flow on the liver tissue. Recipients received cyclosporin in an oral dosage of 10 mg/kg daily. Data are given as mean ± SEM. After testing normality with Shapiro-Wilk statistics, analysis of variance was performed. Otherwise non-parametrical tests were used. Repetitive measurements were analysed with the paired t-test or Kruskal-Wallis test. Differences were considered significant for $p< 0.05$.

Results

The basic characteristics of the different groups are given in table 1.

Table 1. Basic characteristics of the three intervention groups.

	Ligation (N=3)	Banding (N=11)	Free-flow (N=6)
Body weight (kg)	20.0 ± 1.5	20.2 ± 0.7	20.0 ± 0.4
Graft weight (g)	190 ± 16	217 ± 14	201 ± 12
Removed segments (g)	229 ± 11	227 ± 17	191 ± 13
Cold ischemia time (min.)	185 ± 24	209 ± 10	184 ± 13
Warm ischemia time (min.)	70 ± 6	67 ± 4	63 ± 3

One of the dogs died after surgery from incorrectable blood loss. During the procedure the mean arterial blood pressure was 94 ± 4 mm Hg at the onset of surgery, 85 ± 4 mm Hg after reperfusion and 80 ± 4 mm Hg one hour after portal flow intervention for all groups together; there were no significant differences between groups. Cardiac output measured at the same intervals was 3.7 ± 0.2, 3.3 ± 0.2 and 3.2 ± 0.2 l/min. The central venous pressure was 2.6 ± 0.8, 2.5 ± 0.8 and 1.8 ± 1.0 mm Hg respectively, without significant differences between groups. Before transplantation the blood flow through the common portal vein (CPV) was 49 ± 4 ml/kg/min (figure 3), of which 26 ± 3 ml/kg/min (i.e. 52%) went to the left portal vein (LPV). Directly after reperfusion, but before intervention, the total portal inflow decreased to 36 ± 3 ml/kg/min ($p< 0.03$). The flow to the LPV decreased dramatically to 5 ± 1 ml/kg/min: only 16% of the flow in the CPV ($p<0.0001$). The effect of artificial diversion of the portal flow at 1 hour after intervention is given in figure 4. In the banding group (n=11) the flow in the LPV in terms of percentage was restored to 43% (12 ml/kg/min) of the flow in the CPV, while the flow in the free-flow group (n=6) remained as low as 20% (4 ml/kg/min) of that in the CPV. In the ligation group (n=3) the total portal flow was forced towards the graft leading to the highest perfusion: 24 ml/kg/min; $p<0.005$. Adverse effect of the ligation was the development of portal hypertension in all three animals with congestion of the small bowel and spleen. Portal pressure measurements of 11-14 mm Hg (normal 6 mm Hg) (*18*) proved the clinical observation in these animals. In one case the ligature had to be removed not to endanger survival. Simultaneously recorded arterial flow patterns showed a flow of 12 ± 1 ml/kg/min in the common hepatic artery (CHA) before transplantation of which 5 ± 0.7 ml/kg/min went through the left hepatic artery (LHA). After reperfusion this decreased to 8 ± 1 ml/kg/min ($p< 0.01$) and 3 ± 0.5 ml/kg/min ($p<0.0001$) respectively (figure 5). No compensatory effect was observed in the arterial inflow after intervention in the portal

system (figure 6). The arterial flow towards the graft and native liver was 2 ± 0.5 and 4 ± 1 ml/kg/min in the free-flow group, 2 ± 0.8 and 6 ± 1 ml/kg/min in the banding group and 2 ± 0.6 and 5 ± 2 ml/kg/min in the ligation group (p=NS).

The values for the resistance index (RI), pulsatility index (PI) and hepatic blood flow index (HBFI) are given in table 2 for the left lateral and medial liver lobes (before transplantation) and the graft. After reperfusion decreased portal flow to the graft affects the HBFI ($p<0.02$), while the RI and PI, which are parameters of arterial resistance, are relatively unimpaired. Restoration of the portal blood flow normalised the HBFI in the banding and ligation group.

Table 2. RI, PI and HBFI of the native liver lobes before resection and of the graft after transplantation.

	Before Resection	After reperfusion	1 hour after intervention		
			Free-flow (N=6)	Banding (N=11)	Ligation (N=3)
Resistance Index	0.61 ± 0.04	0.65 ± 0.06	0.78 ± 0.08	0.81 ± 0.07	0.63 ± 0.11
Pulsatility Index	1.10 ± 0.15	1.60 ± 0.40	1.56 ± 0.30	2.44 ± 0.87	1.06 ± 0.32
Hepatic blood flow Index	0.66 ± 0.04	0.22 ± 0.15 ($p<0.04$)	0.32 ± 0.29	0.66 ± 0.10	0.82 ± 0.04

Histopathological examination of biopsies taken at the end of the procedure revealed severe damage with swelling, steatosis and moderate to severe necrosis in grafts in the free-flow group, consistent with portal hypoperfusion. Grafts in the banding group only showed evidence of handling damage with mild steatosis and sporadic necrotic hepatocytes. Grafts in the ligation group showed widened hepatic sinusoids and moderate necrosis as signs of congestion. In two dogs (1 banding, 1 free-flow) dilation of the sinusoids was seen, possibly indicating hepatic outflow problems, but clinical congestion was not seen in these dogs. In the dog in the free-flow group metabolic correction of the inborn error decreased 4 days after transplantation, but a biopsy taken on day 7 after transplantation showed normal hepatic architecture.

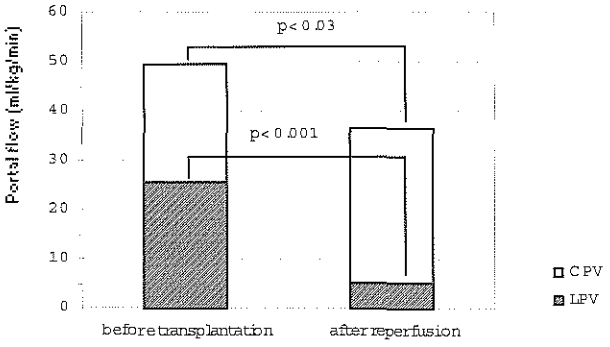

Figure 3. Portal blood flow of the common portal vein (CPV) and left portal vein (LPV) before transplantation and just after reperfusion of the graft. Left portal vein inflow decreased from 52% to 16% of the total portal flow after reperfusion, but before intervention.

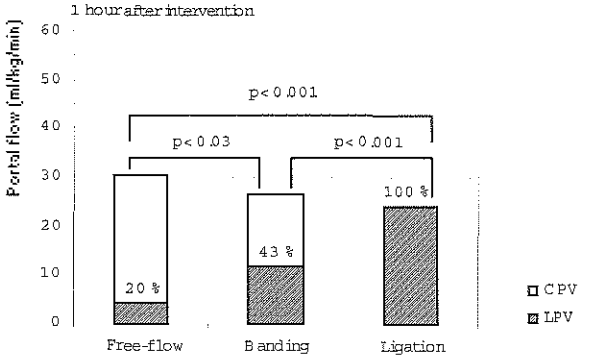

Figure 4. Result of intervention on portal blood flow of the common (CPV) and left portal vein (LPV). 1 Hour after intervention, grafts in the free-flow group received only 20% of the total portal flow.

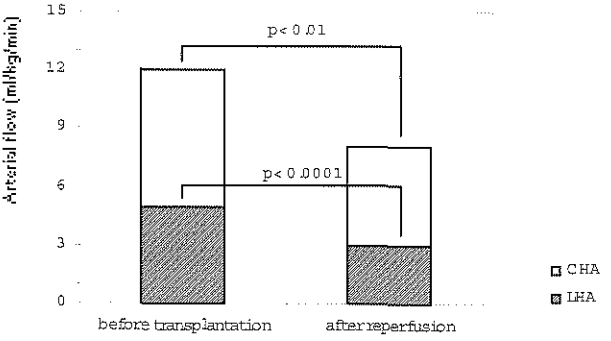

Figure 5. Arterial flow patterns in the common hepatic artery (CHA) and left hepatic artery (LHA). Before transplantation, arterial flow to the left segment was 42 % of the total arterial flow. After reperfusion total arterial flow and left arterial flow decreased significantly, but left arterial portion remained 38% of the total arterial flow

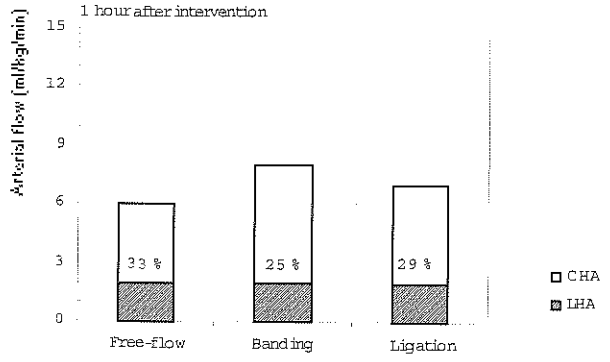

Figure 6. Arterial blood flow in the common hepatic artery (CHA) and the left hepatic artery (LHA), one hour after intervention in the portal blood flow. No compensatory increase in arterial blood flow was observed for loss of portal blood flow in the ligation group or banding group.

Levels of ASAT, ALAT, alkaline phosphatase and γ-GT measured after reperfusion and at the end of the procedure showed no differences between the study groups. Transaminases increased between reperfusion and the end of surgery from 433 ± 73 to 586 ± 79 U/l (ASAT) and 585 ± 110 to 857 ± 123 U/l (ALAT) with similar increment in all three groups.

Discussion

Auxiliary partial orthotopic liver transplantation is an attractive alternative for orthothopic liver transplantation in patients with some metabolic disorders and in patients with acute liver failure. In the latter group regeneration of the native liver is to be expected and auxiliary liver transplantation can prevent life-long immunosuppressive therapy (*20*). The theoretically attractive concept of heterotopic liver transplantation in this situation never gained wide acceptance because it was initially hampered by technical problems and later overcome by the success of the OLT.

In 1990 the first successful clinical APOLT was performed for acute liver failure, in which a segment II / III graft was placed in the orthotopic position after creating space by resection of the corresponding segments in the acceptor (*21*). In this situation no additional precautions are necessary to ensure portal inflow in the graft as the vascular resistance in the swollen native liver is expected to be much higher and gradual atrophy of the graft after native liver regeneration is aimed for. In 1993 APOLT proved to provide total metabolic correction in a patient with Crigler-Najjar syndrome (*6*) and since then APOLT procedures for other inborn errors of hepatic metabolism have been described in 14 patients (*2, 3, 7-11*). It became evident that the complicated hemodynamic situation of the liver graft side by side with the non-cirrhotic native liver led in some of these patients to competition for the portal inflow between the native liver and graft (*3, 6, 9, 11*), requiring banding or ligation of the native portal vein.

In this experimental study we investigated the acute portal redistribution in the situation of APOLT for metabolic disease, in which portal hypertension is absent. The flow distribution in this experiment was studied by direct transit-time ultrasound measurements with perivascular adjustment of the probes, assuring accurate measurements (*22*) and preventing technical problems with the angle of insonation (*19*). In our study the decrease in portal flow to the graft after reperfusion was more marked than in the experimental studies of Then et al. (*17*) and Yabe et al. (*15*), but the values of portal flow towards the graft are comparable to the results found by Kaibori et al. (*11*) in clinical paediatric APOLT. The

decrease was found in all animals without signs of acute rejection on histology. Therefore this was probably caused by the parenchymal swelling in the portal fields due to preservation and handling damage, despite careful dissection technique and standard harvesting procedure with short preservation times. The resistance index - a marker for arterial obstruction and early reperfusion reaction (*23, 24*) – did not increase significantly and the arterial pulsations did not show a dampened waveform in the pulsatility index, indicating good arterial patency (*25*). Without intervention, portal inflow towards the graft remained marginal, whereas ultrasound-guided intervention with an adjustable strapband could restore the pre-transplantation flow distribution in terms of percentage, without compromising the flow in the common portal vein. Ligation of the portal vein of the native liver led to portal hypertension as the total portal flow was forced into a liver graft comprising about 40% of the total liver weight. Remarkable was the absence of a reactive increase in arterial inflow towards the native liver after cessation of the portal inflow in the ligation group, which was described in orthotopic liver transplantation and abdominal surgery (*13, 26, 27*).

In conclusion, this experimental study confirms that in the complicated situation of an auxiliary partial orthotopic liver graft co-existing with a partial non cirrhotic native liver, occlusion of the portal flow towards the native liver is essential for adequate graft perfusion. Acute total ligation of the host portal vein may interfere with the life supporting function of the native liver in case of a primary graft dysfunction and lead to unacceptable portal hypertension in this model. Without intervention the portal flow is deviated from the graft, leading to poor perfusion and severe damage on histology. Partial banding under intraoperative transit-time ultrasonography, aimed at restoration of the pre-transplantation flow distribution, leads to adequate graft perfusion, without compromising the total portal blood flow.

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Portal flow diversion is essential for graft survival in experimental auxiliary partial orthotopic liver transplantation

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Submitted

Chapter 6

Abstract

After auxiliary partial orthotopic liver transplantation for inborn errors of metabolism, finding a balance in portal blood flow distribution between native liver and graft is complicated. We investigated the correction of hypoallantoinuria in the Dalmatian dog with a reduced-size Beagle orthotopic auxiliary liver graft, depending on intra-operative intervention in the portal flow.

There were three groups: a ligation group, where the host portal vein was tied off, a free-flow group with random flow to both livers and a banding group, were the host portal vein was banded with an adjustable strapband. Metabolic correction was initially seen in all groups, but ligation led to portal hypertension and early mortality. In the free-flow group, correction was lost after 7 days, while banding preserved correction until 6 weeks. We conclude that acute ligation can lead to portal hypertension and free-flow leads to hypoperfusion and early loss of metabolic correction. Banding divided the portal blood flow between host liver and graft and prolonged metabolic correction.

Abbreviations

ALT	auxiliary liver transplantation
APOLT	auxiliary partial orthotopic liver transplantation
CI	confidence interval
NS	not significant
OLT	orthotopic liver transplantation
POD	postoperative day
ROI	region of interest

Introduction

Auxiliary liver transplantation, in which a (reduced-size) graft is placed in the recipient and the diseased liver is left in place, has potential advantages over standard whole liver replacement by orthotopic liver transplantation. In children with some life threatening inborn errors of hepatic metabolism, only one enzyme or cell membrane receptor is absent and they have an otherwise normal function of the liver. In this case auxiliary liver transplantation provides the missing metabolic function, while the native liver supports the total liver function.

Should primary graft non-function or severe rejection occur, this does not necessarily lead to an emergency re-transplantation. (1, 2) Furthermore, with partial liver transplantation, future gene therapy is still possible in the preserved native liver (3, 4). The first auxiliary transplantation was performed as an auxiliary partial heterotopic liver graft (APHLT) in 1964 (5), but never gained wide acceptance because of disappointing results. Potential problems of APHLT were the lack of space in the abdomen, causing compression on large vessels and the disturbance of portal inflow and venous drainage (6). Recently, auxiliary partial orthotopic liver transplantation (APOLT), in which a reduced-size graft orthotopically replaces the resected liver lobe in the recipient, has successfully been performed in patients with non-cirrhotic metabolic diseases (2, 7-12).

The complicated division of portal blood between the liver graft side by side with the non-cirrhotic native liver is the critical issue at the time of surgery and of major importance to intermediate and long-term graft survival is. Without an increased vascular resistance in the native liver due to cirrhosis or collapse, the portal flow is preferentially towards the native liver (13). To investigate the requirements for intermediate-term metabolic correction of an inborn error of metabolism, a 60% Beagle liver graft was transplanted into the Dalmatian dog. Graft function was evaluated by correction of the metabolic defect in three groups with different distribution of the portal flow at the time of surgery.

Materials and Methods

Auxiliary partial orthotopic liver transplantation was performed from 20 Beagles to 20 Dalmatian dogs according to the guidelines for animal experiments of the Erasmus University. The Dalmatian dog lacks a normal transport system for uric acid into the hepatocyte, which impedes the conversion of uric acid into allantoin and results in significantly depressed allantoin formation (14). Of this formed allantoin, 94% is excreted in urine by renal clearance (15) and the ratio of allantoin / creatinin in urine proved to be nearly constant, allowing correction for daily variations in renal function. For auxiliary partial grafts, the left lateral and medial segments of the donor were split in situ to mimic a living donor liver transplantation. The graft was isolated, immediately perfused with 1 litre of cold University of Wisconsin solution (Viaspan, DuPont) and stored on melting ice.

In the recipient the left lateral and medial segments of the liver were resected and the partial graft was placed in the orthotopic position. The donor left hepatic vein, left portal vein and left hepatic artery were anastomosed end to end to the preserved vascular pedicles of

the recipient, using 5x loupe magnification. The hepatic bile duct of the graft was anastomosed to a Roux-Y loop. Recipients received a blood transfusion from the liver donor. The effect of diversion of the portal flow on post-operative graft function was studied in three non-randomised groups. In the ligation group (n=3) the host portal vein was tied off, directing the total portal flow towards the graft. The free flow group (n=6) had random flow to both livers. In the banding group (n=11), the host portal vein was banded with an adjustable strap band to restore the pre-transplantation flow pattern in terms of percentage of the total portal flow between native liver and graft segments. The distribution of portal flow was monitored with simultaneous perivascular transit-time ultrasonography (T206, Transonic Systems Inc, Ithaca NY, USA) of the common and left portal vein (13). Recipients received Cyclosporin A (Novartis, Arnhem, The Netherlands) in an oral dosage of 15 mg/kg/day, but rejection was not monitored. Changes in allantoin levels were measured weekly in urine samples, collected over 24 hours. Graft function was determined by urinary excretion of allantoin (16). Liver function of both graft and host liver was further quantitated at 4 weeks after transplantation by the uptake of ^{99M}Tc labelled dimethyl-iminodiacetic acid (^{99M}Tc-HIDA, 74 MBq). Dynamic acquisition was performed with a Pho gamma V camera (Siemens, Gammasonics, the Netherlands) equipped with a low energy all-purpose collimator. Regions of interest were drawn around the native liver and the graft and the uptake of HIDA between 4 and 16 minutes after injection, corrected for background activity, was used to calculate the relative uptake of the graft. Autopsy and registration of the wet liver weight was performed in all animals. Surviving animals were sacrificed 50 days after transplantation. All data are presented as mean ± standard error. Differences between pre- and post transplantation excretion ratio's were detected by paired t-test analysis. Linear regression was calculated with analysis of variance (ANOVA). Graft survival was analysed by the method of Kaplan-Meier with log rank statistics. Loss of graft function was scored when the excretion ratio of allantoin in urine after transplantation reached the 95% confidence interval of the pre-transplantation level (mean ± 2 standard deviations, i.e. 0.15 - 0.58). Differences were considered significant when P was less than 0.05.

Results

Pre-transplantation excretion ratio of allantoin / creatinin was 1.75 ± 0.22 in Beagles and 0.38 ± 0.03 in Dalmatian dogs (95% confidence interval: 0.15-0.58). Operation time was 348 ± 10 minutes, with a mean loss of blood of 1221 ± 192 ml. Cold and warm ischaemia times were 198 ± 9 and 67 ± 3 minutes respectively, with no differences between groups.

Ligation group:

Acute ligation of the host portal vein led in our model to unacceptable portal hypertension with rupture of the capsule of the graft, congestion of the small bowel and diffuse oozing from the splenic area in all dogs in this group. Histology sections taken 5 minutes after reperfusion of the graft showed oedema and necrosis in the portal area (figure 1). Two dogs died from intra-abdominal bleeding at post-operative day 3 and 4 with poor graft function. Excretion ratio of allantoin / creatinin at the first post-operative day was 0.88 ± 0.20, not significantly different from pre-transplantation. In the third experiment again portal hypertension occurred during the procedure after total ligation. It was decided to convert into banding to release portal hypertension and not endanger survival. Hereafter ligation of the host portal vein was no longer performed.

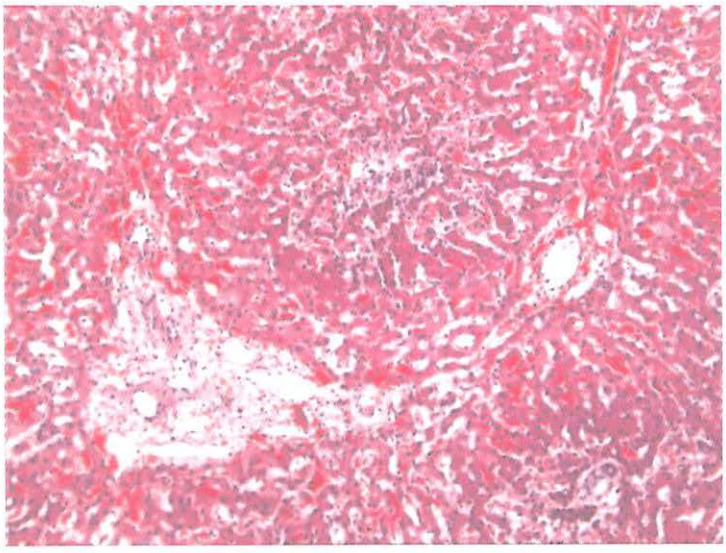

Figure 1. Histology section taken 5 minutes after reperfusion in the ligation group, showing portal oedema and necrosis.

