AUXILIARY PARTIAL LIVER TRANSPLANTATION

AUXILIAIRE PARTIËLE LEVERTRANSPLANTATIE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. M.W. VAN HOF
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
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door

CORNELIS BASTIAAN REUVERS
geboren te Leiden

PROMOTIECOMMISSIE

PROMOTOR:	PROF. DR. D.L. WESTBROEK
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To my parents,
Minke, Bas, Willemijn, Nienke

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Chapter 1

Introduction

1.1 Indications for liver transplantation

Although a wide variety of techniques has been devised to treat patients with end-stage liver disease, none has proven to be very effective. Exchange transfusion, plasmapheresis, cross circulation, extra corporeal liver perfusion, hemodialysis, and hemoperfusion have been unable to improve patient survival significantly[1]. In view of the many complex tasks of the liver, it seems less likely that an effective artificial hepatic support device will become available in the near future. Liver transplantation thus represents at the moment the only treatment which offers hope for survival in children and adults with end-stage liver disease.

Since 1963, when the first transplantation of a human liver was attempted by Starzl, over 1000 liver transplantations have been performed world wide[2]. Until 1980 the one year survival rate was 30% with a five year survival rate of 20%. Improvement in surgical technique and changes in immunosuppressive regimen since 1980 resulted in survival rates of 60% - 70% at one year in patients that were operated by Starzl and associates [2]. Most liver transplantation groups perform orthotopic liver transplantation in which case the damaged liver is removed and replaced by a transplant. There are many liver diseases for which orthotopic liver transplantation is performed (Table 1)[3]. End-stage cirrhosis or biliary atresia is the indication to perform orthotopic liver transplantation in almost 50% of patients reported by the main transplantation groups[3]. Important differences exist among transplantation centers regarding the criteria for acceptance or rejection of individual patients. In the Netherlands selection criteria for orthotopic liver transplantation are rather stringent; only 10% of referred potential candidates for liver transplantation with end-stage chronic liver disease were actually transplanted[4]. Most centers agree that orthotopic liver transplantation bears to many risk in patients with acute hepatic failure. This syndrome occurs by definition in patients with no previous evidence of liver disease, and results in the development of hepatic encephalopathy within eight weeks of the onset of illness[6].

Table 1. Main indications for orthotopic liver transplantation

Primary hepatic malignancy
Non-alcoholic cirrhosis
Alcoholic cirrhosis (Abstentia for 1 year)
Congenital hepatobiliary disorders
Sclerosing cholangitis
Hepatic vein thrombosis

Etiology of acute hepatic failure is summarized in Table 2[7]. The absence of pre-existing hepatocellular disease is an important component of the definition of acute hepatic failure, since it implies that hepatic structure and function could return to normal, provided that: (1) the pathogenetic factor responsible for fulminant hepatic failure could be removed or inactivated; (2) the functions of the failing liver could be adequately replaced; and (3) the liver retains its capacity to regenerate. In the absence of an effective artificial liver support system mortality is 80% in these patients with acute hepatic failure despite of intensive care treatment[8,9]. The frequency of acute liver failure caused by different etiology is not exactly known but is estimated to be 2000 per year in the United States[10].

Table 2. Etiology of fulminant failure.

Viral hepatitis (A,B, and non-A, non-B)
Other viral infections (Epstein Barr, adeno virus, herpes)
Yellow fever
Coxiella burneti
Amanitia phalloides
Drugs (acetaminophen, isoniazid)
Hepatic ischemia
Pregnancy associated
Reye's syndrome

Although liver transplantation is obviously indicated in patients with acute hepatic failure, the major transplantation groups to date have been reluctant to perform orthotopic liver transplantation in the case of acute hepatic insufficiency. The poor clinical condition of these patients and the associated clotting disorders form too great a risk for succesful removal of the diseased liver and replacement by a donor organ.
Patients with acute hepatic failure or with end-stage chronic liver disease that are not accepted for orthotopic liver transplantation might benefit from auxiliary liver transplantation.

1.2 Aim of auxiliary liver transplantation

In auxiliary liver transplantation, a liver is transplanted without the removal of the recipient's own liver.
Indications to perform auxiliary liver transplantation theoretically are the same as in orthotopic liver transplantation (Table 1). The only exception is primary malignancy in the recipient liver where auxiliary liver transplantation obviously is not a realistic solution for the patient.
Long-term survival incidentally has been described after auxiliary liver transplantation[11,12]. However, as initial clinical results with auxiliary liver transplantation were discouraging most centers abandoned this technique in favor of orthotopic liver transplantation[13]. Still the concept of auxiliary heterotopic liver transplantation in non-neoplastic diseases remains attractive.
Compared with orthotopic liver transplantation the auxiliary technique has the following potential advantages.
Limited extent of the procedure. Transplantation without removal of the diseased recipient liver could have the advantage of sparing the critically ill recipient the additional operation time and potential complications of hepatectomy[14].
Temporary metabolic support. In potential reversible liver disease auxiliary liver transplantation could help to keep the patient alive during the time required for the patient's own liver to regenerate. In the event of adequate regeneration and recovery of function of the patient's own liver , the auxiliary graft could, theoretically, be removed[15,16]. Should the host's liver not recover, the graft could serve as a permanent replacement[12].

Residual capacity of the recipient's liver. In auxiliary liver transplantation the patient's own liver could provide some protection for the patient during the period of establishment of graft function, and during rejection periods[11]. The recipient's own liver is maintained as a functional reserve in case the donor liver does not function well.
Stimulation of regeneration in the recipient's liver by the graft Total and partial auxiliary liver transplants produce a factor that stimulates regeneration of the recipient's liver[17]. This factor responsible for the initiation and stimulation of hepatic regeneration has recently been characterized and partially purified[18].

1.3 Results of clinical auxiliary liver transplantation

The first auxiliary liver transplantation in man was performed in 1964 [19]. Only a limited number of such operations have been performed in the United States and Europe since[11]. Indications for which heterotopic auxiliary liver transplantation were performed in man are listed in Table 3[11,20,21,22].

Table 3. Number of patients and indications of auxiliary liver transplants in man.

	Number of patients
Biliary atresia	20
Cirrhosis	17
Primary malignant tumor	4
Acute hepatitis	4
Ideopatic cholangiostatic syndrome	1
Rejection of orthotopic transplant	1
Hepatic failure after extensive liver resection	1

As in orthotopic liver transplantation liver cirrhosis and biliary atresia were the main indications for auxiliary liver transplantation in these patients. The operation was technically feasible in man but experience so far has not been encouraging: of 48 reported cases only two patients have survived more than one year, and most patients died within one month. Fortner reported a patient still alive more than 10 years after transplantation[23], and Bismuth and co-workers recently reported survival

of 6.5 years after auxiliary liver transplantation[20]. The indications for liver transplantation in these two cases were biliary atresia in a child and end-stage cirrhosis in a middle aged man respectively. These two patients demonstrate that auxiliary liver transplantation is possible in man and justify further evalution of this type of liver transplantation.

1.4 Problems in clinical auxiliary liver transplantation

Problems such as preservation of the transplant, reconstruction of the biliary tract, immunosuppression, and management of infectious complications are basically the same in orthotopic liver transplantation and auxiliary liver transplantation. Most of these problems have been overcome in recent years for the orthotopic technique as reflected by improved survival rates[2]. Selection of lower risk patients for transplantation probably also contributed to these better results.
The causes of failure of 44 heterotopic auxiliary liver transplants in man reported by Fortner are summarized in Table 4[11].

Table 4. Causes of failure in human auxiliary liver transplantation.

	Number of patients
Sepsis	12
Liver failure	10
Cardiorespiratory insufficiency	7
Hemorrhage	6
Hepatic artery thrombosis	5
Biliary fistula	1
Pneumocystis	1
Acute renal failure	1
Inanition, monilia	1

One of the problems concerning auxiliary liver transplantation is the size of the organ that has to be positioned in the abdominal cavity. The graft may cause elevation of the diaphragm and subsequent respiratory complications. The use of grafts of reduced size may diminish the frequency of cardiorespiratory insufficiency[20]. Leakage of the biliary enteric anastomosis resulted in sepsis in a significant number of

patients. As in orthotopic liver transplantation this anastomosis is a potential source of complications[13].
It has been suggested that after auxiliary liver transplantation the two livers might function in a balanced state[24], with the graft and the patient's liver functioning simultaneously and being mutually supportive. In a patient with an unresectable liver tumor who survived eight months after auxiliary liver transplantation autopsy indicated that a physiological balance between the transplant and the host liver indeed had been achieved, as the size of the livers appeared to be the same. In that patient there was no indication of recipient liver atrophy, although the tumor mass in the host liver probably did not contributed to total liver function.In clinical auxiliary liver transplantation hypertrophy of the graft and atrophy of the recipient liver has been observed in the two patients that survived more than five years. Both patients are alive and well with no residual host liver function[12,25]. Progressive atrophy of recipient liver could be secondary to long-term preferential portal blood flow through the graft as suggested by Marchioro[25]. Recipient liver recovery after an episode of liver failure with atrophy of an auxiliary liver graft at the same time, has never been reported in man.
Development of a primary liver malignancy in the diseased host liver in the period following the transplantation procedure is a potential hazard in auxiliary liver transplantation. This complication has not been reported in the small series of heterotopic liver transplantations so far performed in man.

1.5 Results of experimental auxiliary liver transplantation

Experimental auxiliary liver transplantation was performed for the first time by Welch in 1955[26]. He demonstrated in the dog that entire livers could be transplanted heterotopically and that these livers continued to function in the recipient animals. Since this first auxiliary liver transplantation extensive experimental work has been performed in this field. Research was mainly focussed on the problems of: (1) space; (2) position of the graft; (3) blood supply to the graft; (4) biliary drainage; and (4) rejection.
The problem of space The placement of a large additional organ into the abdominal cavity may prevent closure of the laparotomy wound[24]. Forced closure results in elevation of the diaphragm causing respiratory complications, kinking of the blood vessels of the graft, compression of

the host's blood vessels and poor wound healing. Gradual enlargement preoperatively of the abdominal cavity[24], removal of other organs such as the spleen or a kidney[19,27], and transplantation of small or partial grafts have been suggested as solutions[27,28,29]. In clinical auxiliary liver transplantation small grafts can only be obtained from children. The number of donor livers available from this age group is limited[30,31]. Therefore the problem of space appears to be solved most practically if only a part of an adult donor liver is used.

The problem of position. In auxiliary liver transplantation the graft may be placed at different sites of the body. Liver transplants have been placed in the right and left upper abdomen[32,33], right and left lower abdomen[34,35], the thoracic cavity[36], the groin[37], and the neck[38]. With the liver in the orthotopic position, the hepatic veins drain into the inferior vena cava in which vessel blood pressure is low and fluctuates with respiration[39,40]. Pressure in the inferior vena cava increases proportionately with the distance from the right atrium[41]. Fluctuations in pressure in the inferior vena cava appear to be inversely related to the distance from the right atrium[42]. It has been shown that pressure in the inferior vena cava, distal to the renal veins, causes hepatic venous outflow obstruction and graft damage[40,43,44]. These findings indicate that an auxiliary liver graft should drain into the inferior vena cava as close to the diaphragm as possible to avoid damage due to outflow obstruction. This will preclude extra abdominal auxiliary grafting and limits intra abdominal sites to the upper abdomen.

The problem of blood supply to the graft. In its normal position a liver has a dual afferent blood supply: hepatic arterial and portal venous. Because the transplanted liver lacks collateral arterial blood flow, transplantation without hepatic arterial inflow results in hepatic infarction. Subsequent liver necrosis will increase the risk of infection. Lack of hepatic arterial blood flow may also cause necrosis of the common bile duct, resulting in bile leakage. These problems may well negate the efforts of those who have in the past attempted auxiliary liver transplantation using a portal venous blood supply only[45,46].

Depriving the liver of portal blood causes atrophy of the liver even if an equivalent volume of systemic venous blood is directed through the liver[25,47,48]. It has been shown conclusively that pancreatic efferent venous blood contains one or more hepatotropic factors essential for the integrity of the liver[25,49,50,51,52]. These observations in non-transplanted normal livers seem to indicate that a liver requires both an arterial and portal venous blood flow for prevention of ischemic complications and atrophy. Starzl and others indeed have shown that atrophy rapidly develops in auxiliary liver transplants lacking this dual

blood supply[27,53,54]. No long-term survival has been reported in hepatectomized dogs with liver transplants receiving arterial blood supply only[28,55].

It thus appears that in auxiliary liver transplantation the graft should have an adequate inflow of arterial blood, as well as inflow of portal venous blood. Anatomically these two anastomoses can be constructed more easily in the upper abdomen than in the pelvis or any extra-abdominal position.

The problem of biliary drainage. Bile drainage can be achieved by external or internal drainage. External drainage of the biliary system of the transplant has been used in some experiments but is a less practical solution than internal drainage[16,28,56]. Most authors perform internal drainage anastomosing the common bile duct of the graft to the common bile duct, the gallbladder, the jejunum or the duodenum of the recipient.

Drainage into the recipient's own distal biliary tract is used successfully in orthotopic liver transplantation[2,57]. Calne has developed a conduit procedure in which the common bile duct of the graft is anastomosed to the gallbladder of the graft, which is subsequentely connected to the recipient bile duct[58]. In orthotopic liver transplantation drainage into the common bile duct of the recipient retains drainage through the sphincter of oddi and prevents cholangitis as has been demonstrated in animal experiments[59]. As the biliary tracts of the transplant and the recipient liver are usually separated, drainage into the common bile duct of the recipient has not been used frequently in experimental auxiliary liver transplantation. The technique of cholecysto-jejuno-cholecystostomy as suggested by Crosier is difficult and time consuming[60].

In experimental auxiliary liver transplantation in large animal models, cholecystojejunostomy was the technique of biliary anastomosis preferred by most authors[27,55,61,62,63]. Animal survival in these forementioned studies, however, is too short to evaluate this type of anastomosis in auxiliary liver transplantation. In orthotopic liver transplantation cholecystojejunostomy appears to be inferior to a direct biliary enteric anastomosis[64,65]. Recent experimental work suggests that in pigs direct choledochoduodenostomy and Roux-en-Y choledochojejunostomy are both successful types of biliary anastomosis[41]. In the case of a direct anastomosis between the intestine and the common bile duct, a cholecystectomy of the transplant must be performed, because in the absence of a sphincter mechanism the gallbladder acts as a diverticulum of the common bile duct which will cause cholangitis[65].

The problem of rejection. For all types of organ transplants prolonged survival of the graft is largely dependent on recognition of rejection and the prompt institution of immunosuppressive therapy. In both clinical and experimental liver transplantation the early detection of rejection, as well as its distinction from cholangitis, cholestasis and other complications has remained a problem[66,67,68,69].

The presence of two livers in auxiliary transplantation complicates the problem of early diagnosis of rejection. The recipient's own liver may modify clinical symptoms and biochemical or haematological results and may complicate assessment of graft function. No single biochemical test exist that reflects specifically the status of the graft. Histological examination of sequential percutaneous liver biopsies of the graft is the only procedure that may correctely indicate graft rejection.

Clinical and experimental orthotopic liver transplantation studies have demonstrated that rejection is a less serious problem compared to rejection encountered in kidney transplantation[70,71,72]. Donor-recipient selection based on tissue-typing appears to be less important. It is not clear if the same holds true in auxiliary liver transplantation. The presence of the recipient liver may modify the rejection process[46]. Liver allografts have been reported to be spontaneously tolerated in the rat and the pig but only after total removal of the recipients' own liver[70.73,74]. This suggests that a healthy recipient liver prevents the induction of a "donor-specific transplantation tolerance" following auxiliary liver transplantation. It has also been shown that the reticulo-endothelial system of the liver participates in graft rejection[46]. In the case of a severely diseased host liver with impaired reticulo-endothelial function the immunological attack on the graft might therefore be less.

In experimental auxiliary liver transplantation either no immunosuppressive therapy has been given or a combination of azathioprine and steroids has been used[33,59,76]. Information about the effect of new immunosuppressive regimens with Cyclosporin A on experimental auxiliary liver graft survival is not available.

The foregoing results on experimental auxiliary liver transplantation enables one to propose theoretical criteria for optimum function of an auxiliary liver transplant: (1) the donor liver should be small or only a part of a donor liver should be used; (2) the graft should drain into the inferior vena cava as close to the diaphragm as possible; (3) the graft should have inflow of arterial blood as well as portal venous blood; and (4) the graft should have a direct anastomosis between the common bile duct of the graft and the duodenum or jejunum of the recipient.

1.6 Objectives of the study

In the following chapters studies in dogs and pigs are described. The aims of these experiments were:

1. To develop a surgical technique of partial auxiliary liver transplantation in which all requirements for optimal graft function were met (chapter 2, and 3).
2. To study the effect of tissue-typing in that model (chapter 2, and 3).
3. To develop a model of acute liver failure (chapter 4).
4. To study the metabolic support of an auxiliary partial liver transplant in that model of acute liver failure (chapter 5).
5. To study technical feasibility, hemodynamic changes, and clotting abnormalities in auxiliary partial liver transplantation in the presence of acute hepatic failure (chapter 6).

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CHAPTER 2

First experiment

LONG-TERM SURVIVAL OF AUXILIARY PARTIAL LIVER GRAFTS IN DLA-IDENTICAL LITTERMATE BEAGLES[1]

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LONG-TERM SURVIVAL OF AUXILIARY PARTIAL LIVER GRAFTS IN DLA-IDENTICAL LITTERMATE BEAGLES [1].

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Summary

Auxiliary heterotopic transplantation of 60% of the liver in the beagle, using a technique in which all requirements for optimal graft survival are met is described.The autologous liver is left in situ . Transplants were performed in both non-tissue-typed and matched donor-recipient combinations.

Postoperatively the recipients were treated with a standard schedule of two mg azathioprine and one mg prednisolone intravenously daily for 75 days, thereafter the immunosuppressive drugs were gradually withdrawn. HIDA-hepatobiliary scanning proved to be useful for the assessment of graft function.

In eight non-tissue-typed donor-recipient combinations median graft survival was 7 days , most transplants being subject to acute rejection. However,in nine experiments where donor and recipient were DLA-identical littermates, the median graft survival was 112 days ($p<0.005$). In these animals signs of chronic rejection developed after tapering off the immunosuppressive drugs.

It is concluded that in this model graft survival is improved by histocompatibility matching. The feasibility of partial heterotopic liver transplantation indicates that this method needs to be reconsidered for clinical application, especially for patients with acute liver failure.

For the recipient it is a relatively minor operation that by its temporary life sustaining function may allow for the regeneration or restoration of function of the recipient´s own liver.

Introduction.

A theoretical advantage of auxiliary liver transplantation is that the recipient does not depend at the outset totally on the homograft function. In patients with potentially reversible hepatic disease temporary life support could be obtained during which recovery of the own liver could be awaited. Technical hazards of recipient hepatectomy, especially in the case of severe clotting disorders as in acute liver failure, are avoided. Despite of these theoretical advantages, results of clinical (1) and experimental (2) auxiliary liver tranplantation have been poor. The appeal of transplanting an auxiliary liver, which can be removed if superfluous, led us to continue to search for a suitable experimental model.

Analysis of the reported failures of auxiliary liver transplantation revealed the following problems. 1) Lack of space in the recipient abdominal cavity after placement of a large additional organ results in compression of the transplanted liver and its blood vessels (3). 2) Insufficient venous outflow of the graft leads to venous congestion and thrombosis (4). 3) Omitting effluent portal blood to the transplanted liver causes atrophy of the graft (5). 4) Omitting arterial inflow to the graft will lead to hepatic infarction and to insufficient blood supply to the bile ducts (6).

Long-term survival of grafts after orthotopic liver transplantation in DLA-identical beagles has been reported (7), but in orthotopic liver transplantation it has been stated that liver allografts are less immunogenic than other tissues in organ transplantation (8). However, rejection phenomena comparable to other transplanted tissues have been described after auxiliary liver transplantation (9). Studies on the beneficial effect of tissue matching on survival of auxiliary liver transplants have not been reported previously.

The present study was aimed at two objectives: 1) to evaluate the technical feasibility of auxiliary transplantation of a part of the liver in dogs as to meet the theoretical requirements essential for optimal graft survival and function and 2) to study the influence of DLA-tissue typing on auxiliary liver graft survival.

Material and methods.

Dogs. In two consecutive series of experiments, 24 heterotopic auxiliary transplantations of part of the liver were carried out in dogs. Beagles of both sexes, obtained from the colonies at the Centraal Proefdierenbedrijf TNO, Austerlitz, The Netherlands, weighing 13.0±0.3 kg (mean±SEM) served as donors; the recipients weighed 13.7±0.4 kg.

Matching. In the first series of experiments (n=14) the donor and recipient were unrelated and not tissue-typed for the DLA system (group A) but in the second group (n=10) donor and recipient were DLA-identical littermates (group B). DLA-identity was established by means of both serological typing and mixed lymphocyte reaction as described previously (10,11).

Donor operation. Hepatectomy was performed using a long midline incision. Peritoneal attachments of the liver and inferior vena cava to the posterior abdominal wall were divided. The portal vein, common bile duct and hepatic artery were dissected. The hepatic artery was carefully isolated and the origin of other arteries of the coeliac axis were individually ligated. The gastrohepatic, triangular and the falciform ligaments were divided. Following exsanguination of the donor animal the liver was perfused in situ through the portal vein with one litre of cooled Eurocollins solution (4° C). The hepatic artery was flushed with 20 ml of the same solution. Thereafter, the graft was prepared by bench surgery. A cholecystectomy was carried out and a small polyethylene tube was inserted into the cystic duct to enable cholangiography after transplantation for follow-up studies. The two left lateral lobes were resected, reducing the weight of the donor liver to 58.4±1.8% of the original weight (mean±SEM).

Recipient operation. During bench surgery another team started operating on the recipient animal. Through an extended right subcostal incision the infrahepatic vena cava, the portal vein and the infrarenal aorta were dissected. Then the suprahepatic vena cava of the graft was anastomosed to the recipient infrahepatic vena cava as close to the diaphragm as possible, proximal to the renal veins, using a running 5-0 prolene suture. After evacuation of Eurocollins by flushing of the donor liver with 500 ml saline, the portal vein of the graft was anastomosed (with 6-0 prolene) end-to-side to the host portal vein. On completion of the anastomosis the portal venous clamp was removed allowing perfusion of the graft and ending the ischaemic period. The infrahepatic part of the donor vena cava was ligated. A shunt for temporary decompression of the splanchnic circulation during portal clamping was not necessary. The recipient

portal vein was ligated and divided between ligatures close to the liver hilum. The hepatic artery with an oval aorta patch was anastomosed end-to-side to the infrarenal aorta using 5-0 prolene. Bile drainage was obtained by choledochoduodenostomy by pulling the common bile duct through a stab wound in the duodenum and securing it to the inner wall of the bowel with a single 4-0 catgut suture (Fig.1). Immediately after recirculation of the graft 400 ml of Haemaccel^{R} was given intravenously. Blood loss exceeding 300 ml was corrected by administration of an equal amount of blood from the donor animal.
Antibiotic prophylaxis with 15 mg lincomycine and 15 mg kanamycine per kg body weight twice daily was given for five days after the operation.