Free-flow group:

In 7 dogs the portal flow distribution between graft and host liver was not altered during the operation. Mortality in this group was high with 5/7 dogs (71%) dying before the end of the experiment. Mean survival of the dogs was 17 ± 8 days, median 7 days. The allantoin / creatinin ratio increased to 1.11 ± 0.14 after transplantation ($P<0.001$ compared to pre-transplantation level), but metabolic correction was not longer significantly different from pre-transplantation levels at day 12 after transplantation (figure 2). Mean graft function lasted 10 ± 4 days with a median of 6 days (figure 3). At autopsy thrombosis of the graft portal vein was seen in 3 of the 7 dogs in the free-flow group.

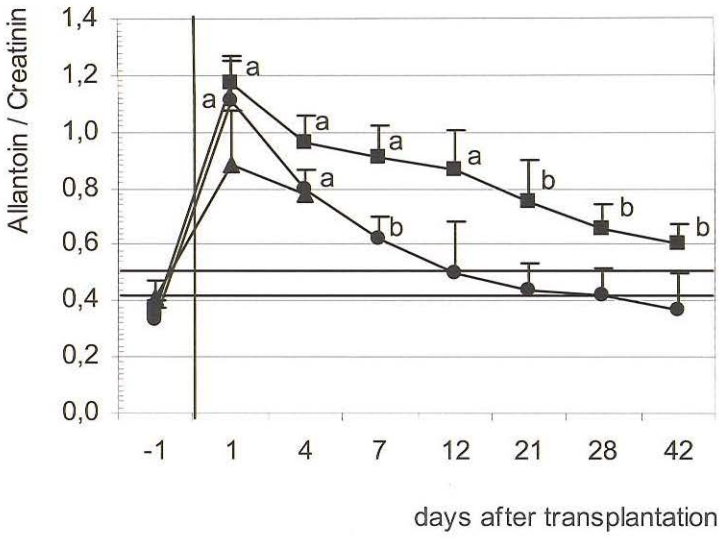

Figure 2. Excretion ratio of allantoin / creatinin in urine.
Metabolic correction lasted until sacrifice in the banding group (■). In the free-flow group (●) a loss of graft function was seen by day 12, where in the ligation group (▲) no significant correction was observed and dogs died of portal hypertension on postoperative day 3 and 4. a: $p<0.01$ versus pre-transplantation ratio, b: $p<0.05$ versus pre-transplantation ratio. ---------: 95% Confidence interval of the pre-transplantation allantoin excretion ratio.

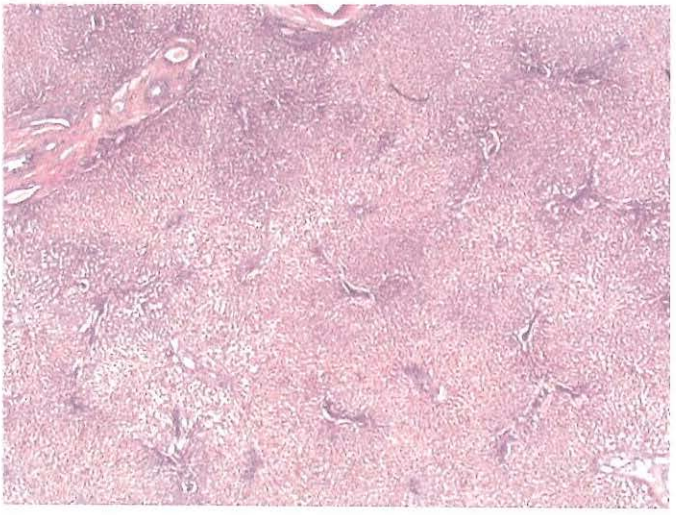

Figure 4. In the grafts lasting until sacrifice, a moderate to severe fibrosis and mainly lymphocellular infiltrate in the portal area was observed

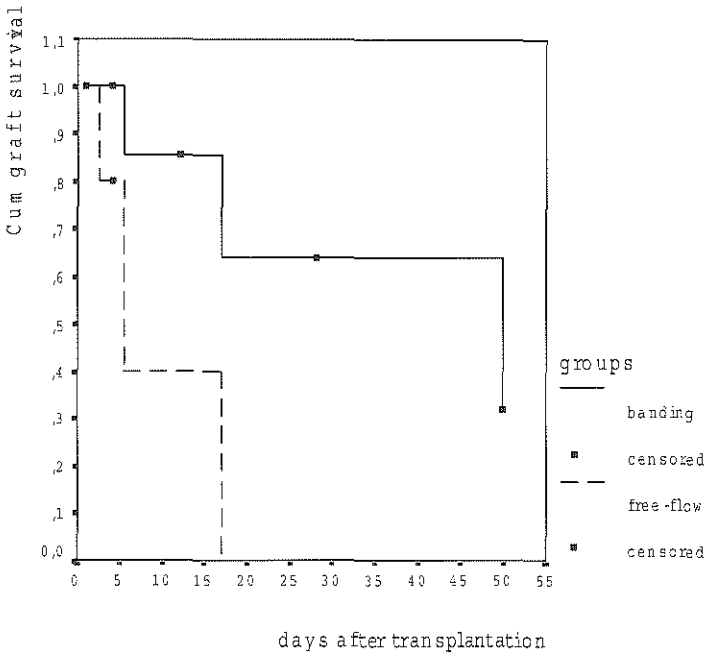

Figure 3. Kaplan-Meier curve of graft survival. Mean graft function lasted 10 ± 4 days in the free-flow group (-----, censoring animals ■) and 37 ± 10 days in the banding group (———, censoring animals ■ : $p<0.05$).

Banding group:

Intra-operative banding of the host portal vein was performed in 11 dogs. In this group, 8 of the 11 dogs died before the end of the experiment (72%). Mean survival was 21 ± 6 days with a median of 14 days, not significantly different from the free-flow group. Metabolic correction increased to 1.17 ± 0.10 ($p<0.001$ compared to pre-transplantation levels) and sustained until sacrifice (figure 2). Mean graft function lasted 37 ± 10 days (median 17, $P< 0.05$ vs. free-flow). At autopsy, portal thrombosis was seen in 4/11 dogs.

In table 1 the wet liver weight at autopsy is shown. There were no significant differences in graft weight between groups. Histopathological examination showed moderate to severe hepatocellular necrosis, mostly with signs of sepsis or congestion of the central vein, in the grafts of dogs that died within a week after transplantation (n=9). In the grafts lasting until sacrifice, a moderate to severe fibrosis and mainly lymphocellular infiltrate in the portal area was observed (figure 4).

Table 1. Wet liver weights at autopsy. All grafts had increased in weight compared to their pre-transplantation weight. Grafts in the free-flow group showed more oedema, but differences were not significant between groups.

	Host liver (g)	Graft (g)	Total (g)
Free-flow	554 ± 90	402 ± 73 (188%)[#]	955 ± 149
Banding	566 ± 52	340 ± 64 (148%)[#]	906 ± 97
Ligation	515 ± 139	333 ± 108 (180%)[#]	815 ± 62

[#] Increment in graft weight compared to the weight at the time of transplantation.

In figure 5 the metabolic correction at the time of 99MTc-HIDA scanning is plotted against the relative graft contribution to the total HIDA uptake. 11 Scintigraphies could be performed in 9 dogs (11 dogs died before the first scan was made). There was a statistically significant correlation between proportional correction of the inborn error and the graft contribution to the total HIDA uptake ($r=0.67$; $p<0.05$).

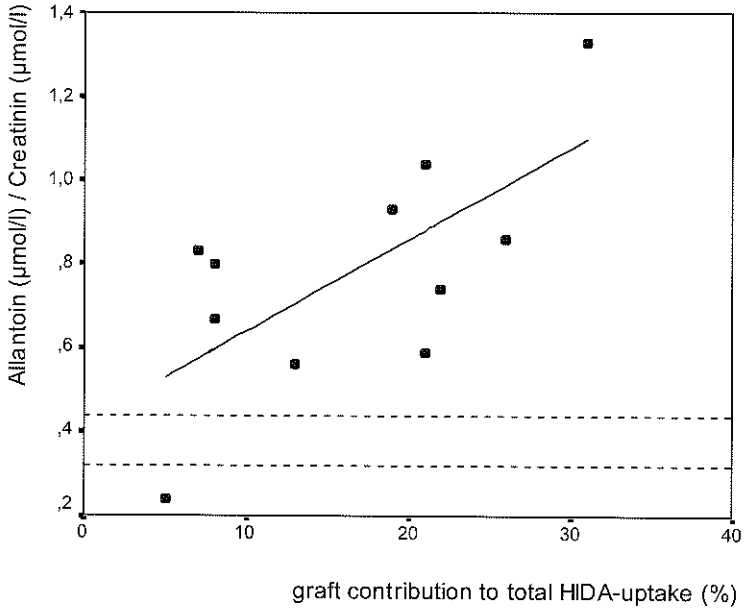

Figure 5. Correlation of HIDA-uptake and allantoin excretion in urine.
11 Scintigraphies were performed in 9 dogs. Graft contribution to the total HIDA-uptake during scintigraphy correlated significantly with the urinary allantoin level; $r=0.67$; $p<0.05$.
---------: 95% pre-transplantation confidence interval of the allantoin excretion ratio.

Discussion

Auxiliary partial orthotopic liver transplantation is an attractive alternative for standard orthothopic liver transplantation in patients with specific disorders of hepatic metabolism and in patients with acute liver failure. In the latter group the graft can provide temporary life support, while regeneration of the native liver is to be expected and thus auxiliary liver transplantation can prevent life-long immunosuppressive therapy (17). In 1990 the first successful clinical APOLT was performed for acute liver failure, in which a segment II / III graft was placed in the orthotopic position (18). In this situation no additional precautions were necessary to ensure portal inflow in the graft as the vascular resistance in the native liver is expected to be much higher and gradual atrophy of the graft after native liver regeneration is aimed for.

In 1993 APOLT proved to provide total metabolic correction in a patient with Crigler-Najjar syndrome (2) and since then APOLT procedures for other inborn errors of hepatic metabolism have been described in 14 patients (7-12, 19). In some of these patients the host portal vein was banded during transplantation (2, 10), in other cases the host portal vein had to be ligated after initial successful transplantation without intervention to guarantee adequate perfusion of the graft (8, 19). The complicated hemodynamic situation of the liver graft - side by side with the non-cirrhotic native liver - leading to competition for the portal inflow between the native liver and graft is still a critical issue at the time of surgery and of major importance to intermediate and long-term graft survival.

In a previous study we assessed the results of intervention in the portal inflow in a model of auxiliary *heterotopic* liver transplantation (20). In this situation a 60% Beagle graft was placed under the native liver and metabolic correction was studied for 180 days. Acute ligation of the portal vein led in this model to moderate portal hypertension, but this had no clinical consequences and metabolic correction was good. Without intervention in the portal flow, metabolic correction was lost after 12 days and mortality was 66% due to sepsis from necrotic grafts. Banding of the portal vein to the native liver led to initial good metabolic correction, which decreased after 84 days due to late portal thrombosis of the graft. Mortality in this group was 25% and chronic rejection (which was not monitored) was found at autopsy.

In the present experimental study the effect of intraoperative intervention in the portal flow on graft perfusion was recorded and the effect on postoperative metabolic correction is for the first time assessed for APOLT. In the free-flow group, allowing free distribution of portal inflow between graft and native liver resulted in early loss of metabolic correction.

Hypo-perfusion of the graft led to portal thrombosis and mortality was high due to sepsis from necrotic grafts, in accordance with the results of our heterotopic experiment.

Acute ligation of the host portal vein led in our model to portal hypertension. Two dogs died of bleeding from the splenic area and showed congestion of the portal vein in histology sections. At autopsy no evidence could be found for stenosis in the hepatic vein anastomosis. At the time of surgery, portal inflow in these grafts was forced from 5 ml/kg/min without intervention to 24 ml/kg/min after ligation. We assume that a hyperperfusion syndrome occurred in which portal inflow exceeded hepatic outflow and this resulted in a relative venous outflow obstruction.

Primary ligation of the native portal vein was clinically performed by Kaibori et al. (19) and further assessed in an experimental study by Yabe et al. without problems of portal hypertension (21). In the study of Yabe, 2/7 dogs died before sacrifice at day 5 from unknown causes but histologic findings were not given. An explanation for the difference with our own results in the heterotopic experiment may be that in the present study the total portal flow was forced into a liver graft comprising about 40% of the total liver weight. According to the results of Yabe et al. (8, 21) we found no evidence that ligation of the native portal vein had detrimental effects on the native liver supplied by arterial flow only.

Banding of the host portal vein provided metabolic correction until the end of the experiment, although the correction decreased within time. Loss of metabolic function during follow-up was accompanied by portal thrombosis 4 of the 11 dogs and can be explained by increased vascular resistance in the graft secondary to infection and rejection. Regarding the problem of late portal thrombosis, a gradual occlusion of the host portal vein as performed by Hirayama et al. (22) may be an attractive solution. This can assure optimal graft function without the risk of portal hypertension. In the banding group we found no evidence for promoted regeneration of the graft liver and atrophy of the native liver. Although all grafts had increased in weight compared to their pre-transplantation weight, there were no differences between groups. The interval after transplantation was probably too short for hypertrophic changes. Grafts in the free-flow group showed more oedema, which might be caused by the shorter interval between transplantation and autopsy in most animals in this group.

Also essential for success in auxiliary liver grafting is the determination of the liver mass that has to be transplanted for correction of the inborn error. In some metabolic diseases probably not more than 1-2% of the total liver volume is required (23). In our study there was no absolute correlation (r=0.67) between HIDA-uptake and allantoin synthesis.

Adequate metabolic correction was seen in a graft that contributed 8% to the total uptake of HIDA, indicating a critical liver mass of about 10% of the functional liver weight for this specific inborn error.

In conclusion, this experimental study shows that an auxiliary partial orthotopic liver graft can correct the inborn error of metabolism, but that an adequate portal flow to the graft is required for sustained graft function. Acute total ligation of the host portal vein leads to portal hypertension with congestion of the parenchyma and early death in our model. Without intervention, the portal flow is preferentially to the native liver, leading to poor perfusion and early loss of metabolic function of the graft. Partial banding, aimed at restoration of the pre-transplantation flow distribution, gives the best metabolic correction.

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Part III

Optimisation of clinical orthotopic liver transplantation: surgical and metabolic aspects

Nitrotyrosine does not impair adenine nucleotide metabolism in primary graft dysfunction after liver transplantation

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Submitted

Chapter 7

Abstract

Background. Primary graft dysfunction (PGDF) occurs in 5-15% of the patients after liver transplantation. PGDF is attributed to cold storage, warm ischemia and reperfusion, which cause damage to the vascular endothelium. Poor tissue perfusion ultimately results in mitochondrial dysfunction with loss of ATP. Recently, direct inhibition of mitochondrial respiration by reaction products of nitric oxide (NO) was described. In this study the expression of the inducible NO synthase (iNOS), the formation of nitrotyrosine and the relation with adenine nucleotide levels was assessed in patients with and without PGDF.

Methods. INOS and nitrotyrosine were determined immunohistochemically, adenine nucleotide levels were measured and transition electron microscopy was performed in liver biopsies of 8 patients with and 16 patients without PGDF.

Results. In patients with PGDF, ATP decreased from 6.1 µmol/g at the end of cold ischemia to 3.5 after reperfusion. In control patients, ATP increased from 3.5 at the end of cold ischemia to 6.8 µmol/g after reperfusion ($P<0.05$). The energy charge was significantly higher in the control patients after reperfusion (0.57 versus 0.47, $P<0.05$). Electron microscopy confirmed poor mitochondrial function in patients with PGDF as soon as 1 hour after reperfusion. iNOS and nitrotyrosine showed abundant staining, mainly in the hepatocellular cytoplasm, without differences between PGDF and control patients .

Conclusions. Mitochondrial dysfunction in patients with PGDF is present after reperfusion, leading to the inhibition of ATP synthesis. Although nitration of cell structures was present during preservation and reperfusion, this does not cause mitochondrial dysfunction, as NO formation and nitrotyrosine residues also were present in patients with excellent postoperative liver function.

Abbreviations

AST	Aspartate amino transferase
iNOS	Inducible nitric oxide synthase
HE	Hematoxylin/eosin
HPLC	High performance liquid chromatography
NO	Nitric oxide
OLT	Orthotopic liver transplantation
$ONOO^-$	Peroxynitrite
PGDF	Primary graft dysfunction
TAN	Total adenine nucleotides
TEM	Transition electron microscopy
UW	University of Wisconsin

Introduction

Primary graft dysfunction (PGDF) occurs after liver transplantation in 5-15% of the patients (1-3), despite the introduction of special preservation solutions like the University of Wisconsin (UW) or histidine-tryptophane-ketoglutarate (HTK) solution (4-6). PGDF is defined as a dysfunction of the donor liver in the first week after transplantation with an AST level > 2500 U/L and a prothrombine time > 16.5 seconds, in absence of vascular complications or acute rejection (7). The pathogenesis of PGDF is attributed to cold storage of the liver, which activates sinusoidal macrophages (Kupffer cells) and induces preservation injury to endothelial cells with progressive loss of viability (8-13). Subsequent reperfusion causes a plethora of reactions involving nitric oxide (NO), reactive oxygen species, cytokines and eicosanoids (14-17). Leukocyte margination and platelet adherence in the damaged sinusoids may lead to poor tissue perfusion and to poor oxygen delivery to the hepatocytes (18-21). Ultimate result of this ischemia / reperfusion damage is mitochondrial derangement with a loss of ATP synthase activity and reduction of ATP levels (22-30).

However, recently an alternative hypothesis for the cause of mitochondrial dysfunction was proposed. In several experimental studies, a direct inhibition of mitochondrial respiration of cytochrome oxidase and complexes I and IV was found by reaction products of NO and the superxide radical $O_2^{\cdot}$ (31-38). The main product of this reaction is peroxynitrite ($ONOO^{-}$), a potent nitrating and oxidizing agent with a high affinity for thiol (R-SH) structures. Peroxynitrite is held responsible for damage to numerous biologic targets by oxidizing thiols and removing iron from the iron-sulfur centres (39). Additionally, peroxynitrite can react with tyrosine residues to produce nitrotyrosine (40-42), which was shown to increase 900% after preservation and reperfusion in an experimental model (43). Ohsima et al. first proposed the stable end product nitrotyrosine as an in vivo indicator of nitration (44) as it is almost exclusively produced by peroxynitrite (45).

In this study we investigated the hypothesis that mitochondrial dysfunction in clinical PGDF is caused by peroxynitrate. The expression of the inducible NO synthase (iNOS) and formation of nitrotyrosine was assessed and related to the adenine nucleotide levels and electron microscopic findings in liver biopsies during cold storage and reperfusion in 24 patients with and without PGDF.

Materials and Methods

From October 1998 to June 2000, 68 orthotopic liver transplantations were performed at the Erasmus University Medical Center Rotterdam. Liver biopsies were taken routinely from the

donor livers in these patients. Biopsies were taken from the left liver lobe at 3 stages during the procedure. 1) At the end of the cold ischemia, when the donor liver was taken from the ice, 2) at the end of the warm ischemia, when all vascular anastomoses were completed and 3) one hour after reperfusion of the donor liver. The biopsies were divided into 3 pieces. One piece was snap-frozen in liquid nitrogen to perform analysis of the intracellular adenine nucleotide content (ATP, ADP and AMP). One piece was fixed in 2% glutaraldehyde to perform electron microscopy. A third piece was formaldehyde fixed and used for normal hematoxylin/eosin (HE) staining and immunohistochemical staining of inducible nitric oxide synthase (iNOS) and nitrotyrosine.

In 8 cases (12%) postoperative primary graft dysfunction (PGDF) was diagnosed by elevation of AST >2500 U/l and a prothrombin time > 16.5 seconds on the 2nd to 7th post-operative day. Vascular occlusion or acute rejection were ruled out in these patients. Biopsies of these 8 patients were compared with the biopsies of 16 control patients without PGDF, transplanted in the same period.

Analysis of adenine nucleotide content.

The nucleotide content was measured using the method of Sellevold et al. (46). Briefly, biopsies were homogenized by 2-3 minutes of shaking in a liquid nitrogen pre-cooled Teflon cup in a mechanical homogenizer (Micro-Dysmembrator, Braun, The Netherlands) with 500 µl of 4% $HClO_4$. The homogenate was neutralized with KOH (2 M) and K_2CO_3 (1 M) to pH 6-6.5. After precipitation, the extract was spun down in an Eppendorff centrifuge for 3 min. at 10x g. The supernatant was kept on ice until injection of 20 µl into the HPLC system. The pellet was used for determination of the protein content according to the method of Lowry. The HPLC system consisted of a Waters model 600E system equipped with a model 484 UV detector and Millenium integration software package (Waters, Milford, MA, USA). Adenosine tri-, di- and mono- phosphates were separated over a C-18 reversed-phase column (µBondapack, ID 3.0 mm, Waters, USA) with a guard column. The mobile phase contained 175 mM potassium dihydrogen phosphate, 2.3 mM tetrabutylammonium hydrogen sulfate and 2.5% acetonitril. After adjusting the pH to 6.25 with KOH, the solution was filtered through a 2µm cellulose filter (Millipore, Bedford, USA) and degassed before use. Eluting peaks were detected at 214 nm and quantified against an external standard. The total adenine nucleotide content (TAN= ATP + ADP + AMP) and the adenylate energy charge (EC = ATP + 1/2 ADP / [ATP + ADP + AMP]) were calculated. The EC denotes the

part of energy directly available for energy demanding cell processes without further use of metabolic pathways.