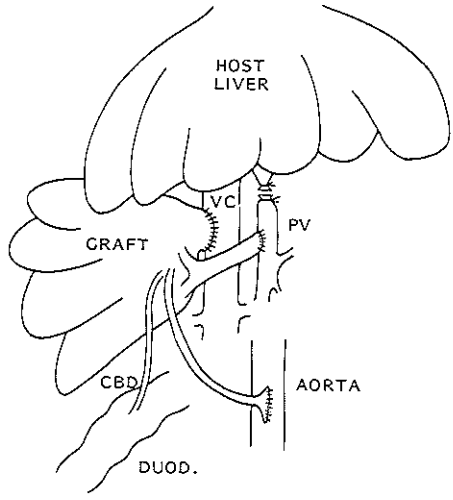

Fig. 1. Diagram of the surgical technique of auxiliary partial liver transplantation.

Immunosuppression. All animals received two mg azathioprine and one mg prednisolone per kg body weight intravenously once daily starting at the end of the operation. The immunosuppressive medication was continued until the 75th postoperative day; thereafter the dosage of the drugs was tapered off gradually until the 150th postoperative day. If the platelet count dropped below $50x10^{9}/l$, azathioprine medication was temporarily discontinued.

Postoperative studies. Weekly blood samples served to determine haemoglobin, leucocytes and platelets. Serum alkaline phosphatase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase and gamma glutamyl transaminase were measured.
Intravenous angiography as described previously (12) was performed at the

fourth postoperative day to visualize the arterial and portal anastomoses. During this procedure a cholangiography of the donor liver was done to exclude stenosis of the choledochoduodenostomy and to detect bile leakage. During the second series of experiments a gamma camera became available and graft function could then be assessed at monthly intervals after the operation by cholescintigraphy. After intravenous injection of 55.5 MBq ^{99m}Tc-HIDA scintigraphy was performed with a Pho Gamma-III camera (Siemens, Gammasonics) with a low energy all purpose collimator.

Histology. At operation and at autopsy wedge liver biopsies were taken. Sequential liver biopsies were obtained with a Tru-cut^{R} biopsy needle in the second post operative week and monthly thereafter. The tissues were fixed in 10% buffered formalin and 5μ paraffin embedded sections were stained with hematoxylin azophloxin and saffron. Examinations of the liver biopsies were performed on a blind observer basis. An assessment was made of the presence and degree of cholangitis, cholestasis, hepatocellular necrosis and rejection as estimated by the degree of periportal lympho-plasma cellular infiltration, bile duct proliferation and vasculitis.

Graft survival was assessed by sequential histopathological studies, visualization of the anastomoses and intrahepatic branches at angiography and by cholescintigraphy. Surviving animals were sacrificed 182 days after the operation or sooner as indicated by clinical condition. Graft survival data were analyzed statistically by means of one sided Wilcoxon rank sum tests.

Results.

Early mortality. No death occurred intraoperatively. In group A (n=14) four dogs died within one week after the operation of technical failures (intra-abdominal hemorrhage, thrombosis at the portal vein and hepatic artery anastomosis). Two other dogs died of unknown causes four and five days after the operation. At autopsy their grafts had a normal appearance and histological examination showed normal liver tissue. The remaining eight dogs provide the data for analysis of graft survival in the non-tissue-typed experiments.

In group B (n=10) one dog died of bile peritonitis caused by puncture of the gall bladder during a percutaneous liver biopsy, 14 days after the transplantation. Histology of the liver graft in this animal was normal. This dog is excluded from the further analysis.

Table 1. Animal and liver allograft survival in eight non-tissue-typed beagles (group A)

Dog No.	Survival (days)	Graft survival (days)	Cause of death	Histological findings in graft	Vascular anastomoses at autopsy* Vena cava	Portal vein	Hepatic artery
1	8	4	Graft necrosis	Acute rejection	Oc	Oc	P
2	12	7	Graft necrosis	Total necrosis	Oc	P	P
3	98	42	Sacrificed in good health	Graft resorbed	Oc	Oc	Oc
4	55	14	Sacrificed in poor condition	Acute rejection	P	Oc	P
5	28	7	Sacrificed in poor condition	Total necrosis	Oc	Oc	Oc
6	13	4	Graft necrosis	Acute rejection	Oc	Oc	Oc
7	182	112	Sacrificed in good health	Chronic rejection	P	P	P
8	18	7	Graft necrosis	Acute rejection	P	P	P
Median value	23	7					

* Oc = occluded, P = patent.

Survival. The median animal survival in group A was 23 days (Table 1). Most animals died or were sacrificed in poor condition caused by graft necrosis. The deterioration in the clinical condition was accompanied by anemia, leukocytosis and thrombocytopenia. Only two dogs remained in good clinical condition until time of sacrifice on days 98 and 182 after the operation.

In group B the median animal survival was 182 days (Table 2). Only two beagles were sacrificed before the end of the observation period because of intraabdominal abscesses in and around the liver graft. Average body weight in these animals diminished to a 92% of preoperative values in the first four weeks following operation but body weight was regained thereafter. In contrast to the first experimental group, the hemoglobin and blood platelet levels remained in the normal range. The initial rise in white blood cell count returned to normal values within three weeks after the operation.

Five animals in each group received a blood transfusion during surgery with blood from the donor animal.

Graft survival. Estimated median graft survival in the non-tissue-typed donor-recipient combinations was 7 days (Table 1), but in the dogs that received a DLA-identical liver transplant median graft survival was 112 days (Table 2). This difference in graft survival time is significant ($p<0.005$).

Table 2. Animal and liver allograft survival in nine DLA-identical littermate beagles (group B)

Dog No.	Survival (days)	Graft survival (days)	Cause of death	Histological findings in graft	Vascular anastomoses at autopsy* Vena cava	Portal vein	Hepatic artery
1	182	112	Sacrificed in good health	Chronic rejection	P	P	P
2	182	182	Sacrificed in good health	Minimal chronic rejection	P	P	P
3	182	42	Sacrificed in good health	Chronic rejection	P	Oc	Oc
4	182	56	Sacrificed in good health	Chronic rejection	P	P	P
5	154	35	Sacrificed, abdominal abscess	Chronic rejection	Oc	Oc	P
6	77	14	Sacrificed, abdominal abscess	Chronic rejection	P	Oc	P
7	182	182	Sacrificed in good health	Minimal chronic rejection	P	P	P
8	182	140	Sacrificed in good health	Chronic rejection	P	P	P
9	182	182	Sacrificed in good health	Minimal chronic rejection	P	P	P
Median value	182	112					

* Oc = occluded, P = patent.

Biochemistry. In group A transaminase and serum lactic dehydrogenase levels rose to high values in the first month after transplantation. Most levels tended to normalize thereafter in all animals. In the two dogs that survived for more than eight weeks, normal values were reached in the end. Serum alkaline phosphatase concentration remained significantly elevated during follow-up period in all dogs.

In group B serum levels of transaminase ,lactic dehydrogenase and alkaline phosphatase were increased in all recipients following the operation. Although restoration to normal was observed in some animals, in others levels remained elevated throughout the experiment whether or not rejection was prominent.

Angiography. Intravenous angiography four days after transplantation was performed in five beagles in group A. The hepatic artery of the graft could be visualized in all cases and no stenosis was detected. The portal vein was depicted in three animals.

In group B seven dogs underwent intravenous angiography. The hepatic artery of the donor liver was patent in all cases while the portal vein was visualized in five dogs.

Cholangiography. Eleven animals underwent an additional cholangiography of the graft. In both groups one animal showed some leakage of the contrast material at the choledochoduodenostomy. At autopsy leakage of

saline injected through the bile duct cannula could not be demonstrated in these two animals. In the other dogs in both groups the choledochoduodenostomy was patent without stenosis or leakage.

Cholescintigraphy. Scintigraphy facilities were not available in group A. In all animals with a DLA-matched transplant (group B) HIDA-hepatobiliary scintigraphy was performed. Three grafts showed normal uptake of the isotope in the liver and excretion into the duodenum; at autopsy these dogs had vital grafts. In two dogs the liver grafts were able to concentrate the radiopharmacon but excretion into the bile system was poor or absent. Scintigraphy showed no graft function in the remaining four animals. All recipient livers could be visualized separately and had normal uptake and excretion .

Histopathological findings. In group A graft biopsies, taken at the end of the surgical procedure, showed minimal changes in the parenchyma consisting of degeneration of groups of hepatocytes. Architecture of liver parenchyma remained intact until necrosis of grafts occurred. One transplant underwent subtotal necrosis in the second postoperative week (Table 1). In five beagles total graft necrosis developed within two weeks. In the other two animals one graft was completely resorbed 12 weeks after the operation, while the other showed an estimated 60% necrosis at sacrifice 182 days after transplantation. In four grafts in this group, acute rejection was demonstrated, characterized by vasculitis and polymorph nuclear infiltration in the portal triads. One donor liver showed chronic cholangiolitis with infiltration of the ductuli by lymphoid cells. Cholestasis was absent in the histopathological slides of the grafts.

Histopathological studies in group B showed a different picture. In three recipients the grafts demonstrated normal hepatocytes with necrosis of less than five percent at the end of the experiment. These three transplants showed only minor signs of chronic rejection characterized by round cellular infiltration in portal triads and pseudo-bile-duct proliferation. Chronic graft rejection resulting in total necrosis of liver parenchyma occurred in four animals (Table 2). In one dog chronic rejection resulted in necrosis of an estimated 50% of hepatocytes at sacrifice. In three transplanted livers in this group signs of ascending cholangitis resulting in early biliary fibrosis were seen. Only one of these animals had a stenosis at the choledochoduodenostomy at autopsy.

The recipient livers in both groups showed no gross abnormalities in liver architecture and parenchyma.

Autopsy findings. Jaundice and ascites were absent in all animals at autopsy. The recipient livers were normal at macroscopic inspection in all cases.

In group A the donor liver was enlarged and congested in four dogs; these animals had died within three weeks after transplantation. The other grafts appeared to be small and partially necrotic, with multiple abscesses in one case. One liver graft was totally resorbed and no remnant could be found. In two animals all the vascular anastomoses were patent; in the other dogs one or more were occluded at autopsy (Table 1). In group B wet weight of the graft had decreased in eight dogs by 53.3±10.9% (mean±SEM) compared with operative values; in one recipient, however, the graft increased in size by 67.8%. The grafts were usually firm and in two dogs abscesses in the transplanted liver were seen. At the choledochoduodenostomy stenosis had occurred in two cases although dilation of the bile duct was only seen once. In six dogs patency of all vascular anastomoses was demonstrated (Table 2).

Discussion.

The results of this study demonstrate that auxiliary partial liver grafting in the dog can be performed without major intraoperative technical problems. The right subhepatic space offers enough room for a transplant consisting of 60% of the donor liver. At the end of the operation the abdomen of the recipient could be closed easily without any tension. The bare resection surface of the graft, created after removal of the two left lateral lobes, caused some blood loss at the end of the operation. Meticulous ligation of all visible vascular and biliary structures during bench surgery is essential.

Most theoretical requirements for well functioning of the graft, as stated by others (3-6), are met in this experimental study. Portal venous inflow was obtained directly from the portal vein. In the early series acute thrombosis of the portal vein, leading to acute death of two recipients, may have been caused by slight torsion of the donor portal vein after completion of the anastomosis. This complication was avoided during later experiments.

In our model the host portal vein was divided close to the liver hilum to ensure optimal portal blood flow through the graft. Although loss of portal blood flow through the recipient's own liver resulted in lack of so-called "hepatotropic" factors and can lead to atrophy (5), this was not apparent in the histopathological examinations in our experiment. In recipients with potentially reversible liver disease, the portal vein of the host liver needs not to be ligated, because an intrahepatic block,

present in most cases of chronic and acute liver failure (13), will probably ensure portal blood flow through the auxiliary grafts.
The histology of the dog liver differs from that of man in respect to smooth muscular sphincters in the hepatic venules (14). Increased vascular resistance in the outflow tract of the graft ascribed to these sphincters has been observed by others after auxiliary (15), as well as after orthotopic (16), liver transplantation. In our experiments this phenomenon of "outflow block" has not occurred once as congestion in the graft was never seen at operation.
The diameter of the common bile duct in the dog is small, not exceeding 4-5 mm. We therefore created a choledochoduodenostomy by using the pull-through technique as described in transplantation experiments in rats (17). Bile duct obstruction could not be demonstrated early after operation on cholangiography. Obstruction probably leading to ascending bacterial infection was demonstrated in one animal, and grafts in only three other dogs showed cholangiolitis without evidence of stenosis at the biliodigestive anastomosis. Therefore it is concluded that the pull-through technique is an adequate method in the dog .
The presence of the healthy liver of the recipient in our model made it difficult to diagnose early rejection or dysfunction of the graft. Biochemical and haematological values are not valid as an index of graft viability. Elevated liver enzym levels indicated ongoing necrosis of hepatic tissue as was demonstrated by histological findings. If the enzym levels return to normal, necrosis is less apparent or the graft is totally resorbed.
Cholescintigraphy with ^{99m}Tc-HIDA is a relatively simple method to assess graft function as has been shown in the second series of experiments. Graft rejection might also be predicted by this method (18).
It has been reported that blood transfusions given on the day of transplantation may have a beneficial effect on allograft survival (19,20). In our experiments an influence of blood transfusions on graft survival could not be demonstrated because the number of animals that received a blood transfusion was too small for statistical analysis.
In experimental orthotopic liver transplantation Calne found that liver allografts in pigs survived for considerable periods without rejection (8). Other investigators concluded that even in the case of a cross-match-positive donor, hyperacute rejection did not occur (21). In contrast to these observations, the auxiliary grafts in the non-tissue-typed combinations from our study were subject to severe immune attack. In the non-tissue-typed group graft survival for longer periods occurred only twice; as tissue typing was not performed in that group an accidental match between donor and recipient cannot be excluded.

It could be argued that operative experience accounts for the difference in the survival times of both experimental groups. However, several pilot-experiments preceded this study and the procedure of auxiliary liver transplantation was well established in our laboratory at the beginning of the reported experiments.Total operation time as well as duration of graft ischemia did not differ between the two series of experiments. Furthermore, the histopathological findings of acute rejection in the first group and chronic rejection in the second group provide in our opinion sufficient proof that the short-term graft survival time in the first group is caused by immunogenetic disparity, similar to that reported for kidney allografts and other tissues so far investigated to that purpose (22).

We think, therefore,that for liver transplants in the orthotopic and auxiliary position, histocompatibility differences are of key importance for the survival of the graft, and that liver tissue transplants obey the laws of immunogenetics just as those of other organs do (23,24). This is in contrast to findings in orthotopic liver transplantation in other models, in which histocompatibility matching seems to be of less importance (25,26).

Gugenheim and co-workers observed long-term graft survival in a non compatible rat donor-recipient combination after heterotopic liver transplantation and excision of the recipient's own liver (27). The contribution of a healthy recipient liver to the whole immune response system is still unknown. Even more obscure is the role of a diseased host liver in this respect. However, our findings indicate that the presence of an intact host liver did not prevent long-term graft survival in DLA-matched beagles.

A partially hepatectomized liver has a stimulatory potential on liver regeneration (28), that is effective even when taken from the perfusate of an isolated liver. In pigs transplantation of a part of the liver in the auxiliary position caused a fourfold increase in the host liver thymidine kinase activity (29), but an intact auxiliary transplanted liver caused less regeneration in the host liver. In view of these reports some regeneration might be expected in the longer-surviving animals in our model. We, however, did not observe an increase in size or numerous mitotic figures at sacrifice. It is a matter for further study whether ligation of the portal vein of the host liver might have prevented regeneration. If partial auxiliary liver transplantation indeed proves to stimulate regeneration of the autologous liver, then this would be an additional argument in support of this method for clinical purposes.

Histocompatibility matching is of key importance in this model, but for clinical purposes grafts that are identical for the antigens of the major

histocompatibility complex will almost never be available at short notice. It is questionable, however, whether such an identity is relevant for the immune-compromised liver failure patient. Furthermore, the use of the new immunosuppressant Cyclosporin A has been shown to overrule the effect of histocompatibility matching in renal transplant recipients (30), and it has improved the results of orthotopic liver transplantation in man (31). Therefore, further research on this subject may prove to provide practical possibilities for auxiliary partial liver grafting in patients with acute liver failure or end-stage chronic liver diseases, where orthotopic liver transplantation bears too many risks.

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CHAPTER 3

Second experiment

REJECTION AND SURVIVAL OF AUXILIARY PARTIAL LIVER GRAFTS IN NON-TISSUE-TYPED PIGS

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REJECTION AND SURVIVAL OF AUXILIARY PARTIAL LIVER GRAFTS IN NON-TISSUE-TYPED PIGS[1]

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Abstract

A technique for auxiliary heterotopic transplantation of 60% of the liver has been developed in the pig to study acute and chronic rejection. Transplantations were performed in 13 non-tissue-typed donor recipient combinations without immunosuppressive medication. Three pigs died in the first postoperative week from technical problems. In the remaining ten animals acute rejection of the graft was not found, but signs of chronic rejection developed in six animals. It is concluded that auxiliary partial liver transplantation is technically feasible in the pig. Although the auxiliary liver graft is subject to immune attack, long-term graft survival without immunosuppressive medication can be achieved.

Introduction

Auxiliary liver transplantation has not met with wide acceptance for the treatment of hepatic failure. Although the method is attractive because it avoids the necessity of removing the patient's own liver, the problems associated with the large size of the transplant and the considerable technical difficulty of establishing a satisfactory vascular circuit have so far limited its use.

Results of experiments in the dog as described previously indicated that auxiliary partial liver transplantation is technically and physiologically feasible in beagles. If 60% of a donor liver is transplanted auxiliary into the right upper abdomen and provided with portal venous and arterial blood, dogs survive for prolonged periods of time with vital grafts (18). In spite of considerable research on liver transplantation in the dog (16,20,22,6), this animal does not provide the ideal model for liver transplantation experiments. The anatomy of the dog liver differs from the human liver both macroscopically and microscopically: the lobes of the dog liver are well defined and separated by deep clefts. Resection of liver lobes aimed at decreasing the size of the graft, therefore, is easy in the dog, but less comparable to the human situation. Furthermore, sphincters are present around the liver venulae in the dog (5), and are absent in man.

Advantages of the use of the pig as laboratory animal in liver transplantation experiments include the similarity of the pig liver to the human liver with regard to the macroscopic and microscopic structure, the large size of the blood vessels facilitating hemodialysis and studies on extracorporeal hepatic support systems as therapy for ischemic liver disease (9), and the lower costs of purchasing the animal.

Our study was carried out to evaluate the feasibility of auxiliary partial liver transplantation in the pig and to assess the severity of rejection in auxiliary liver transplants in pigs, not tissue typed for the major histocompatibility system.

Material and Methods

Pigs. Female Yorkshire pigs, commercially obtained from one farm were used, weighing 28.5±0.9 kg (mean±SEM). Weights of donor and recipient animals were similar. Donors and recipients were not tissue typed for the

major histocompatibility system. Transplantation was performed in 13 donor-recipient combinations.

Surgical technique. Donor hepatectomy was performed using a conventional technique. The donor liver was perfused *ex vivo* by portal vein cannulation with 1 L Euro-Collins (4 °C). During bench surgery the two left lateral lobes of the liver were resected. All vascular and biliary structures in the plane of resection were carefully ligated. In addition, the raw liver surface was compressed by a continuous suture with atraumatic 2-0 Polydiaxanon (Ethicon ®).

The recipient procedure was started by introducing a polyethylene catheter into the internal jugular vein for fluid administration and blood sampling during operation. The catheter was passed subcutaneously to the back of the pig and exteriorized, facilitating postoperative blood sampling. In the first seven animals a Scribner shunt between carotid artery and external jugular vein was inserted as well, serving the same purpose.

The graft was transferred into the recipient´s right subhepatic space, and the following anastomoses were made: an end-to-side anastomosis between the donor suprahepatic vena cava and the recipient subhepatic vena cava, and an end-to-side anastomosis between the donor portal vein and the host portal vein, followed by ligation of the recipient portal vein in the liver hilum, rerouting the splanchnic blood flow through the graft. No

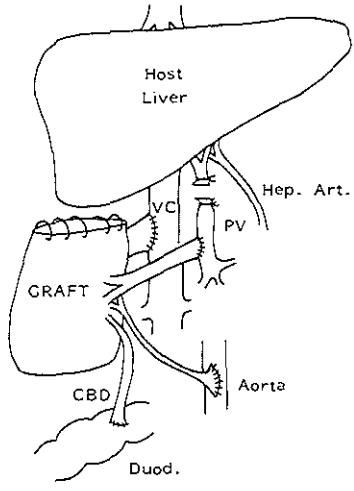

Fig. 1. Technique of auxiliary partial liver transplantation.

porto-systemic decompression shunt was used. The surgical procedure was completed by an end-to-side anastomosis between the donor hepatic artery and the aorta of the recipient, and an end-to-side choledochoduodenostomy (fig.1). In two pigs the host common bile duct was ligated and cut between ligatures as an additional procedure to cause hepatic injury in the host liver.

No immunosuppressive medication was used in these experiments.

Postoperative studies. Hemoglobin, platelets and leukocytes were determined weekly. Serum glutamic-oxaloacetic transaminase (SGOT), gamma glutamyl transaminase, and serum bilirubin were measured weekly.

Intravenous angiography by the same technique as described previously (1) was performed in the first week after surgery. Patency of the biliodigestive anastomosis was investigated by cholangiography.

^{99m}Tc-HIDA scanning under general anesthesia was performed in pigs surviving for at least four weeks. A Pho Gamma-III camera (Siemens, Gammasonics) with a low energy all purpose collimator assessed the uptake of the radiopharmacon after intravenous injection of 111 MBq ^{99m}Tc-HIDA.

Liver biopsies of host liver and the transplant were taken at surgery immediately after revascularization of the graft, in the first postoperative week, and at autopsy. All histological specimens were examined by the same pathologist on a blind observer basis. Cholestasis, cholangitis, vasculitis and inflammatory infiltrates of round cells and polymorphonuclear leukocytes were graded as being absent, mild, moderate, or severe. The degree of hepatocellular necrosis was estimated. Acute rejection was assessed by the degree of vasculitis, while chronic rejection was estimated by the degree of periportal inflammatory lymphoplasma cellular infiltrates and pseudo bile duct proliferation.

Graft survival was estimated by histopathological findings, cholescintigraphy, and angiography.

DNA and RNA content of the transplant at operation and at sacrifice was determined by using the method of Scott et al. (19).

As no alterations occurred in the clinical condition of those animals surviving for more than one month, an average one month observation period was taken as the cutoff point of follow-up. At the end of the experiment the animals were killed, or earlier as indicated by the clinical condition.

Results

Operative results and mortality. All animals recovered well from the surgical procedure. After resection of the two left lateral lobes the graft consisted of 61.2±2.3% (mean±SEM) of the original liver weight. Total ischemic time of the transplanted liver was on average 140 min with a mean warm ischemia time of 57 min. The portal vein was occluded during 14 min. All grafts regained a normal color rapidly after recirculation. Closing of the abdomen was possible without tension in all pigs. The mean loss of blood during transplantation was 480 ml. Transfusion of 400 ml blood from the donor animal was given during surgery.

Postoperative course. Three pigs died in the first postoperative week. One death was caused by arterial bleeding from an accidentally disconnected Scribner shunt. The other two pigs died from air embolism through the central venous line.

Table I. Animal survival, graft survival, and histological findings at autopsy in pigs after auxiliary partial liver transplantation.