Transition electron microscopy (TEM)

TEM was performed on about 1 mm^3 of liver tissue after immersion fixation overnight in 2% glutaraldehyde / 0.1 M cacodylate buffer. After washing with the same buffer, the tissue was postfixed for 2 hours in 1% osmium tetroxide. The specimens were dehydrated through a graded series of ethanol and propylene oxide and embedded in Epon. Ultra-thin sections were stained with uranyl acetate followed by lead citrate. The sections were examined and photographed with a JEOL JEM-200CX electron microscope (Jeol Ltd, Japan).

Immunohistochemistry and histology.

The major portion of each biopsy was fixed in formalin, embedded in paraffin and part of these sections was routinely stained with HE. Specific criteria including cholestasis, severity of steatosis, cellular swelling and inflammation were blindly assessed by the pathologist. In corresponding sections immunohistochemistry was performed after deparaffinisation, dehydration, unmasking with saponin and inactivation of endogenous peroxidase activity with H_2O_2. Tissue sections were stained for inducible nitric oxide synthase (iNOS) and nitrotyrosine. Nitrotyrosine can be formed in the reaction $NO + O_2.^- \rightarrow$ peroxynitrite + tyrosine residue$\rightarrow$ nitrotyrosine. To detect protein tyrosine nitrosylation, a polyclonal rabbit anti-iNOS antibody (Santa Cruz biotechnology) and monoclonal mouse anti-nitrotyrosine antibody, kindly provided by prof. Dr. W.A. Buurman, University hospital Maastricht, the Netherlands (47) were used. Control samples were processed without the second antibody to check for aspecific staining. Specificity of nitration was tested by reducing nitrotyrosyl residues by anaerobic incubation with sodium di-thionite (48) before staining.

Statistical analysis.

Eight patients with PGDF were matched with 16 control patients transplanted in the same period, to correct for confounding factors in the transplantation procedure. A 2-to-1 match was performed to detect a clinical relevant difference of 25%, with a calculated SE of 15%, 90% power and a significance level of 2.5% on both sides following Altman's nomogram for sample size (49). Repeated measurement analysis showed within subject independence of ATP level from sample time. Statistical analysis was performed with Student's T-test and paired T-test after testing normality with the Shapiro-Wilk statistic. If the data were not

normally distributed, the Mann-Whitney U-test was used. For 2x2 table analysis Fisher's exact test was used. A p-value <0.05 was considered statistically significant.

Results

Clinical course

Eight patients with PGDF were compared to 16 control patients, transplanted in the same period as the patients in whom PGDF occurred. Characteristics of the transplantation procedure are given in table 1. There were no significant differences ibetween the two groups, except for the duration of the warm ischemic period, which was about 10 minutes longer in the control group, $p<0.05$. As expected by definition, AST and PT levels were much higher in the patients with PGDF (figure 1a and b). Repeated measure analysis showed significant within-subject dependence, but differences were statistically significant from control patients at day 5 (AST) and at day 4 (PT), $p<0.05$. Three of the 8 patients with PGDF (38%) were scheduled for re-transplantation. Eventually 2 patients received an emergency graft in time, the third patient died after 3 days from cerebral edema. Of the 2 re-transplanted patients, 1 died after 14 days of fungal sepsis and therefore 1-month mortality was 25% in the PGDF patients. Of the control patients, 1 patient died after 28 days from sepsis, another patient was successfully re-transplanted for hepatic artery thrombosis after 4 days. 1-Month mortality was 6%. Patients with PGDF had a 6-month graft and patient survival of both 63%. In control patients these results were better, but with 75% and 82% respectively the differences were not significant.

Table 1. Characteristics of the patients with PGDF and control patients.

	Control patients (N=16)	PGDF (N=8)	P-value
Male / Female	10 / 6	6 /2	N.S.
Recipient age (yr)	46 ± 3	39 ± 6	N.S.
Donor age (yr)	40 ± 3	49 ± 3	N.S.
Donor- acceptor:			
Sex mismatch	8/16	6/8	N.S.
Blood group mismatch	4/16	1/8	N.S.
Cold ischemia time (min)	632 ± 32	621 ± 50	N.S.
Warm ischemia time (min)	79 ± 5	62 ± 7	0.05
Total ischemia time (min)	711 ± 34	683 ± 49	N.S.

Adenine nucleotide metabolism

In patients with PGDF, the total adenine nucleotide (TAN) content at the end of the cold ischemic period was significantly higher than in control patients (33.0 versus 16.8 nmol / mg protein, $p<0.05$). However, this high TAN content decreased significantly to 13.3 nmol / mg protein after reperfusion (40%, table 2). The decrease was caused by a loss of ATP (from 6.1 to 3.5), ADP (from 14.7 to 5.4, $p=0.04$) and AMP (from 12.2 to 4.5, $p<0.01$). The TAN level in control patients initially decreased to 13.1 nmol / mg protein during the warm ischemic period, but tended to increase again to 17.9 nmol/mg after reperfusion.

The amount of ATP present at the end of the cold ischemic period was also significantly higher in patients with PGDF (6.1 versus 3.5 nmol/mg, $p<0.05$), but ATP levels decreased during the warm ischemic period and there was no de novo generation of ATP after reperfusion (figure 2). In contrast, control patients had low ATP levels at the end of the cold ischemic period, but their ATP levels increased after reperfusion, indicating mitochondrial recovery.

Table 2. Adenine nucleotide content in control patients and patients with PGDF during preservation and reperfusion.

	ECI		EWI		Rep + 60'	
	PGDF	Control	PDGF	Control	PDGF	Control
AMP	12.2±1.9	8.1±1.7	11.4±2.1	5.7±1.1#	4.5±0.7	5.0±1.2
ADP	14.7±3.0	5.2±1.1#	10.2±1.2	4.3±0.5*	5.4±1.3	6.1±0.9
ATP	6.1±1.0	3.5±0.7#	3.9±0.8	3.7±0.4	3.5±0.8	6.8±0.8#
TAN	33.0±4.9	16.8±2.4#	25.5±3.1	13.1±1.6*	13.3±2.4	17.9±2.2
EC	0.39±0.03	0.40±0.05	0.37±0.03	0.46±0.03	0.45±0.04	0.57±0.04#

ECI= end cold ischemia, EWI= end warm ischemia, Rep + 60'= one hour after reperfusion. PGDF = primary graft dysfunction. Control = normal function. TAN= total adenine nucleotides. EC= energy charge. Values are nmol/mg protein ± standard error. #: p-value < 0.05 versus PGDF. *: p-value < 0.01 versus PGDF.

One hour after reperfusion, energy restoration was significantly better in control patients, indicated by the higher energy charge in these patients (0.57 versus 0.47, $p<0.05$), due to a decrease in AMP (from 8.1 to 5.0 nmol/mg) and an increase in ATP (from 3.5 to 6.8 nmol/mg).

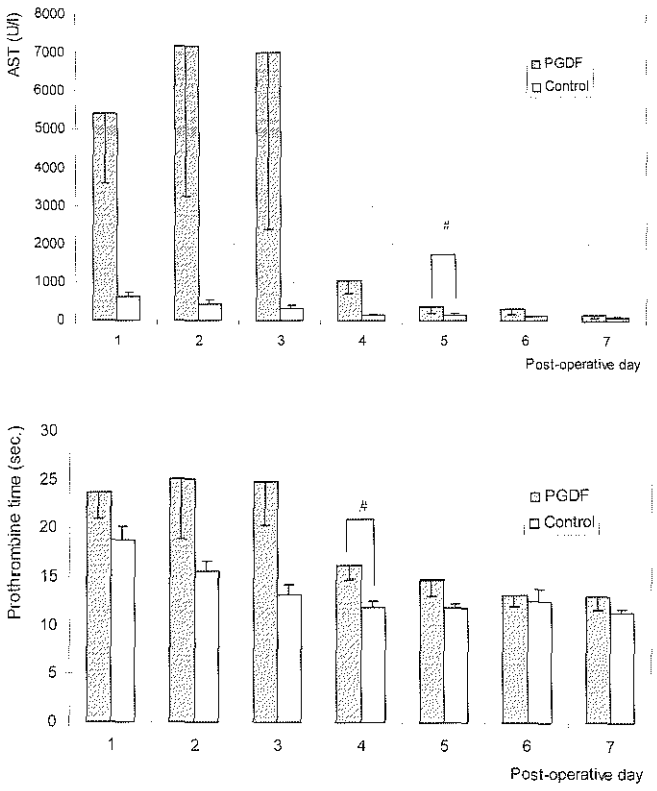
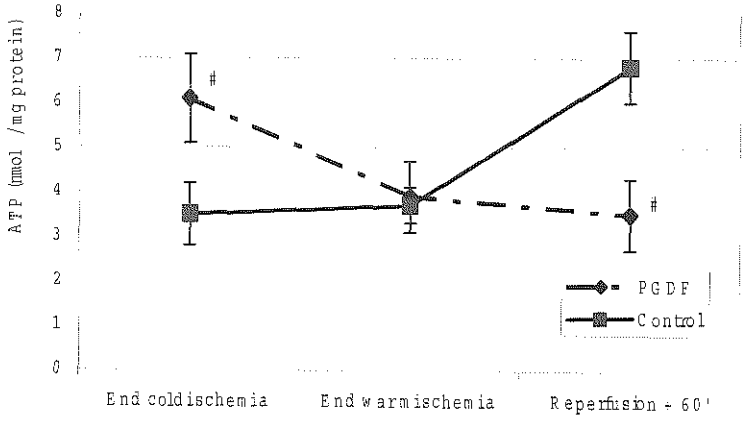


Figure 1a. Post-operative course of AST in patients with (☒) and without (☐) PGDF. Differences between patients were statistically significant on day 5 (#: $p<0.05$). Reference line indicates definition of PGDF, combined with PT > 16.5 sec.
Figure 1b. Post-operative course of pro-thrombine time (PT) in patients with (☒) and without (☐) PGDF. Differences between patients were statistically significant on day 4 (#: $p<0.05$). Reference line indicates definition of PGDF, combined with AST > 2500 U/l.

Figure 2. ATP content of the donor liver at the end of cold and warm preservation and after reperfusion. #: $p < 0.05$ versus control.



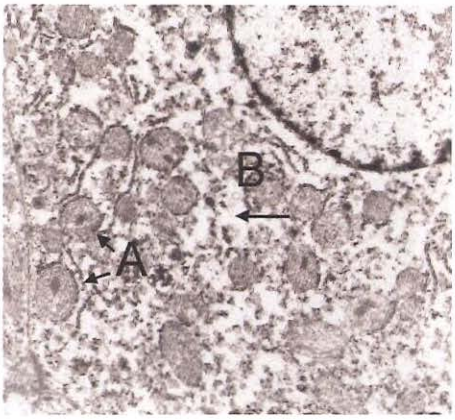


Figure 3. Electron microscopic view of a biopsy taken 1 hour after reperfusion in a patient with PGDF (7000x). Electron lucent hepatocyte with mitochondria showing inclusion bodies (A). The rough endoplasmic reticulum (B) was generally intact and showed only mild swelling. Near total depletion of glycogen particles (arrow).

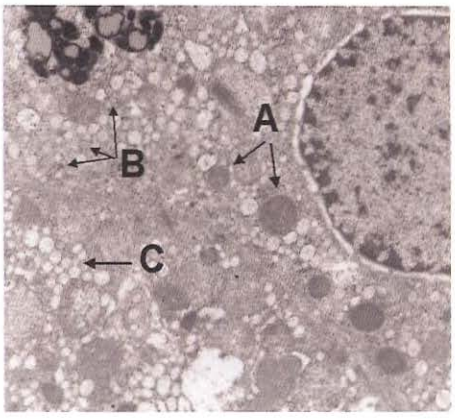


Figure 4. Electron microscopic view of a biopsy taken 1 hour after reperfusion in a control patient (7000x). Electron dense mitochondria showing a bi-layer (A). Glycogen is abundant (B) and fat vacuolisation is present (C).

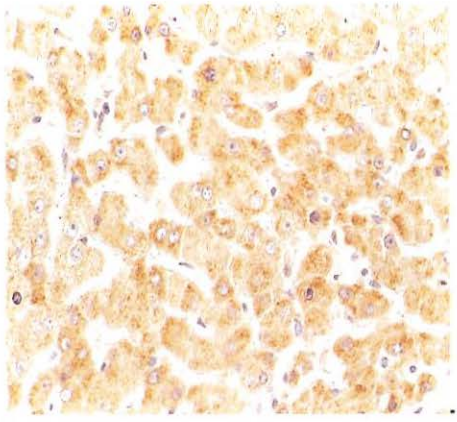


Figure 5. iNOS expression (brown colour) in the cytosol of hepatocytes of a patient with PGDF (400x).

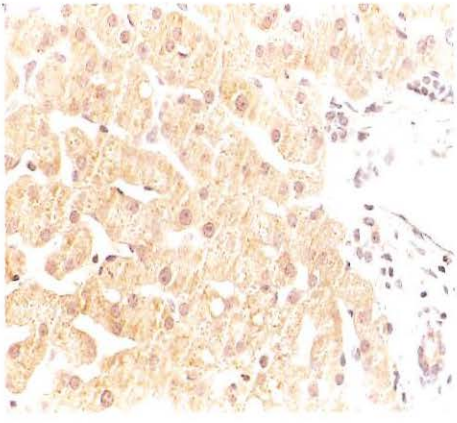


Figure 6. iNOS expression (brown colour) in the cytosol of hepatocytes, bile duct epithelium and portal vein endothelium of a control patient. Note the absence of expression in connective tissue in the portal triad (400x).

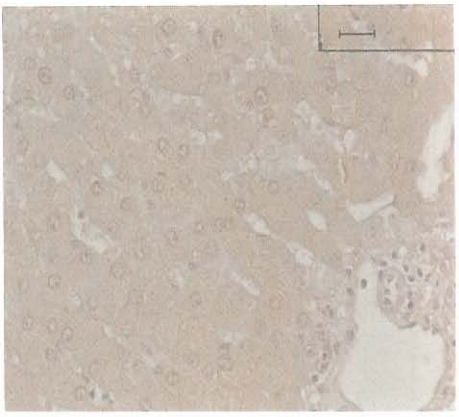


Figure 7. Nitrotyrosine production (red colour) in a patient with PGDF. Note the absence of staining in the connective tissue in the portal triad (400x).

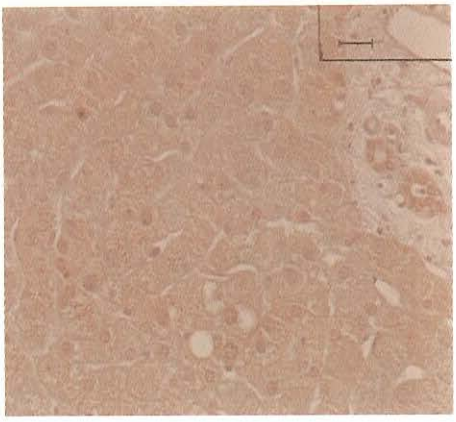


Figure 8. Nitrotyrosine production in a control patient (red colour). There are no differences with the patients with PGDF (400x).

Figure 9. Controle sample of figure 8. The second antibody was omitted, with only standard HE staining (200x).

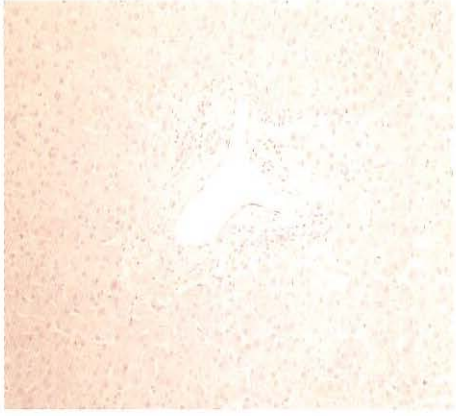


Figure 10. Controle sample of figure 8. Nitrotyrosyl residues were reduced by anaerobic incubation with sodium dithionite. Standard HE staining only (200x).

Histology and electron microscopy

Hematoxylin / eosin stained histology sections were essentially normal at the end of the cold ischemic period, except for mild hepatocellular swelling. Phagocytosis of extracellular matrix material by Kupffer cells was seen in sections, counter stained with periodic acid according to Schiff (PAS), after pretreatment with amylase, which indicates activation of Kupffer cells. This phenomenon was present in patients in both groups at the end of the cold ischemic period, but PAS positive Kupffer cells increased after reperfusion, without differences between groups. Nearly all patients showed micro-vesicular steatosis in 0-15% of the hepatocytes during the cold and warm ischemic period, without differences between patients with or without PGDF. This steatosis could remain present after reperfusion or completely resolve, without relation to post-operative function of the donor liver. After reperfusion a mild reactive hepatitis was seen, characterized by influx of neutrophils into the peri-portal area . Electron microscopy of the biopsies taken 1 hour after reperfusion showed however already marked differences between patients with PGDF (figure 3) and control patients (figure 4). In the former group electron lucent hepatocytes with cytoplasmic fat vacuolization and formation of hepatocellular blebs protruding in the sinusoids were seen. The electron lucent mitochondria showed the onset of condensation with formation of inclusion bodies which indicate protein denaturation and loss of phosphorylating capacity. The rough endoplasmic reticulum was generally intact and showed only mild swelling, but there was a near total depletion of glycogen particles

In control patients, the mitochondria were electron dense and sharp lined, even the bi-layer structure of inner and outer membrane could be recognized. The cytoplasm in these patients contained much more glycogen, but fat vacuolization was present as well. Bile canalicular microvilli were intact in both groups.

Immunohistochemistry

The staining pattern for iNOS and nitrotyrosine showed no differences between the groups: iNOS expression was positive during all stages of the transplantation mainly in the cytosol of hepatocytes and bile duct epithelium and portal vein endothelium of patients with (figure 5) and without PGDF (figure 6). Markedly, Kupffer cells did only show moderate staining, while staining of the sinusoidal endothelium was almost absent. The formation of nitrotyrosine was located in the cytosol with deposits in the nuclei of hepatocytes and bile duct epithelium. Collagen, fibrous tissue and leukocytes in the portal triad were not nitrated. The staining pattern did not show differences between patients with (figure 7) and without PDGF (figure

8). Control samples without the second antibody (figure 9) and after anaerobic incubation with sodium di-thionite (figure 10), showed no staining.

Discussion

In this study we investigated the energy content in hepatocytes during preservation and reperfusion and assessed the role of the nitric oxide derived peroxynitrite radical in mitochondrial dysfunction.

Surprisingly, a higher TAN and ATP content at the end of the cold preservation was observed in patients with PGDF. During the warm ischemic period, the TAN content remained more or less stable in both groups, although there were discrete differences in separate adenosine phosphates. In the control patients, there was some decrease of AMP, little decrease in ADP, but no loss of ATP during this period. In the patients with PGDF, ADP and ATP showed some decrease, with little changes in AMP. These discrete changes became apparent after reperfusion, when the TAN content of the control patients remain stable, but decreased significantly in patients with PGDF. Moreover, control patients showed ATP regeneration after reperfusion, with significantly better energy charging of the hepatocytes, whereas patients with PGDF showed no recovery of ATP.

The high levels of TAN and ATP content at the end of cold preservation in our study are in contrast with the results of others, who described lower or equal TAN and ATP levels in PGDF patients, compared to their control patients (23, 24, 29). The values we found in our control patients are in accordance with previous reports. However, none of these studies did follow the course of the adenine nucleotides content during the warm ischemic period, which makes it difficult to interpret the results.

We hypothesize that the high energy content in our PGDF patients is caused by poor perfusion of the donor liver during organ donation. Optimal rapid hypothermic perfusion leads to cessation of all metabolic processes and enzymatic activity, but poor perfusion gives the donor liver time to induce oxidative phosphorylation, glycogenolysis and glycolysis in a salvage attempt to preserve ATP levels for proton pumping and maintenance of membrane integrity. Due to glucose administration, the glycogen storage of the donor liver is supposed to be filled, which was indeed confirmed by the electron microscopic photographs of the control patients, but absent in patients with PGDF. The glycogen metabolism and glucose output would have been inhibitory on ATP consumption. Also these cell processes are promoted by the presence of stress hormones after brain death, necessity for large

quantities of catecholamines in the liver donor and relative starvation, despite glucose administration (50-52).

The induction of oxidative phosphorylation to form ATP and activation of glucose output in patients with PGDF also prevents the degradation of high energy phosphates to adenosine. This may give another explanation for the association between lower levels of TAN and ATP and good postoperative function, as the formed adenosine can not be further metabolized by dependence of enzymatic activity during the cold perfusion of the donor liver. Adenosine has a strong vasodilative effect, which may be beneficial at reperfusion of the donor liver (53) and omission of this substance from preservation solutions has deleterious effects (54). The functional impairment of the mitochondria found 1 hour after reperfusion as a cause of the decrease of ATP levels in patients with PGDF, was confirmed by electron microscopy in the same biopsies. In these patients we found depletion of glycogen particles and electron lucency of the mitochondria with inclusion bodies already at the end of the cold ischemic period. These results are comparable with the findings of other authors (11, 12, 55, 56).