Pig No.	Survival (days)	Graft survival (days)	Hepatocellular necrosis	Cholangiolitis	Inflammatory infiltrations
1	9	9	0	0	+, L
2	18	18	0	0	0
3	62	62	0	+	+, L
4	8	8	0	+	++, P
5	42	7	++++	impossible to assess	
6	25	25	0	0	+, L
7	33	7	+++	++	+++, L
8	36	14	+++	+	++, L, P
9	30	4	++++	impossible to assess	
10	26	26	++	++	+++, L

0 = absent; + = mild; ++ = moderate; +++ = severe; ++++ = total necrosis; L = lymphoplasmacellular infiltration; P = polymorphonuclear infiltration predominant; L, P = mixed cellular infiltration.

Survival time in the remaining ten animals is depicted in Table I. Median animal survival was 28 days. In both pigs with ligation of the host common bile duct, infectious complications contributed to death. Autopsy

revealed very dilated bile ducts, and biopsies showed cholangiolitis, cholangitis, and an inflammatory infiltrate of polymorphonuclear leukocytes throughout the host liver parenchyma.

Two pigs died 9 and 26 days after operation of intestinal strangulation and pneumonia respectively. Two animals had to be killed because of deteriorating clinical condition; in one case this was caused by graft necrosis while the other pig had bilateral pneumonia. Four pigs were killed in excellent condition.

Biochemistry. The hemoglobin content in the first postoperative week did not change from preoperative levels. Only in two pigs anemia occurred two and four weeks postoperatively. Leukocyte counts rose immediately after transplantation to a maximum of 26 x 10^9/L (normal values 15±5 x 10^9/L). In the week following surgery the values returned to normal. Platelet counts did not change significantly after surgery. Serum transaminase levels were elevated in most animals during follow-up studies. In the first postoperative week three to four times normal values were reached that tended to normalize thereafter. Serum bilirubin was elevated in one of the pigs with ligated common bile duct, but remained normal in the other animals.

Angiography. In eight of the nine animals that underwent intravenous angiography one week after surgery, visualization of the hepatic artery of the graft was obtained without signs of stenosis in seven animals. In one pig stenosis at the site of the anastomosis of the hepatic artery and the aorta was followed by occlusion of the hepatic artery as proven at autopsy. In one animal the hepatic artery was not detectable on the intravenous angiogram, although it was found to be patent at autopsy two days later. The portal vein was visualized in six cases. In the other pigs patency of the portoportal anastomosis was demonstrated at autopsy in three; in one animal, however, the portal anastomosis revealed no abnormalities but the intrahepatic branches of the portal vein were occluded by thrombi.

Cholangiography. By injection of contrast medium through the cannula in the cystic duct of the graft cholangiography was performed in nine pigs. No leakage or stenosis was observed at the choledochoduodenostomy. Passage of contrast medium into the duodenum was shown in all cases.

Cholescintigraphy. Hepatobiliary scanning was performed in four pigs at one month and in one pig two months after surgery. The other five pigs died before a scintigram could be made. Uptake of the radiopharmacon by the graft was excellent or fair in three cases and absent in two animals. Autopsy, however, revealed patent vascular anastomoses and branches in the grafts of these two latter pigs. Excretion of the radiopharmacon into the duodenum was excellent in only one animal.

Histology and autopsy findings. Graft specimens that were taken after recirculation did show only mild histological changes. One week postoperatively a percutaneous needle biopsy was taken from the grafts. Inflammatory infiltration of vessel walls and cholestasis were minor or absent in these biopsies. Only one specimen showed almost complete necrosis of liver tissue. Acute rejection as indicated by vasculitis was absent at this stage of the experiment. In three pigs inflammatory infiltrates of both round cells and polymorphonuclear leukocytes could be seen ranging from moderate to severe.

At autopsy the macroscopic appearance of all host livers was normal except in the two cases of common bile duct ligation (pigs no.2 and 4) where gross dilation of extrahepatic bile ducts was present. Ligation of the host common bile duct caused severe cholangitis and extensive hepatocellular necrosis. In the other host livers histological examination did not reveal major changes, and cholestasis was absent.

At autopsy signs of chronic rejection in liver transplants as indicated by the degree of lymphoplasma cellular infiltration (Table I), could be demonstrated in six animals ranging from mild (fig.2) to severe (fig.3). In two pigs the degree of cholangitis and cellular infiltration could not be estimated because total graft necrosis existed. In the pig that died 18 days after transplantation with a ligated host common bile duct, the transplant appeared to be completely normal without any sign of rejection at all. Different degrees of cholangitis were present in almost all grafts at autopsy, although cholestasis was not prominent.

In only two pigs (no.5 and 9) occlusion of graft vessels had occurred in the follow-up period resulting in total graft necrosis. All other vascular anastomoses and graft vessels were patent at autopsy. Two pigs (no.1 and 4) died from intestinal strangulation and septicemia, but their grafts at autopsy were normal both macroscopically and microscopically.

Graft survival. Estimated graft survival as compared with animal survival and histological findings at autopsy is depicted in Table I. The wet weight of the liver transplant at autopsy had increased 56.1±18.1% (mean±SEM) when compared with operative values in six pigs. Increase in wet weight of the liver transplant correlated well with the viability of the graft. Decrease of the transplant liver wet weight in the other animals was 32.0±9.9%. Median graft survival was 11.5 days (range 4-62 days).

Nucleic acids. DNA and RNA contents of liver grafts at operation and at sacrifice were calculated in the three transplants surviving more than 25 days. In these allografts the total DNA and RNA content of the liver increased with 50.3±28.0% and 33.3±18.7% respectively, indicating regeneration of the graft liver tissue.

Fig. 2. Graft biopsy specimen at autopsy 62 days after transplantation. Mild lymphoplasma cellular infiltration is present, HE, x60.

Fig. 3. Severe plasmacellular infiltration in graft biopsy 26 days after transplantation. HE, x60.

Discussion

Orthotopic liver transplantation in patients with fulminant hepatic failure is rarely successful because of massive intraoperative bleeding, postoperative cardiorespiratory failure, and fulminant sepsis (17). A suitable alternative procedure for such patients is auxiliary liver transplantation. However, experience with auxiliary liver transplantation has so far not been encouraging: of 52 reported cases only two patients have survived for more than 5 years (15,8). The main technical difficulty in heterotopic intraabdominal liver grafting in clinical and experimental studies seems to be the problem of space in the abdomen of the recipient (2). Crosier and co-workers demonstrated that auxiliary transplantation of the whole liver is technically feasible in the pig, if the body weight of the donor animal is about half the weight of the recipient (4). Our results indicate that auxiliary transplantation of part of the liver reducing the problem of space is successful. Furthermore, the partially hepatectomized liver releases regeneration stimulating substances (14). In the presence of a diseased host liver the partial liver transplant might thus help to induce regeneration in the host liver.

Portal inflow and a caval implantation of the graft as close as possible to the heart to ensure a low outflow pressure are essential for long-term graft function (12,13). These two technical requirements were established in our study.

In both experimental and clinical liver transplantations there has been a high incidence of serious and fatal complications arising directly from the reconstruction of the biliary tract (21,23). Many techniques have been invented to resolve this problem. In our experiment no serious complications could be detected after reconstruction of the biliary tract by end-to-side choledochoduodenostomy. This favorable outcome is probably the result of anastomosing a distal part of the duct that has an adequate blood supply; in our opinion it is mandatory to shorten the bile duct till it bleeds before performing the biliodigestive anastomosis.

In two pigs the host common bile duct was ligated to impair host liver function. In both cases serious complications were seen in the postoperative period. Although others have successfully used this technique in different species to place the graft in a favorable position (10,11), septic complications are probable, and we abandoned this procedure.

Relative disadvantages of the use of the pig as experimental animal consist of difficulty in blood sampling by venipuncture and the high incidence of pulmonary infection as reported after liver transplantation

in this animal (24).

Introduction of a Scribner shunt to circumvent problems in obtaining blood samples resulted in accidental bleeding and death of one pig in the postoperative period, although the shunt was placed under a specially designed jacket. In the first days after transplantation shunt occlusion occurred frequently and, therefore, the Scribner shunt as a vascular access was no longer used in the further experiments.

A catheter introduced in the internal jugular vein and exteriorized to the back of the pig functioned adequately in most animals. Blood sampling and administration of antibiotics and transfusions were easy. However, the long-term presence of a central venous line carries with it the hazard of air embolism.

Three animals had bilateral pneumonia after operation, resulting in deterioration of clinical condition and leading to the death of the pigs despite antibiotic treatment. The high incidence of infection will lead to postoperative mortality, especially if immunosuppressive regimens are required.

The value of biochemical results in auxiliary partial liver transplantation in the presence of a host liver that is only deprived of portal blood is limited. Rejection could not be predicted by any of the used biochemical parameters, but must be confirmed by histological examination of liver biopsies.

Intravenous angiographical findings in the early phase of the follow-up period correlated well with the findings at autopsy. Cholescintigraphy proved to be useful in predicting graft function in one pig where uptake of the radiopharmacon in the liver and excretion into the duodenum was seen. In the other animals no stenosis was shown at the biliodigestive anastomosis during cholangiography nor at autopsy. The results of cholescintigraphy, therefore, seem to indicate abnormalities at the cellular level of the hepatocytes.

Although immunosuppressive medication was not given, chronic rejection occurred more frequently than expected on the basis of the results of orthotopic liver transplantation in pigs where liver transplants may survive for considerable periods of time without rejection (3). Acute rejection, however, could not be demonstrated, and this is in contrast to our observations in dog experiments (18) where auxiliary grafts were vigorously rejected in non-tissue-typed donor-recipient combinations receiving immunosuppressive medication. The auxiliary liver grafts in the present experiments, therefore, seem to be less subject to acute rejection than in the dog. If the two pigs that died at eight and nine days with macroscopically and microscopically healthy liver grafts are taken into account, then the number of animals with grafts surviving the period of

acute rejection is remarkably high. Incidental histocompatibility between donor and recipient caused by a high degree of inbreeding in this strain can explain this observation. Tissue typing for the major histocompatibility system in pigs has been reported to be of influence on liver graft survival (7). Matching, therefore, may probably result in better long-term acceptance of liver allografts, reducing the need for immunosuppressive therapy and thus decreasing the risks of septic complications.

As a result of the presented experiments it seems justified to further explore the possibilities of an auxiliary partial liver graft in tissue-typed pigs with induced failure of the recipient liver.

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CHAPTER 4

Third experiment

A REPRODUCIBLE MODEL OF ACUTE HEPATIC FAILURE BY TRANSIENT ISCHEMIA IN THE PIG.

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A REPRODUCIBLE MODEL OF ACUTE HEPATIC FAILURE BY TRANSIENT ISCHEMIA IN THE PIG[1].

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Abstract

A model of transient acute hepatic failure has been developed in the pig. Three days after construction of a functional end-to-side portacaval shunt, 15 ambulant animals underwent total liver ischemia for four or six hours by the closure of a mechanical occluder surrounding the hepatic artery. Four of the eight animals subjected to four hours of ischemia survived. All but one of the animals undergoing six hours of hepatic ischemia developed grade 4 encephalopathy after 24 to 30 hours and died within 50 hours. Quantitative estimation of liver cell necrosis revealed less than 40% necrosis in the survivors, and approximately 62% (range 49-75%) in animals who died of hepatic coma. As far as the putative toxins are concerned, significant differences were found between animals undergoing four and those undergoing six hours of ischemia, especially in the plasma ammonia levels and the plasma ratio's for tyrosine and phenylalanine. Plasma arginine levels had fallen to zero in both groups at 24 hours and only rose to pre-ischemic values in animals who survived. This large animal model fulfills the accepted criteria of potential reversibility, reproducibility and death due to hepatic failure.

Introduction

To evaluate new therapies for human fulminant hepatic failure a suitable animal model is urgently needed. Surgical models such as hepatectomy or a portacaval shunt with permanent ligation of the hepatic artery in one or two stages, are not ideal because they lack potential reversibility[1,2,3,4]. To overcome these problems Misra and Fisher created a model of reversible hepatic ischemia in ambulant conscious dogs and pigs, respectively[5,6]. Sixty minutes of hepatic ischemia were always fatal to dogs. For pigs, the minimum period of hepatic ischemia required to produce death due to hepatic coma could not be estimated. According to other investigators the period of hepatic ischemia tolerated by pigs varied between 35 and 180 minutes[7-10]. Hepatic encephalopathy however could not be induced in these studies, since the animals either survived or died without a definite period of clinically manifest neurological abnormalities.

The aim of our investigation was to develop a model of acute hepatic failure by inducing temporary hepatic ischemia in fully ambulant pigs, and to describe the clinical, biochemical, hemodynamic, histological and electrophysiological features of this model.

Methods

Preparatory surgery. Fifteen healthy Yorkshire pigs, weighing 28-33 kg, were used. One and two days before surgery, the bowel was cleansed by the oral administration of 25 g of magnesium sulfate and 150 ml of lactulose. Anesthesia was induced with an intramuscular injection of ketamine chloride (35 mg/kg). The animal was intubated and connected to a ventilator; anesthesia was maintained with a mixture of nitrous oxyde-oxygen and enflurane. The anesthetized pig was placed on the operating table in a supine position. A Scribner shunt was inserted between the carotid artery and the external jugular vein for pressure monitoring and blood sampling. The internal jugular vein was cannulated with a polyethylene cannula for infusion of fluids. After opening the abdomen with a long midline incision the liver was freed by dissecting the triangular ligaments, the falciform ligament and all peritoneal attachments of the liver. All structures in the hepatoduodenal ligament except the portal vein, the hepatic artery and the common duct were

devided. Blood vessels running along the vena cava into the liver at the level of the diaphragm were interrupted by diathermia.
A side-to-side portacaval shunt was made, followed by ligation and transection of the portal vein close to the hilum to create a functional end-to-side shunt. A specially constructed vessel occluder (fig.1) and a perivascular electromagnetic blood flow sensor (Skalar Instruments, Inc., Delft, the Netherlands) were positioned around the isolated hepatic artery; they were anchored to the abdominal wall and the leads were guided through the skin via separate incisions. The occluder and flow probe were tested during surgery by tightening the occluder, which resulted in a total flattening of the blood flow curve on the oscillosope (fig.2).
The common bile duct was opened and a silicone tube was inserted. This tube was firmly attached by two 2-0 silk ligatures around the proximal and distal ends, thus preventing blood flow through the wall of the common bile duct to the liver.
Subsequently five silver electrodes (diameter:1 mm) were positioned upon the dura, anchored in the skull with acrylic bone cement, and channeled percutaneously; two electrodes were placed above the frontal cortex, one in the vertex and two above the occipital cortex. With this technique artefact-free registration of the electroencephalograms was possible. After discontinuation of anesthesia the animals were kept on the operating table until they were awake, breathing adequately and restoring their body temperature.
Throughout the surgical procedure 0.9% NaCl was administered; just before construction of the portacaval anastomosis an additional dose of 400 ml of Haemaccel^{R} was given intravenously. At the beginning of surgery and immediately afterwards ampicillin (0.5 gr) and kanamycin (0.5 gr) were injected intravenously.
Induction of ischemic necrosis. Three days after construction of the portacaval shunt the normothermic animals, fully awake, were fixed on a table with two cotton sheets. The animals accepted this procedure. Ischemic hepatic necrosis was induced by thightening the occluder around the hepatic artery; arrest of hepatic blood flow was confirmed by total flattening of the flow curve on the oscilloscope (fig.2). Arterial occlusion was maintained for four and six hours, respectively. To validate the total devascularization of the liver, two animals underwent aortography and selective angiography of the hepatic artery. After unlocking the occluder, restoration of blood flow through the hepatic artery was confirmed by continuous registration of the electromagnetic flow measurements.

Fig. 1. View of the vessel occluder used to occlude the hepatic artery of fully alert pigs.

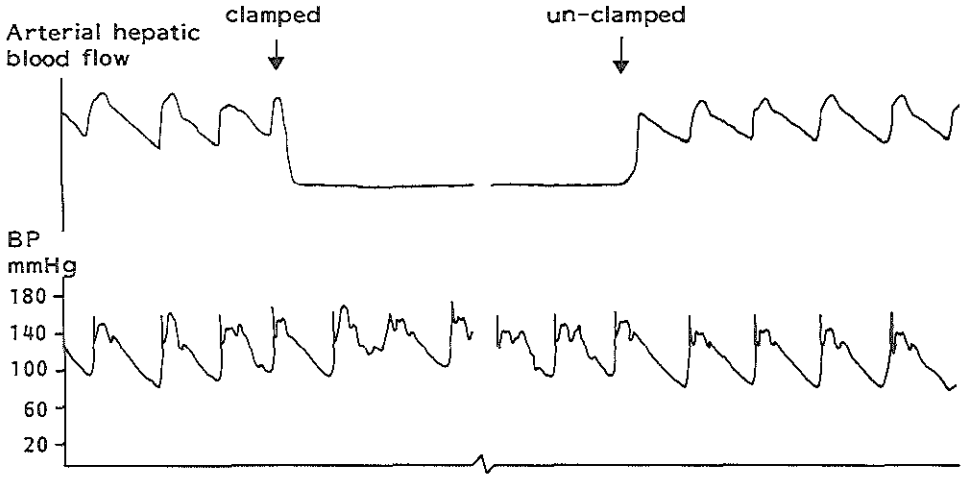

Fig. 2. Blood flow curve of the hepatic artery and systemic blood pressure before, during and after occlusion of the hepatic artery.

All animals received 35 ml of 8.4% sodium bicarbonate within 15 minutes of declamping, followed by a continuous infusion of glucose (12 g/kg/24 hr). Penicillin G (9 mega U/24 hr), kanamycin (3 g/24 hr), potassium and phosphate (37 mmol/24 hr and 45 mmol/24 hr, respectively) were added to the glucose. After the ischemic period, the animals were placed in special cages in which they could move around without disturbing their continuous infusion. Heart rate and mean arterial blood pressure, recorded with an electromanometric transducer, were registered continously. Temperature was measured with a tele-thermometer.

Neurologic assessment. The behaviour of the animals after temporary ischemia of the liver was checked frequently. Standard auditory and pain stimuli were administered regularly and the responses were graded as follows: 0=absent; 1=dubious; 2=present. Spontaneous grunting and muscular rigidity were also noted. The duration of survival was defined as the period between the induction of hepatic ischemia and the time of death.

Electroencephalograms were made before induction of hepatic necrosis and repeated at 24, 30, 48, 54 and 72 hrs. Four bipolar tracings (left and right fronto-occipital, fronto-frontal and occipito-occipital leads) were recorded on a Gogh apparatus (Ahrend van Gogh, Amsterdam, the Netherlands). The EEG recordings were analyzed independently by an electroneurologist; the 5-grade classification described by Opolon was used[11].

Biochemical measurements. Blood samples were taken before and 24, 30, 48, 54 and 72 hrs after induction of ischemic hepatic necrosis. In addition blood for acid-base status and coagulation studies was also drawn 0.5 hr and 1-3 hrs after release of the vessel occluder. Blood glucose, sodium, potassium, urea, pH, pO2 and pCO2, SGOT, bilirubin, bile acids, platelets and clotting factors (fibrinogen, Normotest^{R}, activated partial thromboplastin time) were measured by standard laboratory techniques. Blood samples were cultured in 60 ml of trypticase soy broth at 37° and observed for 2-3 days.

Ammonia was measured by an enzymatic method[12]. Plasma amino acid profiles were determined with a LKB-4400 amino acid analyzer (LKB Biochrom. Ltd, Cambridge, England) in plasma supernatant which had been rendered protein free by treatment with sulfosalicylic acid 75% w/v. The results for the neutral amino acids threonine, valine, leucine, isoleucine, tyrosine, phenylalanine, tryptophan, methionine and histidine are not expressed as simple concentrations but as plasma ratio's. The individual amino-acid ratio (for instance threonine) can be calculated as follows: THR/VAL + LEU + ILE + TYR + PHE + TRY + MET + HIS + THR. These neutral amino acids are transported by the same transport system in the blood-brain barrier[13].

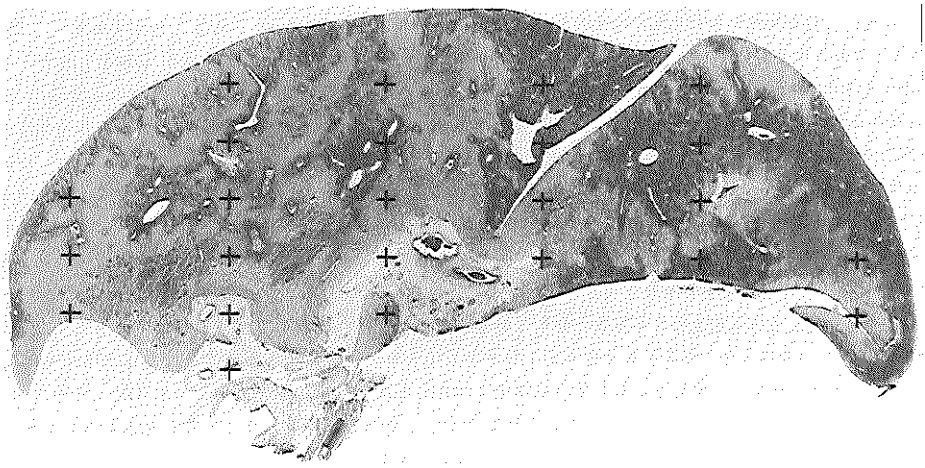

Fig. 3a. A transverse cut of the liver (real size) with 25 points chosen at random for analysis of hepatic necrosis.

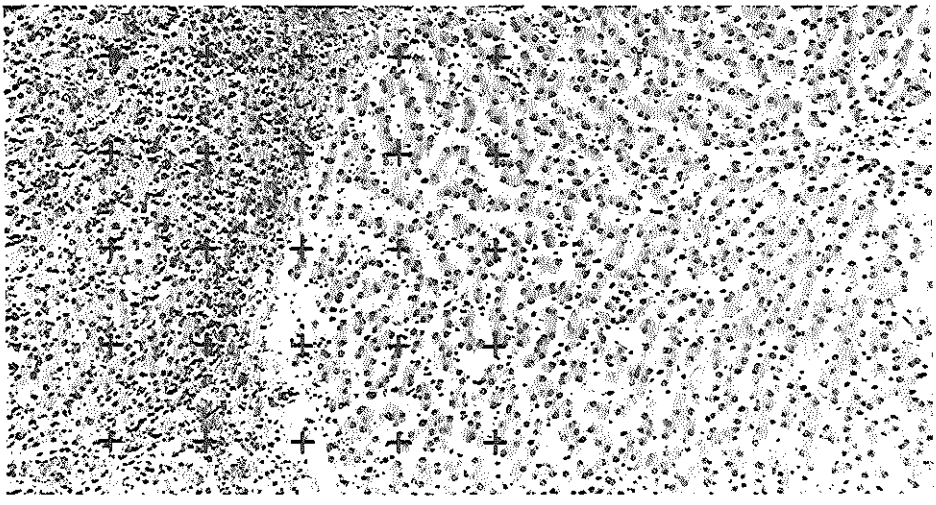

Fig. 3b. A magnification (objective 10x) of one area-point which again is devided into 25 points for analysis.

The ratio of the individual amino acids will reflect the influx of amino-acids into the brain.

Morphology. Postmortem examinations were performed in all cases as soon as the animal died. After macroscopic inspection of the heart, lungs, kidney, stomach and portacaval anastomosis, the liver was removed. Two one centimeter thick transverse slices from the upper and lower part of the liver were fixed in 10% formaldehyde. After fixation, 7μ sections of each complete transversal liver slide were stained with hematoxylin-eosin, PAS or gallocyanine. The degree of hepatic necrosis in each slide was estimated by point analysis (fig.3a,b); for this purpose 625 areas chosen at random were assessed for liver cell necrosis (defined as apparent disappearance of hepatocytes or eosinophilic condensation in the cytoplasm with nuclear pyknosis).