In the same biopsies, nitrotyrosine was used as an indicator to elucidate the cause of mitochondrial dysfunction in PGDF. All hepatocytes and bile duct endothelium stained positive. The level of nitration was high already at the end of the cold ischemic period and remained at that high level during warm ischemia and after reperfusion. Connective tissue in the portal triads did not contain nitrotyrosine. Staining for iNOS revealed hepatocytes as the main source of NO, but Kupffer cells and bile duct endothelium showed, to a lower level, expression as well. We found that in the livers of all patients nitrotyrosine was present and that they did express iNOS already at the end of the cold preservation. Since the necessary NO for tyrosine nitration can not be produced by iNOS during cold ischemia, this indicates that the cellular proteins of the donor liver have been nitrated before the donation procedure.

In conclusion, our study shows that PGDF related mitochondrial dysfunction does not originate from the cold or warm ischemic period, but probably is caused earlier, during the organ donation procedure. Apparently, the high TAN and ATP levels at the end of the cold ischemic period can be used as an early marker of primary graft dysfunction. Although experimental studies indicated that occurrence of nitration of mitochondria is detrimental, the lack of any difference in nitrotyrosine formation between patient with or without PGDF makes nitration of mitochondria a unlikely cause of clinical primary graft dysfunction.

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Fibrinolysis during liver transplantation is enhanced by using solvent/detergent virus inactivated plasma (ESDEP®)

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Chapter 8

Abstract

After introduction of solvent/detergent treated plasma (ESDEP®) in our hospital, an increased incidence of hyperfibrinolysis was observed (75% versus 29%, p=0.005) compared to the use of fresh frozen plasma (FFP). To clarify this increased incidence, intraoperative plasma samples of patients treated with FFP or ESDEP were analysed in a retrospective observational study. During the anhepatic phase plasma levels of D-dimer (6.58 versus 1.53 µg/ml, p=0.02) and FDP (60 versus 23 mg/l, p=0.018) were significantly higher in patients treated with ESDEP. After reperfusion differences increased to 23.5 versus 4.7 µg/ml (D-dimer, p=0.002) and 161 versus 57 mg/l (FDP, p=0.001). The amount of plasma received per packed red blood cell concentrate, clotting tests and levels of individual clotting factors did not show significant differences between both groups. α2-Antiplasmin levels however, were significantly lower in patients receiving ESDEP the anhepatic phase (0.37 versus 0.65 IU/ml, p<0.001) and after reperfusion (0.27 versus 0.58 IU/ml, p=0.001). Analysis of α2-antiplasmin levels in ESDEP itself showed a reduction to 0.28 IU/ml (normal > 0.95) due to the solvent/detergent process. Therapeutic consequences for the use of ESDEP in orthotopic liver transplantation are discussed in view of an increased incidence of hyperfibrinolysis due to reduced levels of α2-antiplasmin in the solvent/detergent treated plasma.

Implications: The use of solvent/detergent virus inactivated plasma is of increasing importance in the prevention of HIV and HCV transmission. Since the use of this plasma during orthotopic liver transplantation an increased incidence of hyperfibrinolysis was observed. Clotting analysis of the patients revealed low α2-antiplasmin concentrations due to the solvent/detergent process.

Introduction

To minimise the risk of transmission of lipid coated viruses (HBV, HCV, and HIV) in blood component substitution therapy, solvent/detergent (SD) treated plasma is used in our hospital since 1996. Since its introduction, an increase in hyperfibrinolysis during orthotopic liver transplantation (OLT) was observed. Enhanced fibrinolytic activity during OLT has been attributed to high levels of tissue-type plasminogen activator (t-PA) (1,2) and contributes to the serious bleeding complications (3). t-PA Plasma levels peak just after reperfusion as result of the absent clearance during the anhepatic phase and the release of t-PA from damaged endothelial cells in the donor liver (4). In vitro studies demonstrated some

alterations in individual clotting factors due to SD treatment, particularly a loss of factor VIII (up to 20%), protein S (35%) and α2-antiplasmin (up to 76%) (5,6).

In the present observational study intraoperative plasma samples of patients treated with SD treated plasma or FFP were analysed to find an explanation for the increased incidence of hyperfibrinolysis observed with the use of ESDEP during liver transplantation.

Methods

From June 1994 to March 1997, 67 patients underwent OLT for end stage cirrhosis, after written informed consent was obtained. In 41 patients, complete coagulation follow-up during the procedure was present. From June 1994 – December 1995 clotting factors were substituted in 21 patients with fresh frozen plasma (FFP, 300 ml, CLB Amsterdam, the Netherlands). Since January 1996 SD virus-inactivated plasma (ESDEP®, 200 ml, CLB Amsterdam), was used in 20 patients. The ESDEP was prepared from pooled plasma of 2000 voluntary Dutch blood donors and virus inactivation is realised by treating the pooled plasma with 1% tri-(n-butyl)phosphate (TNBP) and 1% Triton-X100 (7) by Octapharma (Octapharma, Vienna, Austria).

Anaesthesia was induced with penthotal 3-4 mg/kg, midazolam 0.1 mg/kg and sufentanyl 0.5 µg/kg. Muscle relaxation was achieved with pancuronium 0.1 mg/kg. Anaesthesia was maintained with midazolam 0.1 mg/kg/hr and sufentanyl 0.2 µg/kg/hr and pancuronium 25 µg/kg. Calcium chloride was given by infusion as required. Inotropic support was provided if necessary with dopamine 5-10 µg/kg/min and sometimes epinephrine 0.25 µg/kg/min. Packed red blood cells (PRBC) were transfused to maintain a hematocrit of 25%. FFP or ESDEP was infused 10 ml/kg in the pre-anhepatic phase to correct severe coagulopathy and then in a ratio of 1 ml/ml PRBC or ml salvaged blood. Platelet count was kept above $80x10^9$/l by infusion of platelet suspension. If fibrinogen decreased below 1.0 g/l, purified fibrinogen (Haemocomplettan P, CLB, The Netherlands) was given. A venovenous bypass with heparin coated tubing (Bioconsole, Biomedicus, Minneapolis, USA) was used after trial clamping of the caval vein. A blood cell salvage system (CATS, Fresenius, Schweinfurt, Germany) and rapid infusion system (RIS, Haemonetics, Braintree, USA) was routinely used. Routine coagulation tests (aPTT, PTT, thrombocyte count, thrombine time 5E and 10E, fibrinogen, TT and NT) were performed on arterial blood collected 5 minutes after induction of anaesthesia (but before administration of plasma), 5 minutes after hepatectomy, 5 minutes after reperfusion of the donor liver and at the end of the procedure.

Fibrinolysis was detected with four-channel thrombelastograph (TEG) recordings according to the criteria of Kang et al. (8) standard at sampling times and further when clinically indicated. The following values were measured: reaction time (r), coagulation time (r+k), maximal amplitude (MA) and clot lysis index (CLI) at 30 and 60 minutes ($MA\text{-}MA_{(t)}/MA \times 100\%$). Tranexamic acid (500-1000 mg) was administered when TEG recordings showed a $CLI_{(60)}$ of more than 10% and generalised oozing occurred. After administration of tranexamic acid the result was evaluated with new TEG recording and if necessary the treatment was repeated with dosages of 10-15 mg/kg/h. To confirm the diagnosis of fibrinolysis, D-dimers were analysed afterward in stored plasma using the Miniquant automated agglutination analyser and Miniquant test kit (Biopool Kordia, Leiden, The Netherlands). FDP were also analysed in stored plasma using a Latex agglutination reaction (Diagnostica Stago Roche, The Netherlands). Clotting factors II, VII, VIII, IX and X levels were analysed afterwards by standard clotting assays in stored plasma (-80°C) on an automatic coagulation laboratory (ACL Instrumentation Laboratory, IJsselstein, the Netherlands) using human deficient plasma (Biopool Kordia, Leiden, The Netherlands). AT-III and α2-antiplasmin were determined with a chromogenic assay (Dade-Behring, Leusden, The Netherlands).

All data are presented as mean ± SEM. After testing normality with Shapiro-Wilk statistics, a Student's t-test or Mann-Whitney U-test was used for data analysis. α2-Antiplasmin levels had logarithmic transformation before analysis and the changes from baseline α2-antiplasmin levels were investigated with repeated measures analysis of variance including time period and type of plasma as covariates. For 2x2 table data the Chi-square test with Yates correction or Fisher's exact test was used. Differences were considered significant for P less than 0.05.

Results

The characteristics of the FFP and ESDEP treated patient groups are summarised in table 1. In patients treated with FFP (N=21), hyperfibrinolysis was seen in 6 individuals (29%); in 1 patient during the pre-anhepatic phase, in 1 patient during both pre-anhepatic and anhepatic phase, in 1 patient during both anhepatic and reperfusion phase and in 3 after reperfusion. In the ESDEP treated group (N=20), hyperfibrinolysis was present in 15 patients (75% vs. 29%, p=0.005). In 4 patients an episode of hyperfibrinolysis occurred during the pre-anhepatic phase (20% vs. 10%, NS), in 8 patients during the anhepatic phase (40% vs. 10%; p=0.03) and in 10 patients after reperfusion (50% vs. 19%; p=0.05). In 7 of these 15

patients with hyperfibrinolysis it occurred repeatedly: in 3 patients during both pre-anhepatic and anhepatic phase and in 4 patients in the anhepatic and after reperfusion. Clot lysis indices after 60 minutes (CLI_{60}) were 4 vs. 26% (p=0.03) in the anhepatic phase and 4 vs. 40% (p=0.03) after reperfusion (table 2). The dose of tranexamic acid given in both groups to patients with excessive fibrinolysis was well comparable. In the patients treated with FFP, a total of 6500 mg of tranexamic acid was administered to 6 patients (29%), mean dose 1083 mg/patient. In the ESDEP treated group, 12 patients (60%) received a total of 15250 mg of tranexamic acid, mean dose 1271 mg/patient, p= N.S.

Table 1. Basic characteristics of patients undergoing orthotopic liver transplantation.

	FFP (N=21)	**ESDEP** (N=20)
Age (yr.)	53 (23-65)*	44 (23-62)
Male / Female	9 / 12	15 / 5
Indication (N)		
Viral hepatitis cirrhosis	6	6
Cholestatic liver disease	10	8
Other cirrhosis	5	6
Operation time (min)	419 (14)	436 (21)
Blood loss (ml)	12173 (1907)	15191(3155)
Transfusions		
PRBC (units)	13.1 (1.8)	15.3 (2.9)
Cell salvage blood (ml)	2481 (655)	2655 (981)
Plasma (ml)	4271 (616)	5440 (903)
Fibrinogen (g)	4.7 (0.8)	7.2 (1.6)
Platelets (units)	19.5 (2.4)	20.0 (3.6)
Plasma / PRBC (ml)	342 (30)	383 (26)

Values are mean ± SEM or range. FFP: fresh frozen plasma, ESDEP®: solvent/detergent treated plasma. * *P< 0.05 vs. ESDEP®.*

Analysis of standard coagulation parameters during the procedure revealed no significant differences between patients treated with FFP or ESDEP, except for FVIII after induction and the PT in the anhepatic phase (table 3). However, D-dimer and FDP levels were significantly higher in the ESDEP treated patients in the anhepatic phase and after reperfusion, consistent with the increased fibrinolytic activity recorded on TEG (table 3).

Also determination of α2-antiplasmin levels showed significant differences between treatment with FFP or ESDEP (figure 1). α2-Antiplasmin levels after onset of anaesthesia were not statistically different in the FFP and the ESDEP treated group. In patients who received FFP, α2-antiplasmin levels remained relatively stable and decreased from 0.76 IU/ml to 0.65 IU/ml in the anhepatic phase, to 0.58 IU/ml after reperfusion and levelled at

0.58 IU/ml at the end of the procedure. The corresponding levels in patients treated with ESDEP were 0.64 IU/ml (P=NS), 0.37 IU/ml (P<0.001), 0.27 IU/ml (P=0.006) and 0.40 IU/ml (p=0.03) respectively. Repeated analysis of variance confirmed that type of plasma was responsible for the decrease from baseline value, independent from the time course.

Table 2. TEG parameters during orthotopic liver transplantation in patients treated with FFP and ESDEP.

	After induction		Anhepatic phase		After reperfusion	
	FFP	ESDEP	FFP	ESDEP	FFP	ESDEP
R (mm)	21.7 (0.6)	23.6 (2.7)	15.5 (1.6)	16.1 (2.5)	24.1 (1.0)	29.6 (3.9)
R+K (mm)	31.0 (0.9)	32.0 (3.0)	24.6 (2.1)	23.3 (3.3)	37.5 (1.8)	44.3 (5.0)
Ang (deg)	50.1 (4.7)	42.2 (3.1)	43.7 (4.1)	52.4 (3.6)	41.5 (4.2)	33.7 (3.5)
MA (mm)	47.7 (2.5)	54.5 (3.1)	40.8 (3.0)	48.0 (3.7)	32.2 (3.2)	35.0 (3.9)
$CLI_{(30)}$ (%)	1.6 (0.4)	3.1 (2.1)	1.7 (0.5)*	16.8 (7.5)	2.0 (0.5)*	31.6 (9.9)
$CLI_{(60)}$ (%)	4.3 (0.8)	7.2 (2.2)	3.9 (1.1)*	26.2 (9.6)	4.2 (1.0)*	39.7 (10.7)

Values are mean ± SEM. FFP: fresh frozen plasma, ESDEP®: solvent/detergent treated plasma.
** P< 0.05 versus ESDEP.*

Table 3. Coagulation parameters during orthotopic liver transplantation.

	After induction		Anhepatic phase		After reperfusion	
	FFP	ESDEP	FFP	ESDEP	FFP	ESDEP
APTT (s.)	43 (3)	46 (3)	68 (13)	113 (19)	157 (18)	181 (19)
PT (s.)	17.6 (1.7)	15.8 (0.9)	15 (0.5)*	24.0 (5.5)	17.4 (1.0)	24.0 (5.2)
T 10 E (s.)	17 (1)	20 (2)	19 (2)	25 (4)	26 (4)	33 (6)
Platelets ($x10^9$/l)	108 (16)	113 (17)	89 (19)	75 (10)	90 (14)	79 (9)
Fibrinogen (g/l)	1.8 (0.1)	2.2 (0.3)	1.5 (0.1)	1.6 (0.2)	1.5 (0.1)	1.4 (0.2)
FII (IU/ml)	0.48 (0.05)	0.48 (0.05)	0.47 (0.03)	0.40 (0.04)	0.39 (0.02)	0.37 (0.03)
FVII (IU/ml)	0.43 (0.05)	0.45 (0.05)	0.41 (0.03)	0.37 (0.04)	0.34 (0.02)	0.35 (0.03)
FX (IU/ml)	0.56 (0.04)	0.55 (0.04)	0.49 (0.02)	0.44 (0.04)	0.39 (0.02)	0.38 (0.03)
FVIII (IU/ml)	2.17 (0.25)*	3.41 (0.52)	1.29 (0.21)	1.70 (0.30)	0.80 (0.12)	1.01 (0.24)
FIX (IU/ml)	0.51 (0.07)	0.58 (0.08)	0.36 (0.05)	0.36 (0.06)	0.22 (0.03)	0.19 (0.04)
D-dimer (µg/ml	0.46 (0.14)	1.01 (0.40)	1.53 (0.37)*	6.58 (1.98)	4.70 (1.97)#	23.47 (6.56)
FDP (mg/l)	-	-	23 (5)#	60 (16)	57 (11)#	161 (24)

Values are mean ± SEM. FFP: fresh frozen plasma, ESDEP®: solvent/detergent treated plasma.
T 10E= trombine time. ** P< 0.05 versus ESDEP, # P<0.01 versus ESDEP.*

Analysis of ESDEP itself revealed normal levels of all clotting factors (>0.81 IU/ml), AT-III (>0.81 IU/ml), protein C (0.80 IU/ml) and plasminogen (0.94 IU/ml). Levels of FVIII (0.71 IU/ml) and protein S (0.58 IU/ml) were slightly depressed, but levels of α2-antiplasmin were severely depressed to 0.28 ± 0.02 IU/ml (normal 0.95-1.20 IU/ml) (5). Linear regression showed a statistical significant relation between the level of α2-antiplasmin and the $CLI_{(30)}$ (P<0.01) and $CLI_{(60)}$ (P<0.01), the level of FDP and $CLI_{(30)}$ (P<0.03) and the d-dimer level and $CLI_{(30)}$ (P<0.01) and $CLI_{(60)}$ P<0.01).

Despite the increased fibrinolysis, there was no difference in total blood loss between patients receiving FFP or ESDEP, but blood loss was significantly higher in the 21 out of 41 patients from both groups in whom hyperfibrinolysis occurred (18054 ± 2469 ml vs. 8683 ± 1339, $p<0.001$). In addition the transfusion requirements for PRBC, platelets, fibrinogen and plasma were also higher in patients with hyperfibrinolysis (all $p<0.001$).

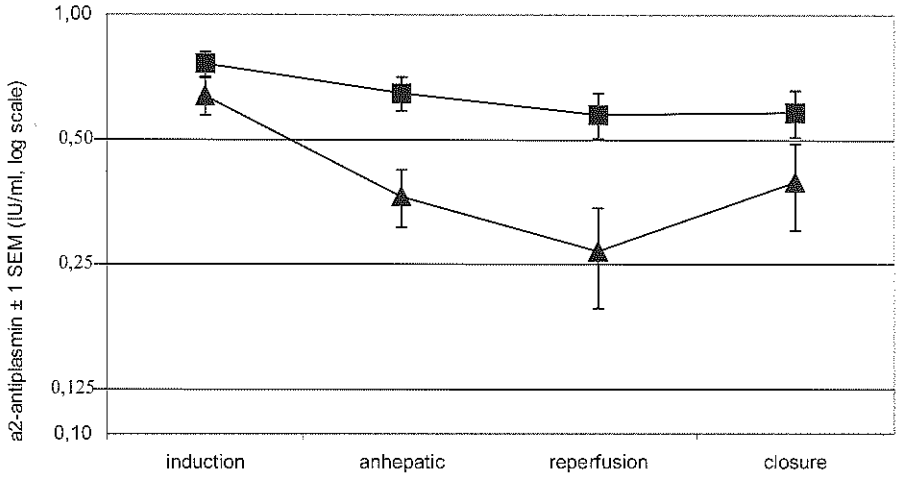

Figure 1. Intraoperative time course of the α2-antiplasmin levels. ■ Patients with chronic liver disease treated with Fresh Frozen Plasma (FFP, N= 21). ▲ Patients with chronic liver disease treated with SD virus inactivated plasma (ESDEP®, N=20).

Discussion

Orthotopic liver transplantation (OLT) has become the standard treatment for end-stage liver disease, but the procedure still can be associated with severe disorders of haemostasis (9).

A key role in the pathogenesis of intraoperative bleeding is played by enhanced fibrinolysis during the anhepatic and reperfusion phase (10,11). To minimise the risk of viral transmission, solvent/detergent (SD) virus-inactivated plasma was introduced in 1996 in our hospital and thereafter an increase in hyperfibrinolysis in patients undergoing OLT was observed. SD treatment of fresh frozen plasma is an effective method to reduce lipid coated viruses by > 5 log 10 (12,13), but it has been reported that because of the SD process the concentration of FVIII, protein S and α2-antiplasmin is reduced (5,6). α2-Antiplasmin is a main physiological inhibitor of t-PA induced fibrinolysis and fibrinogenolysis (14,15) which

are held responsible for uncontrollable intraoperative bleeding. Inhibition of this fibrinolysis may occur at the level of plasminogen activation by plasminogen activator inhibitor (PAI) or at the level of plasmin by α2-antiplasmin (16). Once large amounts of t-PA are released in the circulation of the recipient, α2-antiplasmin plays a key-role in the scavenging of the formed free plasmin. Free plasmin is extreme rapidly inactivated by α2-antiplasmin; the half-life of free plasmin is estimated to be approximately 0.1 s. Without adequate α2-antiplasmin levels, a small amount of t-PA can start the offset of systemic fibrinolysis (17), as the fibrin degradation products can amplify the plasminogen activation (18).

Although the efficacy of SD plasma in clinical studies in patients with coagulation disorders has been established (19,20), the balance between pro- and anti-fibrinolytic activity in our patients seems to be disturbed during OLT by SD plasma substitution. In patients with chronic liver disease enhanced fibrinolysis occurred about 3 times more in the anhepatic phase and after reperfusion in patients who received ESDEP than in patients treated with FFP. Determination of α2-antiplasmin concentrations in ESDEP showed decreased levels in accordance with the results presented in literature (5,6). In 47% of the patients treated with ESDEP hyperfibrinolytic episodes did recur, despite adequate treatment of the first hyperfibrinolytic episode, proven by normalisation of the TEG recordings. We think that this effect is caused by the large plasma exchanges in liver transplantation, which dilute the level of α2-antiplasmin in the patient to the level of ESDEP itself.

In a recent randomised multi-centre study by Freeman et al. (21) no differences were seen in coagulation parameters between patients receiving FFP or SD treated plasma during liver transplantation, but α2-antiplasmin levels were not determined in their study. Hyperfibrinolytic problems were not mentioned in the article, probably because of standard prophylactic use of anti-fibrinolytic drugs in the participating centres in the United Kingdom.

In our patients with chronic liver disease, α2-antiplasmin levels were relatively high (0.70 IU/ml) after induction of anaesthesia, in contrast to the markedly reduced α2-antiplasmin levels reported for patients with advanced liver disease (22,23). Low levels of α2-antiplasmin in the anhepatic phase and after reperfusion in the ESDEP treated patients compared to the FFP treated patients explains the increase in hyperfibrinolysis seen in the former group. At the end of the procedure α2-antiplasmin levels are increasing in the patients treated with ESDEP, probably due to increased clearance of t-PA by the donor liver and starting synthesis of α2-antiplasmin. In accordance with the study of Freeman et al., no

differences were found in other coagulation parameters, use of blood products or blood loss between the FFP and ESDEP treated patients. However, patients from both groups with hyperfibrinolysis had significant more bleeding and transfusion requirements than patients without hyperfibrinolysis.