Statistical analysis. Data are expressed as mean values $\pm$ SD. For unpaired samples with a normal distribution of data points, the Student's t-test was used, while the Wilcoxon rank sum test was used in the event of a skewed data profile. Differences were assumed to be statistically significant when the p-values were less than 0.05.

Results

Adequacy of the ischemic procedure. For all animals, there was a total flattening of the blood flow curve on the oscilloscope during the ischemic period. Aortography and selective angiography of the liver of two animals showed complete devascularization of the liver. After release of the clamp, flow through the hepatic artery was restored in all animals as demonstrated by flow measurements (fig. 2).

Survival. After four hours of ischemia, four out of eight pigs survived (fig.4). Two animals died soon after hepatic ischemia due to technical complications (broken intravenous tubing and bile leakage from the tube); two other animals, that died 26 and 51 hours after ischemia, were found to have a positive blood culture with E.coli. After six hours of ischemia, six of the seven animals died within 50 hours; one animal survived for 72 hours with a grade 4 encephalopathy. In four of the six animals death was due to hepatic coma; in the two remaining animals death was precipitated by a bleeding gastric ulcer.

Neurological assessment. The surviving animals undergoing four hours of ischemia did not show marked abnormalities in behaviour. Immediately after revascularization of the liver most pigs were ambulant and alert, although some appeared excited. The first abnormality observed in animals

that ultimately died was usually an ataxic gait and impaired balance. The animals swayed from one side to the other; this was followed by drowsiness lasting several hours. Within 28 hours, pain sensations and spontaneous grunting had decreased markedly. After loss of sensation coma developed between 24 and 30 hours after hepatic ischemia and was accompanied by muscular twitching of the neck and limbs, and later rigidity. Some hours before death, tachycardia and hyperventilation were noted. Terminally, there was gasping with cyanosis, vomiting and hypotension.

The courses of EEG grades for animals undergoing four and six hours periods of hepatic ischemia are shown in figure 5. The EEG grades became more abnormal in both groups after 24 hours. In general, the EEG changes deteriorated very slowly in 'four-hour pigs'; only one animal had reached grade 4 encephalopathy at 48 hours. 'Six-hour pigs' showed a rapid deterioration of the EEG to grade 4 encephalopathy between 24 and 30 hours after the ischemic period.

General and biochemical measurements. Heart rate and mean systemic blood pressure remained fairly constant, except during a short period immediately after revascularization of the liver (mean arterial blood pressure decreased by 20 mm Hg, and there was a mean increase in the heart rate of 18 beats per minute). Hypothermia did not develop during the experiments. Metabolic acidosis was observed after revascularization, but was easily corrected by administration of sodium bicarbonate (fig. 6). The levels of plasma glucose, potassium and sodium remained within the normal range, also after release of the vessel occluder. Plasma SGOT levels reached a maximum at 24 hours; 'four-hour pigs' had significantly lower levels (2073 $\pm$ 817 IU/l) at 24 hours than 'six-hour animals' (3259 $\pm$ 1600 IU/l).

Coagulation factors (fig. 7). A remarkable decrease in platelets was observed after revascularization of the liver; the lowest value (66 x 10^9/l) was recorded three hours after revascularization. Platelet concentration increased gradually in 'four-hour pigs' but remained low in animals that underwent six hours of ischemia. The same course was observed for coagulation factors (Normotest^R, activated partial thromboplastin time and fibrinogen level).

Putative toxins (fig. 8). Plasma ammonia was only moderately elevated at 24 hrs (162 $\pm$ 86 umol/l) in 'four-hour pigs', in contrast to the levels found in animals undergoing six hours of ischemia: 283 $\pm$ 113 umol/l at 24 hours.

The plasma ratio's for leucine, isoleucine and valine decreased to a minimum level at 24 hrs after the ischemic period, without any difference between the four and six-hour groups (leucine from 20 to 12; isoleucine

from 15 to 8, and valine from 32 to 20). The plasma ratio's for methionine, tyrosine and phenylalanine increased in both groups. In the 'four-hour group', the plasma ratio's for tyrosine and phenylalanine normalized to pre-ischemic values after 48 hours, while the values for the six-hour group remained significantly higher at 30 and 48 hours. Significant differences in the ratio for tryptophan were not found. With this relatively insensitive method, GABA levels could not be detected in plasma exept in two animals (1.6 - 8.0 nmol/l).

Urea cycle amino acids (fig.9). In both groups serum arginine levels had dropped to zero 24 hrs after the ischemic period. The plasma arginine levels gradually returned to the initial values in all but one of the 'four-hour animals', while remaining zero in six of the seven animals that underwent six hours of ischemia. The plasma ornithine and citrulline levels increased in both groups but the rise was only significant in 'six-hour animals'.

Histology (table I). In 'four-hour pigs' a mean of 42% of liver cells showed total necrosis. In those who died, more than 50% of the liver cells were necrotic, and in those two survived less than 40%. Six hours of ischemia resulted in a mean necrosis of 62% (range 49-75%). Necrosis of 50% or more resulted ultimately in hepatic coma in nine out of thirteen animals. It should be mentioned that beyond the necrotic areas large fields of degenerated hepatocytes were always seen; these cells were not included in the quantative assessment of liver cell necrosis.

Table 1. Quantitative assessment of liver necrosis by point analysis of whole liver slices.

	4 hrs of liver ischemia		6 hrs of liver ischemia	
	survival(hrs)	% necrosis	survival(hrs)	% necrosis
pig no 1	72	15	20	70
2	26	68	20	66
3	10	67	29	49
4	72	34	72	50
5	72	36	50	50
6	72	34	43	75
7	6	*	30	69
8	51	*		

* : no histology available

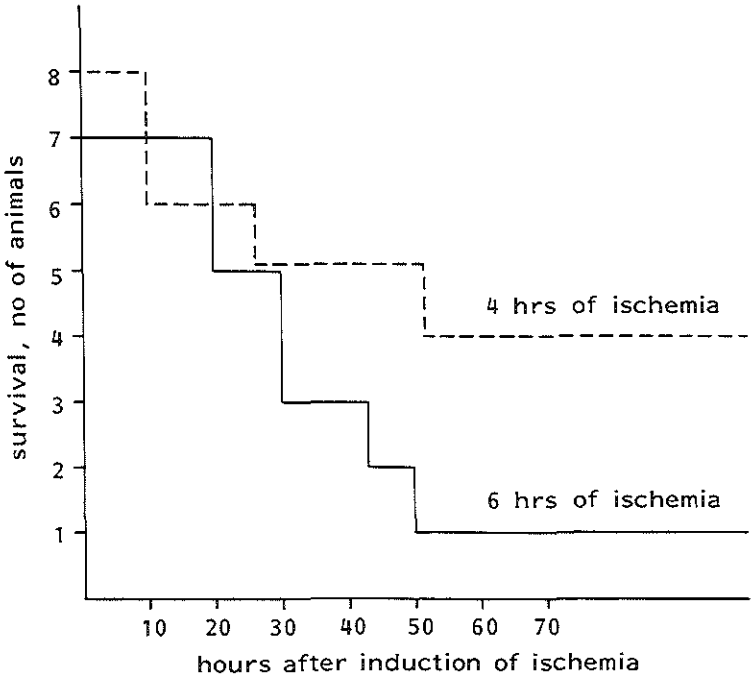

Fig. 4. Survival after induction of transient liver ischemia (4 hrs or 6 hrs of ischemia) in normothermic pigs.

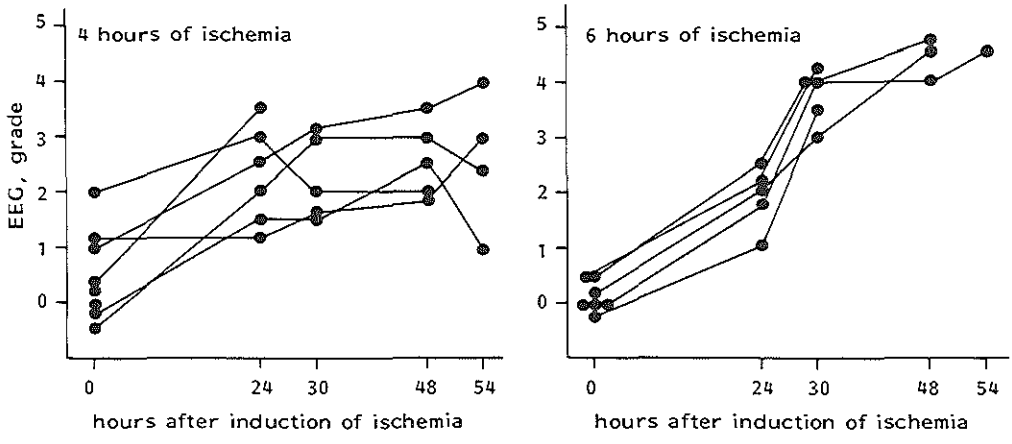

Fig. 5. The course of EEG grades in pigs after 4 and 6 hours of transient ischemia of the liver. The 6-hour animal, that survived for 43 hours, has been included in the 48 hour group.

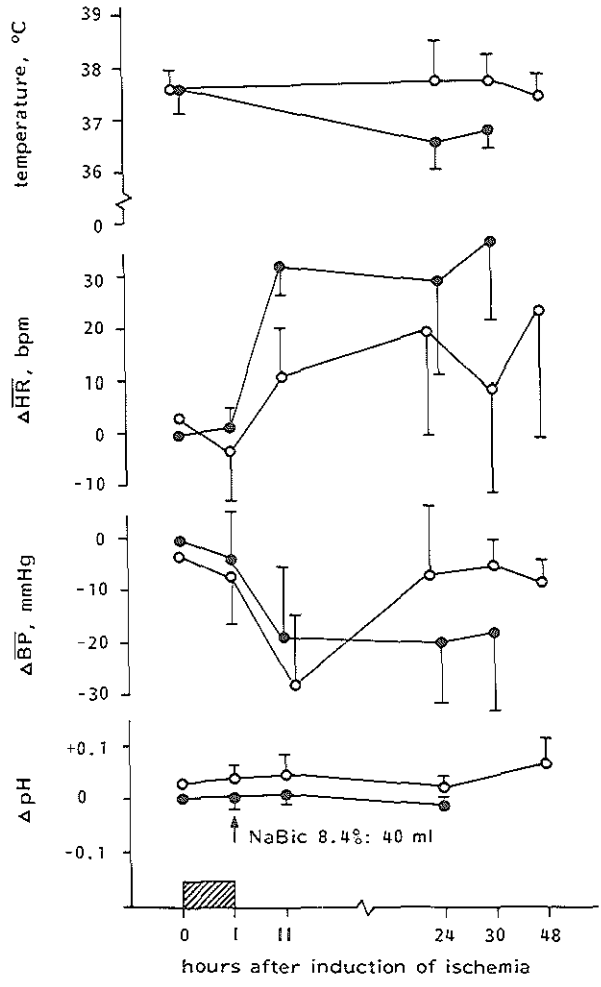

Fig. 6. Effect on temperature. heart rate (HR), blood pressure (BP) and pH (mean ± SD) of 4 hrs of liver ischemia (o-----o), and 6 hrs of liver ischemia (●-----●).
I : just before revascularization of the liver.
II: 30 minutes after revascularization of the liver.