In summary, the results of this observational study have to be interpreted carefully, because potentially other factors could have caused our findings. However, in the time considering this study no changes have occurred in our operating or anaesthesiology techniques, medical staff or transfusion protocol. Also the increased incidence of hyperfibrinolysis was present immediately after switching from fresh frozen plasma to ESDEP. Infusion of SD treated plasma in patients needing massive transfusion may lead to insufficient levels of α2-antiplasmin and subsequently to secondary hyperfibrinolysis. This hyperfibrinolysis can not be corrected with infusion of more SD treated plasma, as this would dilute the α2-antiplasmin concentration in the patient to that of ESDEP itself (0.28 IU/l). Therefore, it should be treated with anti-fibrinolytic medication. The effects of aprotinine on antiplasmin activity, hyperfibrinolysis and subsequent blood loss have been reported with various outcomes (3, 24-27). Considering the significant higher blood loss in patients with hyperfibrinolysis in our study, routine administration of anti-fibrinolytic drugs using SD virus-inactivated plasma is suggested.

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Improved survival after caval preserving liver transplantation

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Submitted

Chapter 9

Abstract

Background: The piggyback technique for orthotopic liver transplantation was introduced to decrease intraoperative blood loss and postoperative complications. A retrospective analysis was undertaken to evaluate the results of the implementation of this technique.

Methods: 45 patients transplanted since 1999 using a modified piggyback technique were compared to 90 controls with a standard anastomosis since 1995. Liver grafts were preserved in UW solution (Viaspan, DuPont). All vascular anastomoses with the vena cava were made with the vascular stapler (Autosuture, Tyco Health Care). The post-operative immunosuppresive regimen consisted of Tacrolimus (FK506, Fujisawa), an IL-2 receptor antagonist (Basiliximab, Novartis or Daclizumab, Roche) and a steroid withdrawal scheme. The impact on transfusion requirements, postoperative course and patient and graft survival was studied.

Results: Warm ischaemic time and total operation time were shortened significantly in the piggyback group: 51 vs. 81 minutes and 5.9 vs. 7.0 hours. Intraoperative blood loss and transfusion requirements were significantly decreased in the piggyback group. No differences could be identified in incidence of infection (57 and 68%) or relaparotomy for bleeding (16 and 10%). Patients in both groups had identical length of stay in the ICU (5 and 6 days) and in hospital stay (27 and 29 days). At 6 months after transplantation graft survival and patient survival were significantly better in the piggyback group (87% and 91%) than in the standard group (66% and 73%).

Conclusion: The introduction of the piggyback technique reduced transfusion requirements and increased graft survival and patient survival at 6 months after liver transplantation.

Introduction

The results of conventional orthotopic liver transplantation continue to improve because of advances in immune suppression therapy, developments in surgical techniques and better perioperative monitoring. During the standard procedure with replacement of the recipient retrohepatic vena cava inferior, the routine use of a veno-venous bypass (VVB) is recommended for decompression of the splanchnic and cava inferior system (1,2). However, the use of a veno-venous bypass is associated with complications including thromboembolism, air embolism, hypothermia and wound infection (3-6). Liver transplantation with preservation of the recipient vena cava ("piggyback" technique) has been proposed as an alternative procedure, allowing normal caval blood flow during the anhepatic stage (7). In a large series of patients the piggyback technique was shown to be a

safe procedure with acceptable technical post-operative complications (8,9). Also the piggyback technique was reported to decrease the need for red blood cell transfusion and administration of platelets and plasma (10-12). Despite these advantages, a beneficial effect on graft or patient survival could not be established (10,12,13). In this study we evaluate the intraoperative transfusion requirements, postoperative complications and 6-month survival after liver transplantation after introduction of the piggyback technique in our hospital.

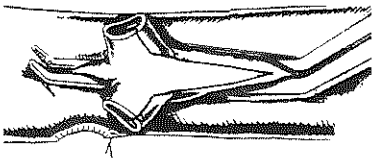

Figure 1a. The middle and left hepatic vein were joined into a cuff for anastomosis. This common orifice was enlarged by a caudal incision of 7 cm.

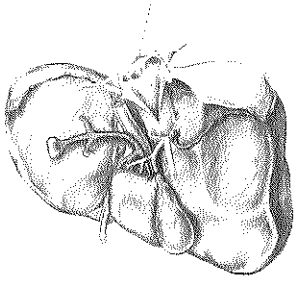

Figure 1b. In the donor liver, the infrahepatic IVC is stapled. The dorsal side of the suprahepatic donor IVC is incised caudally starting at the confluence of the hepatic veins to create a "V shaped" anastomosis. (Compilation of fig 3 and 4, Cherqui et al. Transplantation 1994; 58: 794).

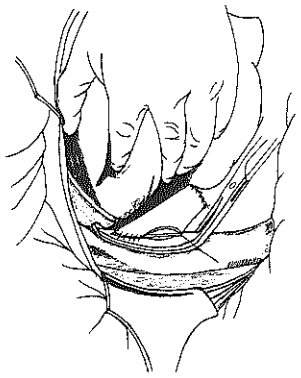

Figure 2. End-to-side cava-caval anastomosis. (Modification fig 5, Cherqui et al. Transplantation 1994; 58: 795).

Patients and Methods

From 1989 to 1999 orthotopic liver transplantation was performed with standard replacement of the recipient's inferior vena cava (IVC) with or without VVB, according to the cardiac index after trial clamping of the IVC. Since March 1999 a modification of the "piggyback" technique described by Belghiti (7) and Cherqui (14) was used for caval anastomosis. After dissection of the porta hepatis, the diseased liver is mobilised from the vena cava with meticulous ligation of all retrohepatic veins originating from segments VI, VII and I. A temporary porto-caval shunt was never used. The IVC of the recipient is preserved and the orifice of the right hepatic vein is stapled. Hereafter a vascular clamp is applied

laterally on the anterior part of the recipient VC and the diseased liver is removed. The middle and left hepatic vein are joined into a cuff for anastomosis. This common orifice is enlarged by a caudal incision of 7 cm in the anterior wall (figure 1a). In the donor liver, the infrahepatic IVC is stapled. The posterior side of the suprahepatic donor IVC is incised caudally starting at the suprahepatic cutting surface of the IVC (figure 1b) This way a "V" shaped end-to-side cava-caval anastomosis is created (figure 2).

From March 1999 to July 2000, 50 adult patients received an orthotopic liver transplantation using the modified piggyback technique. In the early phase, in two patients conversion to conventional liver transplantation with excision of the vena cava was necessary because of uncontrollable bleeding from ruptured hepatic veins. The remaining 48 patients in whom the modified piggyback technique was performed, were matched for indication for transplantation. In three patients no appropriate control patients could be found because of rare indication. Eventually, 45 patients (21 female, 24 male) were matched for indication with 90 control patients. Other variables affecting survival (re-transplantation, recipient age over 60 years, donor age over 55 years, cold ischaemia > 12 hours, ABO incompatibility and donor/recipient sex mismatching (15)) were analysed to be equally distributed. To create a stable control group, minimising the role of a learning effect of the surgeons, regression analysis of blood loss and transplantation date was performed. The 90 control patients (41 female, 49 male) with a standard anastomosis were selected from the period 1995-1999, in which the blood loss during transplantation was stable ($R=0.06$, $p=0.58$).

Statistical analysis

Data are presented as mean with their 95% confidence interval (mean +/- two standard errors), ages as median and range. Differences between the groups were detected with the Mann-Whitney test. Patient survival was calculated using the Kaplan-Meier method. Differences in 6 month survival between groups were detected using the log rank test. For cross table analysis the Fisher's exact test was used. Differences were considered significant for $p< 0.05$.

Results

Indication for transplantation was cholestatic liver disease (31%), viral hepatitis (18%), acute liver failure (18%), post alcoholic cirrhosis (9%), hepatocellular carcinoma (7%), cryptogenic cirrhosis (7%) and other indications (10%, i.e. metabolic diseases, adenoma). Other patient

characteristics are given in table 1. In the conventional group 42 patients (47%) required veno-venous bypass after trial clamping of the IVC. Veno-venous bypass was never used in the piggyback group. There was no difference in duration of the hepatectomy phase between the groups, but warm ischaemia time and total operative time were significantly shortened in the piggyback group: 52 (47-58) versus 79 (76-83) minutes, $p<0.01$ and 5.9 (5.4-6.3) versus 6.9 (6.5-7.3) hours, $p<0.01$ respectively. Intra operative blood loss and transfusion requirements were significantly decreased in the piggyback group (table 2).

Table 1. Patient characteristics for recipients of piggyback technique and conventional orthotopic liver transplantation.

	Piggyback	Conventional	p-value
Donor age	43 (8-68)	38 (13-62)	0.11
Recipient age	48 (19-68)	48 (17-67)	0.96
Previous LTx	3/45	8/90	0.75
Cold iscaemia > 12 hrs	8/45	22/90	0.51
Donor / recipient:			
Sex mismatch	20/45	52/90	0.15
ABO mismatch	4/45	11/90	0.77
Postoperative dysfunction	2/45	4/90	1.0

Post-operative course:

After transplantation, primary graft non function occurred in 2/45 patients in the piggyback group and in 4/90 patients in the standard group (NS).

Levels of ASAT at the first post-operative day were higher in the piggyback technique group than after standard liver transplantation: 782 (135-6214) U/l versus 680 (115-7127) U/l, $p=0.43$. At the third post-operative day ASAT levels were decreased to 214 (35-3540) U/l in the piggyback group and 178 (9-17300) U/l in the conventional group, $p=0.55$. Serum creatinine levels were comparable at the third post-operative day in the piggyback group 76 (42-460) µmol/l and in the conventional group: 84 (20-464) µmol/l, $p=0.81$. The median length of stay in the ICU and in hospital stay was 5 (range 1-119) and 27 (range 13-162) days in the piggyback group, patients transplanted with the standard technique were discharged after 6 (range 1-101) days from ICU and after 29 (range 14-320) days from hospital. No significant differences were found in the number of patients that acquired an infection in the first month after transplantation: 29 patients (64%) in the piggyback group and 55 patients (61%) in the conventional group. However, in the patients treated with conventional liver transplantation more severe infections (septicaemia, deep wound infection and pneumonia) occurred in 27 patients (30%) compared to 6 (13%) in the piggyback group, $p<0.05$. Post-operative bleeding requiring laparotomy occurred in 7 patients (16%) after

piggyback technique and in 9 patients (10%) in the conventional group, p=0.40. Incidences of hepatic artery thrombosis, portal vein thrombosis and vena cava stenosis were 6/45 (13%), 2/45 (4%) and 1/45 (2%) after piggyback transplantation and 8/90 (9%), 2/90 (2%) and 3/90 (3%) after conventional technique. Stenosis of the vena cava was treated with balloon angioplasty in 3 patients, one patient with a conventional liver transplantation received an intra vascular stent. Actuarial survival rate of the graft at 6 months after transplantation was 87% in the piggyback group and 68% (p<0.03) in the standard liver transplantation technique group (figure 3). Patient survival rate was 91% in the piggyback group and 73% (p<0.02) in the standard liver transplantation group (figure 4).

Table 2. Intra operative blood loss and transfusion requirements of recipients of piggyback technique and standard orthotopic liver transplantation. PBRC: packed red blood cell concentrate.

	Piggyback	Conventional	p.value
Blood loss (L)	5.7 (4.6-6.7)	12.4 (10.1-14.8)	<0.01
PRBC (U)	7 (6-8)	12 (10-14)	<0.01
Cell saver blood (L)	0.6 (0.4-0.9)	2.1 (1.4-2.7)	<0.01
Fresh frozen plasma (L)	2.8 (2.2-3.3)	4.9 (4.2-5.7)	<0.01
Thrombocyte concentrate (U)	9 (7-11)	18 (15-21)	<0.01
Fibrinogen (g)	1.1 (0.5-1.6)	4.2 (3.2-5.3)	<0.01

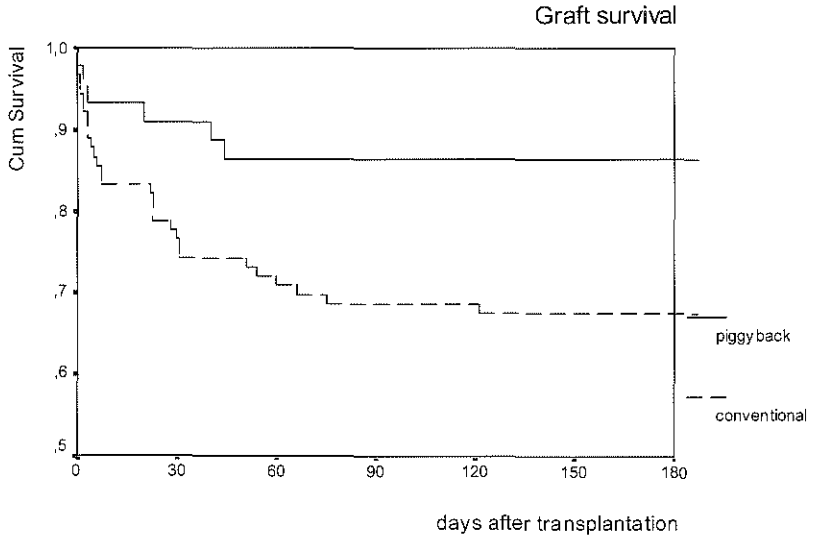

Figure 3. Grafts at risk:

Piggyback	40	38	38	38	38	38
Conventional	68	63	61	61	60	60

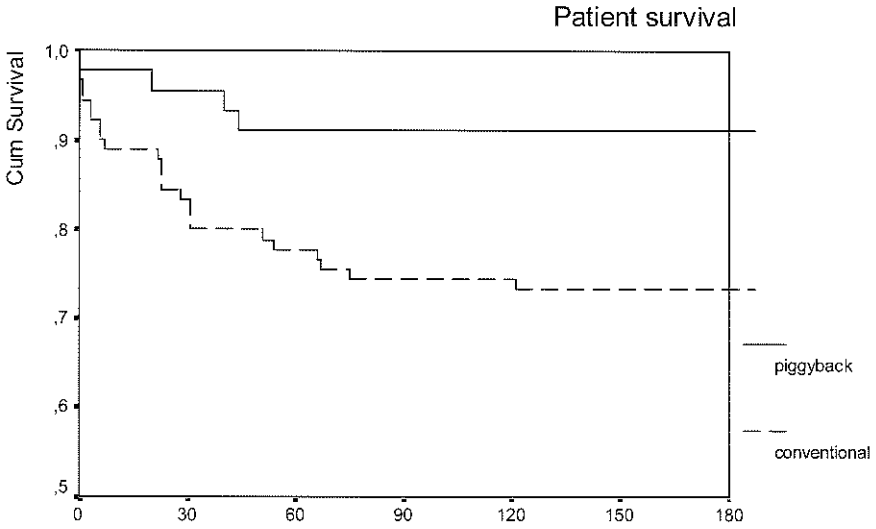

Figure 4. Patients at risk:

Piggyback	43	41	41	41	41	41
Conventional	75	70	67	67	66	66

Discussion

Conventional orthotopic liver transplantation requires crossclamping of the inferior vena cava, reducing venous return and resulting in decreased cardiac output and systolic blood pressure and increased systemic vascular resistance (16-18). These changes are poorly tolerated in hemodynamic unstable recipients and may contribute to renal impairment. The introduction of veno-venous bypass by Shaw in 1984 (2) improved the results of adult liver transplantation as it corrected hemodynamic and renal disturbances and decreased intra operative blood loss. The use of the piggyback technique for orthotopic liver transplantation in which the retrohepatic vena cava is preserved, was first mentioned by Calne in 1968 (19) and fully described by Tzakis et al. in 1989 (20). In these first cases the hepatic veins were crossclamped and a veno-venous bypass was used in adult recipients. The concept of side clamping of the retrohepatic vena cava, introduced by Belghiti in 1992 (7), allowes preservation of caval blood flow during the hepatectomy phase and overcame the need for veno-venous bypass, which was associated with complications including hypothermia and embolism (5,6). Since then, (modified) piggyback techniques have been compared to the

conventional orthotopic liver transplantation technique by several authors (10-13, 21-24), reporting reduced operative time, blood loss, transfusion need and hospitalisation. So far no survival benefit has been established, but in these retrospective studies patients were not matched for other variables affecting outcome. In the prospective, but not randomised study by Hosein Shokouk-Amiri et al. (12), outcome in both groups was excellent and no differences could be established.

In the present study we compared our results after introduction of a modified piggyback technique to a matched historical group of patients transplanted with the conventional technique. This necessitates careful interpretation of the results, as selection or time bias may be present. To minimise these effects, control patients were selected from a recent time period, in which the surgical team and surgical techniques were not changed in our hospital. Donor and recipient characteristics contributing to graft and patient survival according to the European Liver Transplantation Registry were analysed and equally distributed in both groups. In accordance with most previous studies we found a significant decrease in intra-operative blood loss and transfusion requirements due to absence of retrocaval dissection and caval encirclement. The observed survival benefit of the piggyback technique can not easily be explained as we found no differences in postoperative course between both groups, except for the incidence of severe infections. The decreased blood loss and blood component transfusion may be directly responsible for the improved survival, as these factors were proven to increase graft and patient survival (25-27). Finally the reduction of the warm ischaemic period with more than 30 minutes may contribute to graft quality and patient survival (27-28). In summary, the modified piggyback technique could be used in all patients without use of a veno-venous bypass. It reduced the warm ischaemia time, intra-operative blood loss and the need for transfusion of blood components. In our series these factors seemed to improve patient and graft survival at 6 months after liver transplantation.

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Orthotopic partial liver transplantation:

Strategies to overcome donor shortage

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Chapter 10

Abstract

Since the introduction of the split liver transplantation procedure now 15 years ago a variety of partial liver transplantations have been developed. The earliest form consisted of a reduction of a whole liver graft to the left lateral segment or the left liver lobe, small enough to transplant to a young child. The rest of the liver was discarded. This method solved part of the great need of liver grafts for children but as the remaining part of the liver was lost for transplantation, the method was in fact detrimental for adults on the waiting list.

Further surgical development resulted in splitting of the liver *ex-vivo* into two transplantable partial grafts, the left part to a child and the right lobe to an adult. The procedure was successfully introduced but the complicated logistics resulted in prolonged cold ischaemia times of the grafts. In order to keep the cold ischaemia time as short as possible Rogiers *et al.* developed the *in-situ* split liver technique in which the liver was split in the post-mortem donor. Refinement of this operation led to results, which were superior to the *ex-vivo* method. Moreover, it opened the gate to living donor liver transplantation. The first successful procedure was performed from a mother to a child who received the mother's left liver segment. The introduction resulted in a decrease over the years on the paediatric waiting list to almost zero. As the demand for organs increases every year and the numbers of donors are constant in western countries, the right lobe living donor liver transplantation for adults was introduced.

Introduction of all forms of partial liver transplantation has relieved the pressure on waiting lists, especially for children but also for adults. There is however serious concern of the well being in terms of morbidity and mortality of the living donor donation procedure.

Introduction

World-wide shortage of donor livers was the main obstacle for further expansion of liver transplant programs during the last decade. During this period the waiting lists for adults showed a steady increase all over the world but the death rate on the waiting list for children increased at an alarming rate. To solve this problem several alternative techniques of partial liver transplantation were developed.

In the first split liver techniques a donor liver was reduced mostly to the left lateral segment, the segments 2 and 3 or to the left lobe, the segments 2, 3 and 4. As most attention and care was paid to the graft and as all vascular and biliary structures were kept to the graft including the vena cava, the right part of the liver could not be used as a liver graft. This first important step forward over the years eventually has led to transplantation of

living donor transplantation of the right liver lobe to an adult recipient in a high urgent situation.

REDUCED SIZE LIVER TRANSPLANTATION.

The first reduced size liver transplantation (RSLT) was performed by Bismuth and Houssin in 1984 (1). They transplanted a left lateral liver segment to a child. In their consecutive series of paediatric liver transplantations, morbidity and mortality did not differ from the early results of whole liver transplantation at that time in the Paris program. Emond and Broelsch reported larger series of RSLT in children and, with the refinement of surgical technique and better patient selection, the results gradually improved. Initially RSLT was applied in desperate situations only and complications as hepatic artery thrombosis and portal vein thrombosis lead to inferior results. With increasing experience patient and graft survival approached 70% at one year and these results were comparable to the results of whole liver transplantation in children (2,3). Reduced size liver transplantation significantly reduced the number of children dying on the waiting list.

SPLIT LIVER TRANSPLANTATION.

Post mortem ex vivo SLT

Reduced size liver transplantation alleviated the mortality on the waiting list for children but its introduction was detrimental for the waiting list of adults as only a small part of a whole liver was transplanted to a child and the remaining part was discarded. Realising this, further attempts were made to preserve both parts for transplantation. This requested a new approach and a meticulous dissection technique during preparation on the backtable.

Already in 1957 the French pathologist Couinaud proposed a new concept of functional division of the liver in 8 segments, according to their portal and arterial blood supply and bile drainage. It took however until 1988 before this knowledge could be applied by Pichlmayr to transplant one donor organ to two recipients (4). The donor liver was divided in such a way that the left part, the segments 2 and 3 without the caval vein could be transplanted successfully into a child and the right part, the segments 1,4,5-8, into an adult. Common bile duct and common hepatic artery remained with the left part of the liver and the portal vein with the right one. In the recipient of the left part of the liver the vena cava was preserved and anastomosed with the left hepatic vein, the other anastomoses were carried out in the typical way. In the recipient of the right part of the liver the right hepatic artery was anastomosed with the recipient's common hepatic artery using a saphenous interponate.

Two separate intrahepatic bile ducts were anastomosed with a Roux-en-Y loop of the jejunum. The other anastomoses were carried out in the typical way. Shortly afterwards the first emergency split liver transplantation in two patients using one donor was reported.

Using the same technique as Pichlmayr, Bismuth et al. grafted two patients with fulminant hepatitis when only one donor was available. Both recipients recovered from coma and regained normal liver function. However, both patients died due to causes not directly related to the transplantation, one from multiple organ failure and one from diffuse cytomegaly virus infection on the 20^{th} and 45^{th} postoperative day respectively (5).

Especially in the early phase of this new development the complication rate regarding thrombosis of the small hepatic artery was higher than in whole liver transplantation. Stevens *et al.* reviewed the early Chicago experience and concluded that the use of a cadaveric left lobe or left lateral segment graft and an aortic arterial anastomosis were less prone to hepatic artery thrombosis than right sided grafts and an arterial anastomosis on the acceptor hepatic artery or celiac axis (6). Biliary leakage and obstruction were the most common complication but were similar in reduced size and full size grafts (7).

The European experience was reviewed by de Ville de Goyet (8). The European Split Liver Registry included the early results of 9 European centres over a 5 year period from 1988 to 1993. From 50 donor livers, 100 grafts were prepared, 2 grafts were discarded and the other 98 were transplanted in 53 children, two times in 2 children and 43 adults.63 grafts were transplanted in an emergency situation. Portal vein thrombosis, hepatic artery thrombosis, biliary complications and retransplantation rate were 4%, 11.5%, 18.7%, and 18.7%, respectively. Most of these complications were unrelated to the technique itself. Actual 6-month graft survival of elective and urgent orthotopic transplants was 88.9% and 61.3% in children, and 72.2% and 55.6% in adults. Actual 6-month patient survival for similar groupings was 88.9% and 61.1%, and 80% and 67.7%, respectively. These results were comparable to the results of whole liver transplantation at the time.

One of the major drawbacks in partial liver transplantation is the long cold ischaemia time due to the logistics around the ex-vivo splitting procedure on the back table which has to be performed in the donor hospital or in the transplant centre. The procedure itself takes 2 to 2.5 hr. in experienced hands and there is a possibility of warming up during the back table procedure. There are some reports of early graft dysfunction and post transplant complications due to the cutting edge of a right hemi-liver graft, which were attributed solely to the partial aspect of the transplantation. To overcome the logistic as well as the functional

problems of the ex/vivo procedure *Rogiers et al.* developed the in-situ liver splitting operation.

Post mortem in-vivo SLT

In the in-situ split liver operation the liver is split in the post mortem donor. The procedure is demanding especially during a multi organ donation. It has to be performed with the greatest surgical skill under constant perfusion of the liver preventing the Pringle manoeuvre in which the vascular structures in the hepato-duodenal ligament are clamped in order to prevent blood loss but resulting in warm ischaemia. Rogiers reported the first case in 1995 (9) and analysed their first in-situ split liver procedures one year later. The 14 split livers resulted in 14 transplanted patients with a 6 months patient and graft survival of 92.8% and 85.7% Biliary complications were absent. Postoperative courses were mostly uneventful and characterised by lower peak transaminases compared to the standard techniques (10).

These extremely good early results were superior to the 19 ex-vivo split liver transplantations performed during the same time period. It rendered graft reduction alone obsolete and opened a donor pool for adults to receive right lobes safely.

Goss *et al.* procured 15 in-situ split liver grafts, which resulted in 28 liver transplants. In-situ splitting of selected livers from hemodynamically stable post mortem donors was performed at the donor hospital without any additional work up or equipment being needed. The procedure took an extra 1 to 1,5 hour of donor hospital operating room time and resulted in 14 left lateral segments, the segments 2 and 3, and in 14 right trisegmental grafts, the segments 1 and 4-8. The 1-year actuarial patient and graft survivals were 92% and 86%. The 1-year survival of patients who received a left lateral graft was 100% (11).

From the same centre, Ghobrial analysed the predictors of survival after in-vivo split liver transplantation. During a 3.5 yr.-period, 55 right and 55 left lateral in vivo split grafts were transplanted in 102 paediatric and adult recipients. Overall survival rates were not significantly different from those of patients who received a whole organ transplant at the same median follow up time. Survival of left lateral segment recipients was 76% versus 80% receiving a trisegmental graft. 50 of 102 patients were high-risk urgent recipients and this group had a significantly lower survival rate than that of non-urgent patients. Multivariate logistic regression analysis identified UNOS status and length of donor hospital stay as independent predictors of survival (12).

After and simultaneously with this pioneering work other groups introduced the ex-vivo and the in-situ split liver techniques in their liver transplantation programs and published

equal results. This virtually solved the problem of children dying on the waiting list (13-15). Moreover, the in-situ split liver technique opened the possibility of living donor liver donation, in the beginning the transplantation of left lateral lobes for pedantic transplantation but in a later phase, transplantation of right lobes to adults.

LIVING DONOR LIVER TRANSPLANTATION

The first successful liver transplantation from a living donor to her son was performed in Australia and reported in 1990 (16). The success of the procedure had a major impact on liver transplantation especially in countries in which post mortem organ transplantation was not available or rarely performed. The first series, of 20 children, was published by the Chicago group one year later (17). All children were less than 2 years old or weighed less than 15 kg. Volunteer related donors were selected after medical and psychiatric evaluations, and the suitability of the donor liver was established by functional and radiological criteria. The donor group for the initial 20 LDLT procedures comprised 12 mothers, 7 fathers and 1grandmother. In addition one father and one uncle, who was an identical twin to the recipients father, who did not qualify for anatomic reasons, were used in repeat LDRT. All donors survived and were in normal health 3 and 18 month after donation. Nineteen infants received LRT as first grafts and one as a second graft. Four patients underwent retransplantation, all for hepatic artery thrombosis. All other complications were in the first 3 transplantations during which a full left liver graft was used, including segment 4. After a median hospital stay of 27 days 17 of the recipients were alive 3 to 8 months after LDRT.

As liver transplantation from brain death donors has not yet been accepted in Asia and especially in Japan, the only alternative method at present is transplantation from a living donor. One of the first reports comes from Makuuchi et al. who transplanted 16 children with mainly biliary atresia using 16 living donated partial livers. These early results were unsatisfactory as 5 of these children subsequently died. Much care was taken to ensure the safety of the donor. A preoperative banking of the donor's own blood and plasma was performed. In addition, selective vascular occlusion minimised blood loss of the donor during resection. Using intra-operative Doppler ultrasound, the optimal position of the graft for maximal vascularisation could be established. They concluded that living related liver transplantation is an acceptable procedure for small children but that post mortem donation is absolutely necessary for adult recipients (18).

One of the mayor causes of morbidity and mortality in LDLT in small children is thrombosis of the hepatic artery and this complication was solved to a great extend by the introduction of microvascular surgery into the reconstruction of the hepatic artery. Mori et al. from the Tanaka group from Kyoto introduced this modality at an early stage in their paediatric living related liver transplantation program. The time required for completion of the microvascular anastomosis was not different from that in the loupe magnifying assisted anastomosis. More important, there were no patients with hepatic artery thrombosis after introduction of this technique (19). Between 1989 and 1994 more than 150 living related liver transplantations were performed world wide, mostly in the United States and in Japan. The first series of living related liver transplantation in Europe came from Hamburg (20). Twenty LDLT were performed in children transplanting the left liver lateral segment in the majority of cases and with an overall patient survival of 85%. For the 13 patients who underwent elective transplantation the survival was 100%. Technical complications included one arterial thrombosis necessitating re-transplantation and five bile leaks requiring surgical revision. All procurements resulted in excellent graft function. No intraoperative complications occurred, and no re-operations were necessary. No heterologous blood transfusion was given. In 2 patients an incisional hernia developed after wound infection.

Only a small proportion of the potential living donors-to-be donates eventually. In a retrospective analysis of 75 identified potential donors for 38 paediatric candidates with an age range from 17 days to 14 years the donor process was evaluated. Twenty three percent of potential donors declined evaluation. Of the 75 donors only 10 (13%) were found to be acceptable for donation. The leading causes for donor declination were significant medical history (23%), ABO incompatibility (23%), and psychosocial history (20%). Of the 38 recipient candidates, 9 (23%) were offered living-donor liver transplantation. These results suggest that, especially in the early phase of LDLT, donor criteria were the limiting factor in the application (21). When the post operative donor liver function is analysed, donation of a left lobe (the segments 2,3 and 4 with the middle hepatic vein) leads to significantly higher post-operative liver enzyme levels in the donor than donation of a left lateral segment (the segments 2 and 3 without the middle hepatic vein) (22).

This is important, as donor safety is the most prominent ethical issue in living donor transplantation of solid organs. In the literature there are scarce long-term follow-up data available about the medical, social and psychological complications of living donors, but their long-term well being is all-important and this should be further established.

ADULT LIVING DONOR LIVER TRANSPLANTATION

Since its introduction in 1988 LDLT using the left lateral liver segment has become the standard procedure for children with end stage liver disease. In larger children and in adults a left sided liver graft will not require the metabolic needs of the recipient as the amount of transplanted liver tissue will be less than 40-50% of the original liver. The first successful report of a right lobe LDLT was by Yamaoka using the right lobe in a 9-year-old with biliary atresia. The decision to use the right lobe was made intraoperatively when the vascular anatomy of the left lobe was found incompatible with transplantation (23). Since then large experience has been developed in Hong Kong and in Kyoto.

The shortage of livers for adult transplantation prompted transplant centres to investigate right lobe LDLT as left lateral segmentectomies or left lobectomies provided insufficient liver mass for an average size adult patient. Wachs et al. described right lobe LDLT in two adult patients with chronic liver disease. The first recipient was a 44 year old female with end stage liver disease due to hepatitis C infection. Her deteriorating condition prompted discussion regarding the possibility of living donation. Her sister was ABO compatible and was evaluated as a potential donor. Her medical, psychiatric and social evaluation revealed no contraindication for living donation. The donor operation took 3,5 hr. The LDLT was uneventful, as was the postoperative course in both donor and recipient.
The second adult recipient of a right lobe LDLT was a 50-year-old female with Child's B cirrhosis and recurrent sepsis due to primary sclerosing cholangitis. Recurrent bouts of cholangitis led to consideration of living donor liver transplantation. Her 31-year-old son was ABO compatible and wished to donate. Both donor and recipient operation went well and even more important, both donors stated that they would make the decision to donate again (24).

Marcos et al reported early results of right lobe liver transplantation. Twenty-five right lobe LDLT´s were performed between adults, with no significant complications in the donors. Recipient and graft survival was 88%, with 3 recipient death due to uncontrolled sepsis in high-risk patients, all with functioning grafts. They stressed the value of the intra operative cholangiogram of the donor as it avoided unnecessary dissection near the common bile duct and defined the number of bile ducts draining the right lobe. Intra operative ultrasound was used to map the course of the middle hepatic vein and its relationship to the right hepatic vein. Accessory veins contributing to the outflow of the right lobe to the vena cava were identified and preserved (25).

As cadaveric liver donors are scarce in Hong Kong, Lo *et al.* evaluated the use of living donors in high urgency patients. 49 patients in intensive care with acute or chronic liver failure were placed on the high urgency list for liver transplantation. Family members were not solicited for living donation and the initiation for living donation was based on the donor's voluntary intent. In 25 of 49 patients (51%) no family member volunteered as living donor, 23 died awaiting donor organs, and 2 received a cadaveric organ. The other 24 patients had 36 family members who volunteered as living donors. During the evaluation of the donors another 2 patients received a cadaver graft. LDLT was not performed in 9 patients because of recipient contraindications. Eight of these patients died and one received a cadaveric liver transplant. The remaining 13 (27%) patients received grafts from living donors. Eleven of these 13 (85%) who underwent LDLT survived. The authors concluded that emergency liver transplantation from living donors can be applied to high-urgency adult patients (26).

The Kyoto group of Tanaka confirmed these initial experiences in 26 patients. Although 6 patients died after transplantation only one was related to graft dysfunction due to an outflow block while the other death were due to poor pre-transplant condition (27). Meticulous surgical technique and attention to detail is all important in LDLT and much attention is given to this topic in the literature. With growing experience the results further improved (28-30).

With the growing numbers there is real concern regarding the safety of the donor (31). Until now there are in the literature two donors who died after living liver donation but the real figure, after more than 1000 living donor liver transplantations is expected to be higher judged by experts (32,33). Transplant programs are reluctant to publish a donor death but the exact figures should be available to the clinician but above all the donor-to-be should be given detailed information regarding morbidity and mortality before his or her decision is made (34).

CONCLUSION

Since the first partial liver transplantation 15 years ago several initiatives have been undertaken to seek alternatives for conventional post-mortem liver transplantation. The post-mortem reduced size liver transplantation for children was favourable for the paediatric waiting list but, as the major part of the liver was discarded, the procedure was in fact detrimental for adult patients waiting for an organ. The split liver technique in which the liver was *ex-vivo* divided in 2 transplantable hemi-livers solved part of this problem. Negative effects however were a longer cold ischaemia time due to difficult logistics resulting in more

post operative complications and, not unimportant, only a small percentage of donor livers were suitable for this procedure. After introduction of the *in-situ* technique, the donor procedure became longer but the results improved due to more surgical experience and to shorter cold ischaemia time. The *in-situ* technique opened the possibility of living donor liver transplantation. The first experience gained in paediatric patients who received a left lateral segment of, in most cases, mother or father and in selected cases from other family members or even unrelated donors. The excellent results in experienced centres led to the first right lobe living donor liver transplantation, a decision taken during the donation procedure because of surgical anatomical reasons. The procedure was quickly introduced for elective but also for acute adult liver transplantation. There is concern about safety of the living donor and a world wide registry of morbidity and mortality of living donors would give the key information needed to take a well informed decision regarding live donor donation.

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Summary and discussion

Chapter 11

Introduction

Four decades ago, patients with acute hepatitis, chronic hepatitis and cirrhosis were treated conservatively. If cirrhosis progressed to end-stage liver failure, only supportive treatment could be provided. Accordingly, attention was focussed at the complications of end-stage liver disease like ascites, encephalopathy and variceal bleeding. The provision of a new liver, with or without removal of the diseased liver was considered the ultimate, yet impossible therapeutic step.

In nearly forty years, liver transplantation has become an effective and successful therapeutic modality in the treatment of end-stage liver diseases with an one-year patient survival of more than 80%. Due to the success of liver transplantation however, new challenges are brought to optimise the existing procedures and expand the use of donor organs.

Chapter 1 of this thesis is focussed on the current problems in experimental auxiliary liver transplantation and clinical non-auxiliary liver transplantation. The aims and outline of the thesis are described here.

In **chapter 2** the history and development of the auxiliary liver transplantation is described. Since the first experiments both introduction of an extra organ in the abdominal cavity and whole organ replacement have been studied. The idea of auxiliary transplantation of the liver was primarily born from the technical difficulties to remove the diseased liver in a safe way. Although experimental and clinical successes were reported, the consequences of increased abdominal mass caused complications to the donor liver. Increased abdominal pressure caused insufficient portal inflow and hepatic outflow with graft dysfunction as a common result. When resection of the cirrhotic liver became technical feasible with progression in surgical skill and the use of technical innovations like the venous bypass, the interest in auxiliary liver transplantation decreased. However, the concept of preserving part of the liver remained interesting for selected patients. With the possibility to devide post-mortem livers into two pieces (1), transplantation of an auxiliary graft in an orthotopic position came into focus and interest for auxiliary transplantation is now growing again since the early nineties.

Chapter 3 addresses the history of and improvements in surgical technique, patient monitoring, post-operative care and immunosuppressive management of the orthotopic liver transplantation procedure.

Optimisation of portal flow in experimental auxiliary liver transplantation

In **chapter 4** we analysed the technical requirements for long-term metabolic correction of an inborn error of hepatic metabolism provided by a *heterotopically* placed graft.
It has become apparent from previous experiments that a liver graft should receive both arterial and portal blood flow to prevent atrophy. In case of an inborn error of metabolism, portal hypertension is mostly absent and finding a balance in portal blood distribution between native liver and graft is complicated. The effect of four different techniques to supply the graft with portal blood on the post-operative metabolic correction was studied in the Dalmatian coach dog, which has a metabolic defect leading to hyperuricosuria and hypoallantoinuria. Separate from the initial results of our study (2), no experimental studies have been published which investigate the requirements for long-term graft function in correction of an inborn error.

Metabolic correction increased in all groups after transplantation, but remained significantly better in the *split-flow* and *ligation* groups. These results are in line with the findings of Lilly in 1971 and Ducerf in 1995, who described superior graft function after split portal inflow in a pig model without portal hypertension (3, 4). Interestingly, Lilly described graft atrophy when the graft was perfused with systemic venous blood, but regeneration if pancreatico-splanchnic portal blood was used.

We found no impact of the source of the portal blood on the regeneration capacity of the liver graft during the experiment and this contradicts the hypothesis of need for hepatotrophic growth factors in pancreatico-duodenal venous blood. The native livers in the ligation groups, which were deprived of portal blood, did show significant atrophy compared to the native livers in the free-flow group, but there was no significant difference with the banding and split-flow group. This confirms the hypothesis of autoregulation towards a required functional hepatic mass of about 3% of the total body weight of the dog, which was described previously by Terpstra (5).

In **chapter 5** the experiment was modified with the graft in the orthotopic position, after resection of the left lateral and medial segments of the recipient. In this experiment there were three groups of portal blood inflow: ligation of the portal vein to the native liver, banding of the portal vein to the native liver and a free-flow group. Separate portal blood inflow for the native liver and graft was technically impossible in this model, because the grafts were harvested as living donor grafts without large vascular pedicles from the donor. Portal and arterial flow was quantitated using Doppler ultrasonography before resection of the left lateral segments in the recipient, after reperfusion of the donor lateral segments and after

intervention in the portal blood flow. Until then, only one attempt was made to investigate the distribution of portal and arterial blood flow between graft and host liver after APOLT (6), but in this study no interventions in the portal flow pattern were made.

After reperfusion the blood flow through the common portal vein decreased significantly to 73% of the initial portal flow, probably caused by the size of the graft, which comprised only 40% of the liver weight. Also a "small for size" phenomenon was present in the ligation group, where the inflow was resticted by the hepatic venous outflow.

Without intervention in the portal blood distribution, inflow towards the graft remained marginal. Ligation of the portal vein to the native liver resulted in the best graft perfusion, but adverse effect was the development of portal hypertension with periportal haemorrage and necrosis. Banding of the right portal vein restored the flow in the left portal vein in terms of percentage of the total portal blood flow.

We conclude that diversion of the portal flow is necessary for adequate graft perfusion in APOLT and that ultrasound-guided banding of the native portal vein is the preferable technique to achieve this.

In **chapter 6** the consequences of the intervention in the portal inflow on graft function were assessed. Significant metabolic correction was initially seen in the banding and free-flow group, but not achieved in the ligation group. In this group, two dogs died of bleeding from the splenic area. Histo-pathology confirmed massive portal and sinusoidal congestion and focal hemorrhagic necrosis in these dogs. At autopsy no evidence could be found for stenosis in the hepatic vein anastomosis. We assume that the portal inflow exceeded the hepatic outflow in this group and resulted in a relative venous outflow obstruction. In an experimental study model without portal hypertension, Yabe et al. described a mortality of 2/7 dogs before sacrifice at day 5 after acute ligation of the portal vein of the native liver. Histologic findings were however not given (7). An explanation for the difference with our own results in the *heterotopic* experiment may be that in the present study the total portal flow was forced into a liver graft comprising only about 40% of the total liver weight, where in the previous experiments a 70% liver graft was used. According to previous reports, we found no evidence that ligation of the native portal vein had detrimental effects on the native liver supplied by arterial flow only.

In the free-flow group, allowing free distribution of portal inflow between graft and native liver resulted in loss of metabolic correction within 2 weeks. Hypo-perfusion of the graft led to portal thrombosis in 3 of the 7 dogs and mortality was high due to sepsis from necrotic grafts, in accordance with the results of our heterotopic experiment.

Banding of the native portal vein provided metabolic correction until the end of the experiment, although metabolic correction decreased in time, which was comparable to the results that we found in our *heterotopic* partial liver transplantation model. Loss of metabolic function during follow-up was accompanied by portal thrombosis 4 of the 11 dogs. It might be explained by increased vascular resistance in the graft secondary to infection and rejection. Regarding the problem of late portal thrombosis, a gradual occlusion of portal vein to the native liver, as proposed by Hirayama et al., may be an attractive solution (8). This assures optimal graft function without the risk of portal hypertension.

In conclusion, an adequate portal flow to the graft is required for sustained graft function. Acute total ligation of the host portal vein led to portal hypertension with congestion of the parenchyma and early death in our model. Without intervention, the portal flow is preferentially to the native liver, leading to poor perfusion and early loss of metabolic function of the graft. Partial banding, aimed at restoration of the pre-transplantation flow distribution, gives the best metabolic correction.

Optimisation of clinical orthotopic liver transplantation

Since the introduction of liver transplantation, the one-year patient survival improved from approximately 30% to better than 80%. Although excellent results can be achieved, there are still some subjects that need to be elucidated. In part III of the thesis, three current problems in orthotopic liver transplantation are addressed.

Primary graft dysfunction

In **chapter 7** we investigated the role of nitric oxide in the pathogenesis of primary graft dysfunction after liver transplantation (PGDF). Although nitric oxide has received much attention and became "molecule of the year" in 1992 (9), it's role in ischaemia/reperfusion injury remains unclear. The vasodilatation that results from produced nitric oxide may be beneficial in ischaemic parts of the liver, but recently also a direct toxic effect of nitric oxide derived radicals on the mitochondrial function has been reported.

In this study we investigated the energy content in hepatocytes during preservation and reperfusion and assessed the role of the nitric oxide derived peroxynitrite radical in mitochondrial dysfunction.

We found higher energy levels at the end of the cold preservation in patients with PGDF. After reperfusion, this pattern reversed and control patients showed ATP regeneration, whereas the ATP and TAN levels in patients with PGDF decreased.

Other investigators described lower or equal TAN and ATP levels in PGDF patients at the end of the cold ischaemic period, compared to their control patients. However, none of these studies did follow the course of the adenine nucleotides content during the warm ischemic period, which makes it difficult to interpret the results.

We hypothesise that the high energy content in our PGDF patients is caused by poor perfusion of the donor liver during organ donation. Optimal rapid hypothermic perfusion leads to cessation of all metabolic processes and enzymatic activity, but poor perfusion gives the donor liver time to induce oxidative phosphorylation, glycogenolysis and glycolysis in a salvage attempt to preserve ATP levels for proton pumping and maintenance of membrane integrity. The induction of oxidative phosphorylation to form ATP and activation of glucose output in patients with PGDF also prevents the degradation of high energy phosphates to adenosine. As adenosine has a strong vasodilative effect, it may be beneficial at reperfusion of the donor liver and omission of this substance from preservation solutions has deleterious effects.

Electron microscopy confirmed the functional impairment of the mitochondria as soon as 1 hour after reperfusion in patients with PGDF. In the same biopsies, nitrotyrosine was used as an indicator of peroxynitrite mediated damage. The level of nitration of hepatocytes and bile duct endothelium was high already at the end of the cold ischemic period and remained at that high level during warm ischemia and after reperfusion. Staining for iNOS revealed hepatocytes as the main source of NO, which was also present at the end of the cold ischemic period. Since the necessary NO for tyrosine nitration can not be produced by iNOS during cold ischemia itself, this indicates that the cellular proteins of the donor liver have been nitrated before the donation procedure

In conclusion, our study shows that PGDF related mitochondrial dysfunction does not originate from the cold or warm ischemic period, but probably is caused earlier, during the organ donation procedure. Apparently, the high TAN and ATP levels at the end of the cold ischemic period can be used as an early marker of primary graft dysfunction. Although experimental studies indicated that occurrence of nitration of mitochondria is detrimental, the lack of any difference in nitrotyrosine formation between patient with or without PGDF makes nitration of mitochondria a unlikely cause of clinical primary graft dysfunction.

Hyperfibrinolysis and blood loss

In **chapter 8** the problems of impaired coagulation and blood loss during the liver transplantation procedure were investigated. Patients with preceding liver disease often have profound disturbances in the haemostatic system, including a low platelet count, low levels of clotting factors and increased levels of fibrinogen degradation products due to dissiminated intravascular coagulation and subsequent fibrinolysis. The blood loss during the procedure has to be minimalised as recent publications indicate that perioperative blood loss still affects postoperative outcome.

To minimise the risk of viral transmission during blood component substitution therapy, solvent/detergent (SD) virus-inactivated plasma (ESDEP) was introduced in 1996 in our hospital. Since it's introduction however, hyperfibrinolysis occurred more frequent in patients undergoing liver transplantation than before, when fresh frozen plasma (FFP) was used.

SD treatment of fresh frozen plasma is an effective method to reduce lipid coated viruses, but it has been reported that because of the SD process the concentration of FVIII, protein S and α2-antiplasmin is reduced. To clarify this increased incidence, intraoperative plasma samples of patients treated with FFP or ESDEP were analysed in a retrospective observational study.

Hyperfibrinolysis occurred about 3 times more in the anhepatic phase and after reperfusion in patients who received ESDEP than in patients treated with FFP. Determination of α2-antiplasmin concentrations in ESDEP showed decreased levels in accordance with the results presented in literature. In 47% of the patients treated with ESDEP hyperfibrinolytic episodes did reoccur, despite adequate treatment of the first hyperfibrinolytic episode, proven by normalisation of the thromboelastograph recordings. We think that this effect is caused by the large plasma exchanges in liver transplantation, which dilute the level of α2-antiplasmin in the patient to the level of ESDEP itself.

Our results are in contrast with the study of Freeman (10), who found no differences in coagulation parameters between patients receiving FFP or SD treated plasma during liver transplantation. α2-Antiplasmin levels were however not determined in their study and hyperfibrinolytic problems were not mentioned in the article. This may well be explained by the standard prophylactic use of anti-fibrinolytic drugs in the participating centers in the United Kingdom.

In summary we feel that the use of solvent/detergent virus inactivated plasma is of increasing importance in the prevention of HIV and Hepatitis C virus transmission. Clinicians

should however be aware that infusion of solvent/detergent treated plasma in patients needing massive transfusion may lead to insufficient levels of α2-antiplasmin and secondary hyperfibrinolysis. This hyperfibrinolysis can not be corrected with infusion of more solvent/detergent plasma, but should be treated with antifibrinolytic drugs like aprotinine or tranexamic acid. Considering the significant higher blood loss in patients with hyperfibrinolysis in our study, routine administration of antifibrinolytic drugs using solvent/detergent virus-inactivated plasma in patients with massive transfusion requirements is advised (11).

Piggyback technique

In **chapter 9** the technique of liver transplantation with preservation of the vena cava is described. Preservation of the vena cava obviates the use of a veno-venous bypass and reduces blood loss from the retroperitoneal cavity. It has been reported to reduce warm ischaemic period, blood loss and transfusion requirements. Since then, (modified) piggyback techniques have been compared to the conventional orthotopic liver transplantation technique by several authors, reporting reduced operative time, blood loss, transfusion need and hospitalisation.

So far no survival benefit has been established, but in these retrospective studies patients were not matched for other variables affecting outcome. In a prospective, but not randomised study by Hosein Shokouk-Amiri (12), outcome in both groups was excellent and no differences could be established (13). A retrospective analysis was undertaken to evaluate the results of the implementation of this technique in our institute.

In accordance with most previous studies we found a significant decrease in intra-operative blood loss and transfusion requirements due to absence of retrocaval dissection and caval encirclement. We also found a survival benefit at 6 months after liver transplantation with the caval preserving technique. This benefit can be attributed to a reduction in severe infections, a shorter warm ischaemic period and decreased blood loss during the procedure, which was proven to be a important factor for graft and patient survival before (14, 15).

In summary the modified piggyback technique can be used in all patients without use of a veno-venous bypass. In our series these factors improved patient and graft survival and the piggyback technique has become the preferred technique for liver transplantation in our transplant centre.

Strategies to overcome donor shortage

There is a reverse to every medal and the success of liver transplantation caused an increasing number of patients, waiting for a donor organ.

In the Netherlands the number of donor livers hat is retrieved is stable since 1994 with about one hundred per year. The number of patients entering the waiting list is however doubled from 85 in 1994 to 159 in 2000 (16). In the United States there are now three times more patients on the waiting list for a liver transplantation than there are livers transplanted per year. Especially in paediatric liver transplantation, there was an alarming shortage in suitable liver donors for the growing population of transplantable patients.

In **chapter 10** new solutions to solve the problem of donor shortage are described. In 1989 the first liver from a non-heartbeating liver donor was used for transplantation after initial success with non-heartbeating kidney donation (17). Results of non-heartbeating liver transplantation were however inferior to those of post-mortem heartbeating donation (18, 19).

Therefore another solution was sought to solve the problem of donor shortage. With the introduction of the split liver transplantation procedure now 15 years ago, a variety of partial liver transplantations were developed to split the donor liver and transplant the parts into two recipiens.

The earliest form consisted of a reduction of a whole liver graft to the left lateral segment or the left liver lobe, small enough to transplant to a young child. The rest of the liver was discarded. This method solved part of the great need of liver grafts for children but as the remaining part of the liver was lost for transplantation, the method was in fact detrimental for adults on the waiting list. Further surgical development resulted in splitting of the liver on the bench table into two transplantable partial grafts, the left part to a child and the right lobe to an adult. The procedure was successfully introduced but the complicated logistics resulted in prolonged cold ischaemia times of the grafts. In order to keep the cold ischaemia time as short as possible, Rogiers *et al.* developed the *in-situ* split liver technique in which the liver was split in the post-mortem donor.

Refinement of this operation led to results, which were superior to the bench table method. Moreover, it opened the gate to living donor liver transplantation. The first successful procedure was performed from a mother to a child who received the mothers left liver segment. The introduction resulted in a decrease over the years on the paediatric waiting list to almost zero. As the demand for organs increases every year and the numbers of donors are constant in western countries, the right lobe living donor liver transplantation

for adults was introduced. Introduction of all forms of partial liver transplantation has relieved the pressure on waiting lists, especially for children but also for adults. There is however serious concern of the well being in terms of morbidity and mortality of the living donor donation procedure.

Conclusions of the thesis

1. Long-term metabolic correction can be provided by an auxiliary placed liver graft, but intervention in the portal blood flow is essential for graft survival in both heterotopically and orthotopically placed auxiliary liver grafts in absence of portal hypertension (chapter 4, 5 and 6).
2. Ligation of the portal vein to the native liver does not result in histological atrophy of the native liver, but only reduces the liver mass and is well tolerated if it does not produce portal hypertension (chapter 4 and 6).
3. Division of the portal blood flow between the two livers by ultrasound guided banding of the native portal vein is an accurate possibility (chapter 5 and 6). Banding based on elevated portal pressure in the graft portal vein did not guarantee long-term metabolic correction (chapter 4).
4. There is a striking difference in resistance to portal blood flow deprivation between the native liver and the liver graft. The native liver tolerates ligation of the portal vein well, while insufficient portal inflow of the graft immediately leads to necrosis of hepatocytes with fibrosis and atrophy (chapter 4 and 6).
5. Mitochondrial dysfunction in grafts with primary graft dysfunction is already present during the cold ischaemic period and is not caused by nitrosylation of mitochondrial membrane by nitric oxide derived peroxinitrite (chapter 7).
6. TNBP and Triton X-100 treated plasma (ESDEP®) is deficient for α2-antiplasmin and standard antifibrinolytic medication should be administered when ESDEP is used for large plasma volume substitution to minimise the risk of hyperfibrinolysis (chapter 8).
7. The implementation of a vena cava preserving technique for orthotopic liver transplantation decreased blood loss and transfusion requirements and increased 6-month graft and patient survival.
8. Partial liver transplantation succeeded in reducing the waiting list for paediatric liver transplantation to practically nil, but initially increased donor shortage for adult liver

transplantation. With split liver techniques two recipients can now be helped with one donor liver, without compromising one-year patient survival.

Future perspectives

The widening indications for liver transplantation and more liberal patient selection result in an increasing shortage of donor organs. At this moment, patients with liver cancer, who should be transplanted early, die on the waiting list because of low urgency status caused by their good functioning liver. The emerging technique to overcome this problem in the near future is living (related) donor liver transplantation, which gives equal results to post-mortem liver transplantation because of careful planning and short ischaemia periods. In the near future there will be attempts to split the donor liver during a laparoscopic procedure to minimise the extent of the operation for the living donor. The first steps are recently taken with sucessfull laparoscopic liver resection (20).

As the use of partial grafts becomes more widespread, the interest for auxiliary liver grafting in selected indications will be renewed.

Especially patients with inborn errors of metabolism and patients with acute liver failure will benefit from this development. In patients with inborn errors of metabolism the partial graft will provide metabolic correction, until gene therapy becomes available. If in the meanwhile the graft fails, it can electively be replaced with another graft. In these patients the portal flow has to be deviated away from the native liver to allow adequate portal blood inflow for the graft. The use of intra-operative ultrasound with Doppler sonography and computed resistive indices will allow accurate distribution of the portal flow between the two livers. Separate portal blood flow for liver graft and native liver seems to be the best guarantee for long-term metabolic correction. If separate blood flow is not possible and banding of the native portal vein is applied, the portal flow should be visualised non-invasively with ultrasound in the port-operative period to detect changes in the equilibrium due to infection or rejection.

Patients with acute liver failure will be bridged to recovery with an auxiliary liver graft as the processes that promote liver regeneration will be better understood and regeneration can be predicted more accurately. With increasing safety of living donor liver transplantation it will become ethically acceptable to propose this therapeutic option in an early stage of liver failure, when the prognosis is substantially better and long-term admission to an intensive care unit can be prevented.

When whole or partial livers are used for transplantation, the piggyback technique will become the standard procedure for vascular anastomosis. The piggyback technique allows

the use of vascular stapling devices, which are rapid, reliable and decrease anastomotic problems. Also the use of a lateral cavo-cavostomy obviates the use of a veno-venous bypass and even enables liver transplantation in haemodynamically instable patients. The standard use of antifibrinolytic therapy will decrease per-operative blood loss, while still allowing the use of virus safe plasma. The combination of the piggyback technique and routine antifibrinolytic therapy will decrease the impact of the liver transplantation and make it a less extraordinary surgical procedure.

To reduce the incidence of primary graft dysfunction of post-mortem donor livers, intensive research should be directed at the donor liver before and during harvesting. Our results indicate that changes leading to dysfunction are already present during the cold ischaemic period and probably not only result from the quality of the liver, but also from the procurement procedure. Haemodynamic and hormonal instability of the brain-dead liver donor, hypoperfusion during harvesting of the liver due to technical imperfection or bad communication between the organ procurement teams probably determine the faith of the graft before transplantation is started. In the pathogenesis of graft dysfunction activation of the Kupffercell seems to plays a key role. Therefore the behaviour of Kupffer cells during organ procurement and reperfusion is currently studied in the Laboratory of Experimental Surgery of the Erasmus University Rotterdam in an attempt to down regulate the activation of Kupffer cells.

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Samenvatting

Chapter 12

Introductie

Tot veertig jaar geleden werden patiënten met acute en chronische hepatitis en levercirrose conservatief behandeld. Wanneer levercirrose voortschreed naar leverinsufficiëntie, kon slechts een ondersteunende behandeling worden gegeven. Daarom was de therapie gericht op de behandeling van complicaties van het eindstadium van leverinsufficiëntie, zoals ascites, encephalopatie en bloeding van slokdarmvarices. Transplantatie van de lever, met of zonder de verwijdering van de zieke lever werd gezien als de uiteindelijke, maar op dat moment onhaalbare therapeutische oplossing.

Sindsdien is levertransplantatie in bijna veertig jaar echter een effectieve en succesvolle behandeling geworden van leverinsufficiëntie met een patiënt-overleving van meer dan 80% na één jaar. Het succes van de levertransplantatie heeft, door een toenemend tekort aan donororganen, echter tot nieuwe uitdagingen geleid.

Dit proefschrift beschrijft de aanpak van enkele problemen bij experimentele en klinische lever transplantatie om de bestaande procedures en het gebruik van donororganen te optimaliseren.

Hoofdstuk 1 van dit proefschrift is gericht op de thans bestaande problemen bij experimentele auxiliaire levertransplantatie en klinische niet-auxiliaire levertransplantatie. De doelstellingen en indeling van het proefschrift worden hier beschreven.

In **hoofdstuk 2** worden de geschiedenis en ontwikkeling van de auxiliaire levertransplantatie beschreven. Sinds de eerste experimenten zijn zowel de toevoeging van een extra lever in de buikholte (auxiliaire transplantatie) als totale vervanging van de lever bestudeerd. Het idee om een extra lever in de buik te plaatsen, in een niet-anatomische positie (heterotoop) kwam voort uit technische problemen om de zieke lever op een veilige manier te verwijderen. Hoewel experimentele en klinische resultaten werden gerapporteerd, veroorzaakte implantatie van een extra orgaan in de buik complicaties. Toegenomen druk in de buik veroorzaakte onvoldoende doorbloeding van de v. Portae (poortader) en afknikken van de levervenen met disfunctie van de donorlever als resultaat. Toen resectie van de cirrotische lever technisch mogelijk werd door verbeteringen in de chirurgische technieken en het gebruik van de veneuze bypass, verminderde de interesse voor de auxiliaire levertransplantatie. Toch blijft behoud van een deel van de lever interessant voor geselecteerde patiënten. Met de mogelijkheid om een donorlever te scheiden in twee gedeelten, werd transplantatie van een auxiliaire donorlever in de anatomische (orthotope) positie mogelijk, na resectie van een deel van de zieke lever. Sinds de jaren negentig groeit daarmee opnieuw de interesse in auxiliaire lever transplantatie.

Hoofdstuk 3 beschrijft de geschiedenis van de orthotope levertransplantatie en de verbeteringen in de chirurgische technieken, postoperatieve zorg en immunosuppressie-therapie.

Optimalisering van de bloedstroom door de v. Portae in experimentele auxiliaire levertransplantatie

In **hoofdstuk 4** worden de technische voorwaarden voor een langdurige correctie van een aangeboren stofwisselingsziekte van de lever met een heterotoop geplaatste transplantatielever bestudeerd. Het is reeds langer bekend dat de donorlever zowel van arterieel bloed als van veneus bloed uit de v. Portae moet worden voorzien om atrofie te voorkomen. Bij aangeboren stofwisselingsziekten bestaat er meestal geen portale hypertensie en het is daarom moeilijk om het bloed uit de v. Portae zo te verdelen tussen de eigen lever en de donorlever, dat er geen atrofie van de donorlever optreedt. Het effect van 4 verschillende technieken om de donorlever van bloed uit de v. Portae te voorzien werd daarom bestudeerd in een model met honden met een aangeboren stofwisselingsziekte.

De beste metabole correctie werd gezien in de groep met een gescheiden bloedtoevoer voor de eigen lever en donorlever en in de groep, waar de v. Portae-tak naar de eigen lever werd onderbonden, zodat al het portale bloed ten gunste kwam aan de donorlever. In de groep, waarin de bloedstroom naar de eigen lever kunstmatig werd vernauwd, bestond metabole correctie gedurende het hele experiment, maar nam de hoogte van deze correctie af na 70 dagen. Dit ging gepaard met trombose van de v. Portae in 4 van de 6 honden in deze groep. Vrije verdeling van de bloedstroom in de v. Portae leidde tot competitie tussen de eigen lever en de donorlever met een totaal verlies van metabole correctie na 3 maanden.

In onze studie werd geen aanwijzing gevonden dat de oorsprong van het veneuze bloed van belang is voor regeneratie van de donorlever. Dit pleit voor de hypothese die eerder door Terpstra werd geponeerd, dat er een autoregulatie bestaat naar een vereiste functionele levermassa van ongeveer 3% van het totale lichaamsgewicht van de hond.
Donorlevers die meer dan 15% activiteit opnamen tijdens nucleair onderzoek, vertoonden totale correctie van de stofwisselingsziekte, hetgeen in overeenstemming is met eerdere publicaties, waarbij transplantatie van ongeveer 12% van de totale levermassa nodig was voor correctie van ongeconjugeerde hyperbilirubinemie. De resultaten van deze studie geven aan dat auxiliaire heterotope levertransplantatie een goede langdurige correctie van een aangeboren stofwisselingsziekte kan verzorgen, wanneer een adequate

bloedvoorziening van de donorlever wordt gegarandeerd. De beste resultaten werden bereikt met een gescheiden bloedstroom voor de eigen lever en de donorlever.

In **hoofdstuk 5** werd dit experiment gemodificeerd herhaald met de donorlever in de orthotope positie, na resectie van de linker laterale en mediale segmenten van de ontvanger. Doordat het experiment werd uitgevoerd als levende-donor levertransplantatie was het technisch niet mogelijk om de groep met een separate bloedstroom voor de eigen lever en de donorlever te bestuderen. De linker v. Portae-tak van de donorlever werd geanastomoseerd op de vaatstomp van de linker v. Portae-tak van de geresecteerde leversegmenten. Tijdens het experiment werd de bloedstroom door de a. Hepatica en v. Portae van de eigen lever en de donorlever gemeten met Doppler-echografie.
Na reperfusie van de donorlever nam de doorbloeding van de gemeenschappelijke v. Portae af tot 73% van de initiële waarde vóór transplantatie, met name veroorzaakt door een afname in doorbloeding van v. Portae van de donorlever. Deze afname was opmerkelijk, aangezien de levende-donor procedure een optimale kwaliteit van de donorlever garandeert. Tijdens de levende-donor levertransplantatie kan slechts zo'n 40% van het totale levergewicht veilig worden afgestaan. De kleine afmeting van de donorlever die tijdens deze experimenten gebruikt werd kan daarom een beperkende factor zijn voor de doorbloeding. Ook kan de instroom gelimiteerd zijn door de beperkte uitstroom in de v. Hepatica, het zogenaamde "small for size" fenomeen.

In de groep met vrije verdeling van de bloedstroom in de v. Portae tussen de eigen lever en de donorlever bleef de doorbloeding van de donorlever marginaal. Kunstmatige vernauwing van de v. Portae naar de eigen lever herstelde de doorbloeding naar de donorlever. Totale onderbinding van de v. Portae naar de eigen lever gaf de beste perfusie van de donorlever. Ongewenste bijwerking hiervan was echter een verhoging van de druk in de gezamenlijke v. Portae, met bloedingen in het darmpakket en in de miltregio tot gevolg.
In deze studie werd aangetoond dat kunstmatige interventie bij APOLT in de bloedstroom in de v. Portae tijdens de operatie noodzakelijk is voor een adequate bloedvoorziening van de donorlever.
In **hoofdstuk 6** worden de consequenties van de peroperative interventie in de bloedstroom op de postoperatieve correctie van de stofwisselingsziekte beschreven.
Significante metabole correctie werd initieel gezien in de groep met kunstmatige vernauwing van de v. Portae naar de eigen lever en in de groep met vrije distributie van bloed tussen eigen lever en donorlever. In de groep met totale onderbinding van de v. Portae naar de eigen lever overleden twee honden aan de gevolgen van de portale hypertensie met

bloeding uit de miltregio. Histologisch onderzoek bevestigde massale congestie van de lever met hemorragische necrose. Bij sectie konden geen aanwijzingen voor een stenose van de v. Hepatica-anastomose worden gevonden, zodat een "small for size" fenomeen met een overtollig aanbod ten opzichte van de afvloed de meest waarschijnlijke oorzaak van de hypertensie is.

Dit verklaart ook het verschil met het experiment met de donorlever in de heterotope positie, waar ligatie van de v. Portae geen portale hypertensie gaf. In het eerste experiment bedroeg de getransplanteerde massa ongeveer 70% van de totale lever, terwijl nu slechts 40% van de lever werd getransplanteerd. In de groep met vrije distributie van de bloedstroom over de eigen lever en donorlever trad een verlies van metabole correctie op na twee weken. Trombose van de v. Portae naar de donorlever werd gezien in 3 van de 7 honden in deze groep.

Kunstmatige vernauwing van de v. Portae naar de eigen lever zorgde voor metabole correctie tot het einde van het experiment, hoewel de hoogte van de correctie met de tijd afnam. In 4 van de 11 honden trad op langere termijn een porta trombose van de donorlever op, waarschijnlijk ten gevolge van de verhoogde vasculaire weerstand in de donorlever door infectie en afstoting.

Deze studie bevestigt de eerdere bevindingen, dat voor een langdurige metabole correctie kunstmatig ingrijpen in de portale bloedstroom vereist is. Met een kleine donorlever leidt totale ligatie van de v. Portae van de eigen lever tot het ontstaan van onacceptabele portale hypertensie. Zonder interventie stroomt het portale bloed daarentegen preferentieel naar de eigen lever en gaat de donorlever te gronde aan hypoperfusie. Partiële vernauwing van de v. Portae naar de eigen lever garandeert langdurige correctie van een aangeboren stofwisselingsziekte.

Optimalisatie van de klinische orthotope levertransplantatie

Sinds de introductie van de orthotope levertransplantatie is de 1-jaars patiëntenoverleving verbeterd van ongeveer 30% naar meer dan 80%. Hoewel zeer goede resultaten kunnen worden bereikt, is er toch een aantal punten waarop verbetering mogelijk is. In de hoofdstukken 7 tot 11 van dit proefschrift wordt een viertal problemen besproken, die zich thans bij klinische orthotope levertransplantatie voordoen.

Primaire disfunctie van de donorlever na transplantatie

In **hoofdstuk 7** wordt de rol van stikstof oxide (NO) in de pathogenese van primaire disfunctie van de donorlever (PGDF) onderzocht. De rol van NO in het ontstaan van PGDF is nog onduidelijk. De vasodilatatie die door NO veroorzaakt wordt kan gunstige effecten hebben op ischemische gebieden in de donorlever, maar recent werd ook een schadelijk effect van NO op de functie van mitochondria beschreven.

In deze studie werden ATP- (adenosine tri-fosfaat) en TAN- (totaal adenine nucleotiden) gehaltes in hepatocyten tijdens de preservatieperiode en na reperfusie gemeten. De aanwezigheid van nitrotyrosine, een radicaalproduct van NO, dat grote schade aan mitochondria kan aanbrengen werd aan de energie-inhoud gerelateerd. Tevens werden elektronenmicroscopische foto's van de levers gemaakt om schade aan mitochondria te visualiseren.

In patiënten met PGDF werd een hogere ATP- en TAN-inhoud gemeten aan het einde van de koude ischemische periode, dan in controle patiënten. Na reperfusie van de donorlever namen zowel de TAN- als ATP-gehaltes in patiënten met PGDF significant af, terwijl in controle-patiënten deze waarden juist stegen na reperfusie. De hogere ATP- en TAN-waarden in patiënten met PGDF werden niet eerder in de literatuur beschreven. De stijging in controle patiënten na reperfusie is conform enkele eerdere bevindingen, waarbij echter niet het beloop tijdens de gehele transplantatieprocedure werd gevolgd. De hoge energiewaarden in patiënten met PGDF zijn waarschijnlijk te wijten aan een slechte koude perfusie van de donorlever. Hierdoor valt de biochemische activiteit niet direct stil en is er tijd voor inductie van oxidatieve fosforylatie, glycogenolyse en glycolyse, waarbij ATP gevormd wordt om de celmembraanpotentiaal in stand te houden. Tijdens dit proces wordt ATP niet afgebroken tot adenosine, dat een sterke vaatverwijdende eigenschap heeft en een gunstig effect kan hebben op de reperfusie van de donorlever. Nitrotyrosine werd gevonden in leverbiopten van patiënten met PGDF, maar ook bij alle controle patiënten tijdens de koude en warme ischemische periodes en na reperfusie.
Dit geeft aan dat de cellulaire eiwitten reeds tijdens de donorprocedure genitreerd zijn, omdat de voor deze reactie benodigde NO niet tijdens de koude ischemie door het enzym 'inducible NO synthase' kan worden geproduceerd.

In conclusie laat deze studie zien dat PGDF gerelateerde disfunctie van de mitochondria niet ontstaat tijdens de koude of warme ischemische periode en reperfusie, maar reeds eerder wordt veroorzaakt, waarschijnlijk tijdens de donorprocedure. De hoge

ATP- en TAN-waarden kunnen mogelijkerwijs als indicator voor het optreden van PGDF worden gebruikt.

Het ontbreken van enig verschil in nitrotyrosine vorming tussen patiënten met PGDF en controle patiënten maakt nitratie van de mitochondria een onwaarschijnlijke oorzaak van PGDF.

Hyperfibrinolyse en peroperatief bloedverlies

In **hoofdstuk 8** wordt het probleem van hyperfibrinolyse en bloedverlies tijdens de levertransplantatieprocedure beschreven. Patiënten met een leverziekte hebben voorafgaand aan de transplantatie reeds vaak een ernstige verstoring van de hemostase met een laag thrombocytenaantal, lage niveaus van stollingsfactoren en een gedissimineerde intravasale stolling met toegenomen fibrinolyse (voortijdig oplossen van stolsels). Tijdens de transplantatieprocedure dient het bloedverlies geminimaliseerd te worden, omdat er een verband bestaat tussen het peroperatieve bloedverlies en de postoperatieve overleving.

Om het risico van virale transmissie tijdens bloed(componenten)transfusie te verkleinen werd in 1996 in het Academisch Ziekenhuis Rotterdam ESDEP® geïntroduceerd, een nieuw plasma-product waarin virussen door middel van solvent/detergent (SD) behandeling worden geïnactiveerd. Sinds de invoering van ESDEP nam echter de incidentie van hyperfibrinolyse bij patiënten die een orthotope levertransplantatie ondergingen drievoudig toe. Om de oorzaak van deze stijging te achterhalen werd een groep patiënten die met ESDEP behandeld was vergeleken met een groep patiënten, die met het oude plasma (FFP) behandeld was.

Er werden behoudens een toename in factor VIII geen verschillen gevonden in de individuele stollingsfactoren. Wel werden tijdens de anhepatische fase en na reperfusie van de donorlever significant hogere waarden gevonden van de fibrinogeenafbraakproducten en d-dimeren, samen met een toegenomen fibrinolyseactiviteit op de thrombo-elastograaf. Dit is bewijzend voor een toegenomen fibrinolyse. Tevens bleek op deze tijdstippen de concentraties van het α2-antiplasmine, de belangrijkste inhibitor van fibrinolyse in het bloed, verlaagd te zijn in patiënten die met ESDEP waren behandeld. Oorzaak van deze lage concentraties is het ESDEP plasma zelf, dat ten gevolge van de SD behandeling slechts 25% van de normale hoeveelheid α2-antiplasmine bleek te bevatten.

Het gebruik van virus-veilig plasma wordt steeds belangrijker in de preventie van HIV, hepatitis B en C infecties. ESDEP-gebruik in situaties van massale transfusie kan echter

leiden tot insufficiënte concentraties van α2-antiplasmine en secundaire fibrinolyse. Deze fibrinolyse kan niet worden behandeld door infusie van meer ESDEP, maar moet met anti-fibrinolytische medicatie (zoals aprotinine of cyclokapronzuur) worden bestreden. Het standaard gebruik van anti-fibrinolytische medicatie ter preventie van hyperfibrinolyse wordt aangeraden bij gebruik van ESDEP bij massale transfusie behoefte.

De v. Cava sparende transplantatietechniek

In **hoofdstuk 9** worden de resultaten van de levertransplantatietechniek beschreven, waarbij de v. Cava van de ontvanger wordt behouden tijdens de transplantatie. Het niet afklemmen en niet vervangen van de v. Cava inferior tijdens de transplantatie staat toe dat het veneuze bloed uit het mesenteriale vaatbed en de onderste extremiteiten via de v. Cava inferior wordt teruggevoerd naar het hart. Deze zogenaamde piggyback techniek maakt daardoor het gebruik van een veno-veneuze bypass overbodig en vermindert het bloedverlies uit de retroperitoneale weefsels. In klinische onderzoeken is bij gebruik van de piggyback techniek een verkorting van de warme ischemische periode en vermindering van het peroperatieve bloedverlies en transfusiebehoefte beschreven. Tot nu toe werd echter nog nooit een voordeel in patiëntenoverleving van de techniek aangetoond.

In deze studie wordt de overleving van patiënten, die met de piggyback techniek geopereerd zijn retrospectief vergeleken met die van patiënten die een standaard levertransplantatie ondergingen. In de beginfase van de piggyback techniek moest in 2 patiënten worden overgegaan tot de standaard transplantatieprocedure met vervanging van de v. Cava wegens technische problemen. Tijdens de studie kon de techniek in alle 45 patiënten worden toegepast. Er werd in de piggyback groep inderdaad een significante vermindering gezien van het peroperatieve bloedverlies en de transfusiebehoefte. Daarnaast was in de piggyback groep zowel het aantal ernstige infecties verminderd als de overleving van de donorlever en van de getransplanteerde patiënten significant beter.

De piggybacktechniek blijkt in alle patiënten goed uitvoerbaar en leidt tot een vermindering van het peroperatieve bloedverlies en (bloed)transfusiebehoefte. Gezien de verbetering van de overleving na 6 maanden wordt de piggyback techniek als standaard techniek bij orthotope levertransplantaties gebruikt in het Erasmus Medisch Centrum Rotterdam.

Strategieën om het tekort aan donoren te overbruggen

Het succes van de levertransplantatie heeft als keerzijde dat er een groeiend aantal patiënten op de wachtlijst staat voor een donorlever. In **hoofdstuk 10** wordt een overzicht gegeven van nieuwe strategieën beschreven om het tekort aan donorlevers te verminderen.

In Nederland wordt sinds 1994 een stabiel aantal van ongeveer honderd donorlevers per jaar verkregen. Het aantal patiënten dat jaarlijks op de wachtlijst wordt geplaatst voor een transplantatie is echter gegroeid van 85 in 1994 tot 159 in 2000. Dit leidt tot een toenemend tekort aan geschikte donorlevers voor de groeiende populatie transplantabele patiënten.

In een poging om het aantal voor transplantatie beschikbare donorlevers te vergroten, werd in 1989 in Japan voor het eerst een donorlever getransplanteerd van een patiënt met een circulatiestilstand. Enthousiastme voor deze techniek was ontstaan na eerdere bemoedigende resultaten van niertransplantatie van donoren met een circulatiestilstand. De resultaten van deze transplantatietechniek vor levertransplantatie bleven echter achter bij die van de gebruikelijke postmortale donatie van een hersendode donor zonder circulatiestilstand. Daarom werden andere oplossingen gezocht om het donortekort op te lossen. Met de opkomst van de gesplitste-lever transplantatieprocedure werden de afgelopen 15 jaar technieken ontwikkeld om één donorlever naar twee ontvangers te transplanteren. Aanvankelijk werd een donorlever van een volwassen donor gereduceerd tot linker laterale leverkwab, klein genoeg om naar een kind te transplanteren. Deze methode bracht verlichting in de wachttijd voor kindertransplantatie, maar had als ongewenste bijwerking dat de hoeveelheid beschikbare donororganen voor volwassenen afnam. Verbetering in de chirurgische technieken resulteerde in de mogelijkheid om de donorlever te splitsen buiten het lichaam en beide helften voor transplantatie te gebruiken: de linker leverkwab voor een kind en de rechter leverkwab voor een volwassene. Later werd de techniek verfijnd en kon de lever reeds in de orgaandonor gesplitst worden, waardoor de koude ischemische periode met enige uren werd bekort. De techniek maakt inmiddels een 1–jaars patiëntenoverleving mogelijk, die minstens vergelijkbaar is met die van de conventionele levertransplantatie.

Tevens opende dit de deuren naar de levende-donor levertransplantatie. De eerste succesvolle procedure werd in Australië uitgevoerd bij een meisje, dat de linkerleverkwab van haar moeder ontving. Door de bovengenoemde ontwikkelingen verdween in drie jaar tijd de wachtlijst voor kinderen bijna geheel.

De vraag naar donorlevers voor volwassenen nam echter elk jaar toe en recent werd levertransplantatie van de rechter leverkwab van een levende-donor geïntroduceerd. Ondanks de voordelen en gerapporteerde successen van de levende-donor levertransplantatie bestaat er zorg over de morbiditeit en mortaliteit van de gezonde leverdonor.

Conclusies van dit proefschrift

1. Langdurige metabole correctie kan door een auxiliair geplaatste transplantatielever worden verkregen, maar in afwezigheid van portale hypertensie is interventie in de bloedstroom van de v. Portae essentieel voor overleving van de donorlever, zowel bij heterotoop als bij orthotoop geplaatste donorlevers (hoofdstuk 4, 5 en 6).
2. Totale onderbinding van de v. Portae van de eigen lever leidt niet tot histologisch bewezen atrofie van de eigen lever. Het vermindert slechts de levermassa en kan goed worden verdragen als het niet in portale hypertensie resulteert (hoofdstuk 4 en 6).
3. Verdeling van de bloedstroom van de v. Portae tussen de donorlever en eigen lever op geleide van echogeleide banding is een accurate methode (hoofdstuk 4 en 6). Banding op geleide van de drukverhoging in de v. Portae van de donorlever kon metabole correctie op lange termijn niet garanderen (hoofdstuk 4).
4. Er bestaat een opmerkelijk verschil in afhankelijkheid van bloedvoorziening via de v. Portae tussen de eigen lever en de donorlever. De eigen lever kan totale ligatie van de v. Portae goed verdragen, terwijl insufficiënte portale doorbloeding van de donorlever onmiddellijk leidt tot necrose van de levercellen met fibrose en atrofie (hoofdstuk 4 en 6).
5. Disfunctie van de mitochondria in patiënten met een primaire disfunctie van de donorlever (PGDF) bestaat reeds tijdens de koude ischemische periode. Het wordt niet veroorzaakt door nitrosylering van de mitochnodriale structuren door peroxynitriet, gevormd uit stikstofmonoxide (hoofdstuk 7).
6. Solvent/detergent behandeld plasma heeft een α2-antiplasmine deficiëntie. Anti-fibrinolytische therapie zou dan ook standaard gegeven moeten worden, wanneer ESDEP gebruikt wordt bij massale transfusie om het risico van hyperfibrinolyse te minimaliseren (hoofdstuk 8).
7. Het gebruik van een v. Cava sparende operatietechniek bij de orthotope levertransplantatie leidde tot een afname van het peroperatieve bloedverlies en de transfusiebehoefte en verbeterde de 6-maands overleving van patiënten (hoofdstuk 9).

8. Door partiële levertransplantatie verdween de wachtlijst voor kinderlevertransplantatie bijna geheel, maar dit ging ten koste van donororganen voor volwassen patiënten. Met de komst van technieken om de donorlever te splitsen in twee volwaardige transplantabele gedeelten, kunnen nu twee ontvangers met één donorlever worden geholpen, zonder de 1–jaars patiëntenoverleving in gevaar te brengen.

Toekomstige ontwikkelingen

Het toenemend aantal patiënten dat in aanmerking komt voor levertransplantatie zal tot een toenemend tekort aan donororganen leiden. Op dit moment overlijden reeds patiënten op de wachtlijst. De levende-donor levertransplantatie techniek is in opkomst en kan een bijdrage leveren aan de oplossing voor dit probleem. De resultaten van deze procedure zijn momenteel gelijk aan die van de standaard levertransplantatie. Recent werd de eerste succesvolle poging gemeld om de donorlever op laparoscopische wijze te splitsen en dit zal op den duur resulteren in een verdere afname van operatierisico's voor de leverdonor.

Met de toename in ervaring met partiële levertransplantatie zal ook de *auxilaire* partiële orthotope levertransplantatie voor geselecteerde patiënten meer in de belangstelling komen. Met name patiënten met een aangeboren stofwisselingsziekte van de lever en patiënten met acuut leverfalen zullen hiervan profiteren. In patiënten met een metabole ziekte kan de partiële donorlever de ziekte corrigeren, terwijl de eigen lever beschikbaar blijft voor toekomstige gentherapie. Als intussen de donorlever faalt, kan deze electief vervangen worden, terwijl de patiënt overleeft op de eigen lever.

Een deel van de patiënten met acuut leverfalen kan met een auxiliaire donorlever naar herstel van de eigen leverfunctie overbrugd worden, waarna de donorlever verwijderd kan worden en de anti-afstotingsmedicijnen kunnen worden gestaakt. Het proces van leverregeneratie moet echter nog beter begrepen en voorspelbaar worden, om te bepalen welke patiënten baat zullen hebben bij een auxiliaire donorlever. Met toenemende veiligheid van levende-donor levertransplantatie zal het ethisch acceptabel worden om deze techniek aan te bieden in een vroeg stadium van de ziekte, wanneer zich nog geen complicaties van het leverfalen hebben voorgedaan en de overlevingskansen aanzienlijk beter zijn.

De combinatie van de piggyback transplantatie techniek en standaard anti-fibrinolytische medicatie tijdens de orthotope levertransplantatie zal het peroperatieve bloedverlies doen dalen, de duur van de ingreep verkorten en het operatietrauma verminderen.

Om het rendement van de schaarse donororganen te verbeteren zal primaire disfunctie van de donorlever verder moeten worden teruggedrongen. Onze resultaten geven aan dat de oorzaken van deze disfunctie mogelijk al tijdens de orgaandonatie-procedure ontstaan. Activatie van de macrofagen in de lever (Kupffercellen) tijdens de perfusie van de donorlever lijkt een sleutelrol te spelen in het ontstaan van primaire disfunctie. Het gedrag van de Kupffercellen tijdens de orgaandonatie en preservatieperiode en mogelijke therapeutische interventies daarop wordt daarom momenteel bestudeerd in het Chirurgisch Laboratorium van het Erasmus Medisch Centrum Rotterdam.

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Curriculum vitae auctoris

Jeroen de Jonge was born on June 12th, 1973 in Haarlem, the Netherlands. After finishing high school at the Stedelijk Gymnasium in Haarlem in 1991, he continued his studies at the Faculty of Medicine of the Erasmus University in Rotterdam. During medical school he worked as a student assistant at the department of Urology of the Erasmus University Medical Centre Rotterdam (prof. dr. F.H. Schröder). In the second year of the study, his interest in surgery was aroused when he started participating in clinical research as a research student in the Rotterdam liver transplantation team. Before starting his internship, a two-year position as PhD student was offered by prof. dr. H.W. Tilanus at the Laboratory of Experimental Surgery in Rotterdam (Head dr. R.L. Marquet), which formed the basis for this thesis. Substantial parts of the research work have been carried out at the laboratory of Haematology of the Erasmus University MC Rotterdam (dr. H.H.D.M. van Vliet, prof. dr. B. Löwenberg) and the Department of Biochemistry of the Erasmus University Rotterdam (dr. W. Sluiter, prof. dr. J.F. Koster). In 1999 he obtained his medical degree *cum laude*. His experimental work was awarded with the "Studieprijs van het Bataafsch Genootschap der Proefondervindelijke Wijsbegeerte" in 2000. From December 1999 until May 2001 he worked as an intern at the Surgical Intensive Care Unit, Erasmus University Medical Centre Rotterdam (prof. dr. H.A. Bruining).

In May 2001 he started his residency in general surgery at the Sint Franciscus Gasthuis in Rotterdam (dr. C.H.A. Wittens and dr. A.J.H. Kerver), to be completed at the department of Surgery of the Erasmus University Medical Centre Rotterdam (prof. dr. J. Jeekel, head of department and prof. dr. H.J. Bonjer).