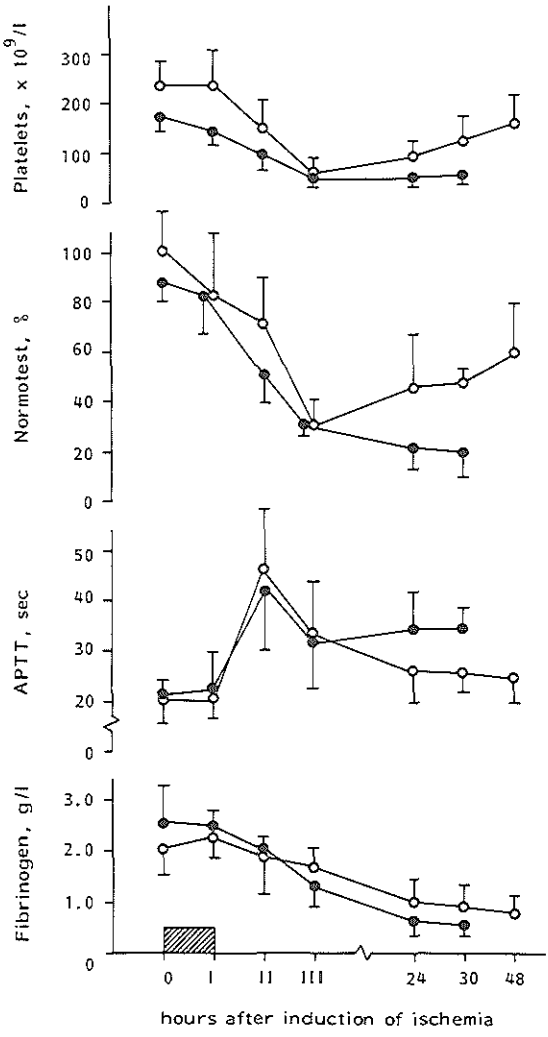

Fig. 7. Platelet counts, Normotest^{R}, and APTT and fibrinogen levels (mean ± SD) after 4 hrs of liver ischemia (o-----o), and 6 hrs of liver ischemia (●-----●).
I : just before revascularization of the liver.
II : 30 minutes after revascularization of the liver.
III: 3 hours after revascularization of the liver.
* $p < 0.05$, comparison between '4 hour animals' and '6 hour animals'.

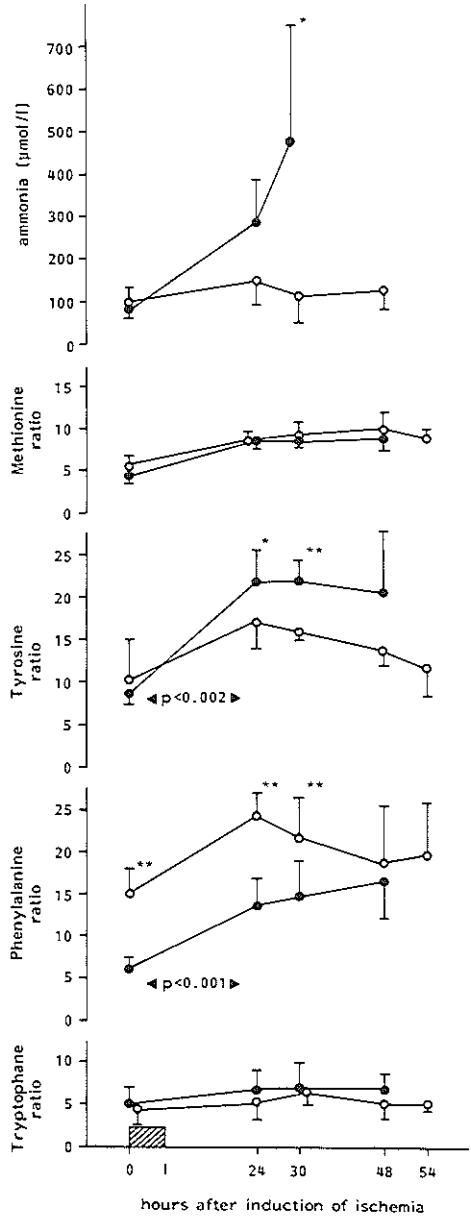

Fig. 8. Plasma ammonia concentrations, and methionine, tryosine, phenylalanine and tryptophane ratio's (mean ± SD) in pigs subjected to 4 hours of liver ischemia (o-----o), and to 6 hours of liver ischemia (●-----●).
* $p < 0.05$, ** $p < 0.01$, comparison between '4 hour animals' and '6 hour animals'.

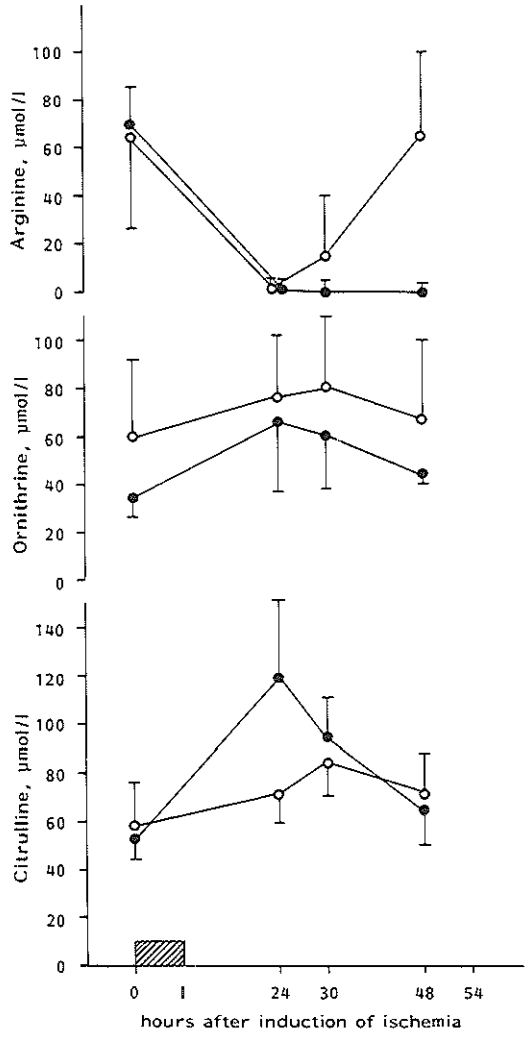

Fig. 9. Plasma arginine, ornithine and citrulline concentrations (mean ± SD) in pigs after 4 hours of liver ischemia (o-----o), and after 6 hours of liver ischemia (●-----●). * $p < 0.05$, ** $p < 0.01$, comparison between '4 hour animals' and '6 hour animals'.

Discussion

Our experiments show that ischemia of the liver in fully ambulant normothermic pigs was often tolerated for four hours, but that six hours of hepatic ischemia was usually followed by hepatic coma and death. Previous studies had failed to identify the minimum ischemic period required to produce hepatic coma in non-anesthetized pigs mainly because of poor reproducibility[6]. Our results, however, confirm several recent reports that the normothermic liver appears to be more resistant to ischemia than previously appreciated[9,10].

Except for the duration of transient hepatic ischemia other factors may influence the effect of hepatic ischemia on survival. Anesthesia with its variations in duration and depth and the variable metabolic disturbances associated with surgery may modulate the extent of liver damage and thereby the final outcome[5]. Therefore, in contrast to most previous studies[7,10], we induced transient hepatic ischemia in the pig after the effects of anesthesia and surgery had disappeared.

The time interval between initial surgery and the induction of liver ischemia is said to influence the extent of liver cell necrosis due to the formation of collaterals[6,14]. Since we had nearly always observed encephalopathy and death due to massive necrosis of the liver in earlier experiments with non-surgical induction of permanent hepatic ischemia[15], we have continued to use a time interval of three days between initial surgery and the non-surgical induction of hepatic ischemia. A potential source of collateral circulation in a model based on transient ischemia is the wall of the common bile duct. This structure contains a network of blood vessels which can supply an appreciable amount of blood to the liver. Therefore a short piece of tubing was placed in the common bile duct and the blood flow along the common duct was interrupted by two ligatures around the tube ends.

Another factor that could affect liver cell necrosis and thus the duration of tolerable liver ischemia is the amount of putative toxins that appear in the anhepatic state. Extensive bowel cleansing before induction of hepatic ischemia and an adequate supply of calories afterwards were included in our protocol in an attempt to minimize the disturbances of the 'milieu intérieur'.

Is this an adequate model of acute hepatic failure? The requirements for a satisfactory animal model of acute hepatic failure, as compiled by Terblanche include: (1) potential reversibility; (2) reproducibility; (3) death due to hepatic coma after elapse of a time period sufficiently long to allow hepatic support procedures to be instituted; (4) the use of

a large animal; (5) induction of liver necrosis without biohazard[4].

Reversibility. Since hepatic circulation is restored in our model (albeit only through the hepatic artery), the potential for recovery and regeneration is present. Histologically six hours of hepatic ischemia resulted in necrosis less than 75% of the liver cells. None of the animals had a totally (90-100%) necrotic liver. Therefore, at least 25% of the liver tissue remained available for recovery and possibly regeneration, assuming that the majority of cells in various stages of degeneration retain the potential of recovery in a normal 'milieu intérieur'[17,18].

Reproducibility. All animals subjected to six hours of ischemia developed severe encephalopathy (EEG grade 4) within 30 hours and died within 20 to 50 hours, except for one animal that survived for 72 hours. The histological data showed necrosis of 50-75% of the liver and all biochemical measurements, including analysis of putative toxins were fairly uniform. The variations in observations which are inherent to any biological experiment appear less prominent in our model than in most models for drug-induced acute hepatic failure[19,20].

Death from liver failure. Within 30 hours all animals that underwent six hours of hepatic ischemia developed severe encephalopathy which was followed by death. In two animals liver failure was complicated by gastric hemorrhage that resulted in early death. With respect to this complication, stress induced by insufficient freedom of movement might be of pathogenetic importance in this animal species. Endotoxemia and bacteremia were excluded as non-hepatic causes of death in our earlier studies[16]. The time between induction of ischemia and the development of encephalopathy and death is about 24 and 48 hours respectively; such a period is sufficiently long for introduction of an experimental treatment and evaluation of its effects.

Other requirements for an appropriate animal model are the use of a *large animal* and *minimal hazards to personnel*. In our model highly inbred pigs were used. The model of hepatic ischemic necrosis does not require the use of dangerous toxic substances.

We think therefore that our animal model fulfills all the criteria proposed for an appropriate animal model of fulminant hepatic failure[4].

Several other interesting observations with regard to this animal model were made.

1. Clinical neurological assessment by means of semi-quantitative measurements was of restricted diagnostic value and distinguished only non-coma from coma. In contrast EEG assessment identified all grades of encephalopathy in pigs with ischemic hepatic necrosis. Automated EEG analysis showed that objective measurement of encephalopathy in pigs is

feasible.

2. Hepatic ischemia for six hours did not, in itself, induce marked abnormalities in coagulation tests. However, as soon as revascularization of the ischemic liver was established severe disturbances developed. A marked drop in platelet count and in the levels of fibrinogen and other clotting factors was observed, suggesting intravascular coagulation. Exposure of the blood to damaged sinusoidal cells within the ischemic liver seems a likely explanation for the observed findings[21]. No reduction in platelet counts was observed in earlier experiments in pigs with permanent ischemia of the liver that showed a decrease in the levels of clotting factors to 20% at 24 hours[15,22,31].

3. Tyrosine and phenylalanine ratio's clearly increased in our model. Elevation of the tyrosine ratio was greater for the 'six hour animals' and therefore seems to be related to the degree of hepatic insufficiency[23-27]. Correlations between the degree of liver cell necrosis and other amino acids ratio's were not found. Assuming that disturbances in the transport of amino acids across the blood-brain barrier are best expressed by plasma ratio's, tyrosine appears to be the major abnormality of neutral amino acids in hepatic encephalopathy in the pig. These observations contrast with the findings in rats and dogs of a concurrent rise of tyrosine, phenylalanine and tryptophan.

4. Associated with the liver failure was a decrease in plasma arginine levels of more than 90%. In animals who survived, the plasma arginine levels normalized, but they remained zero in those who died. Arginine is required for effective utilization of ammonia in the urea cycle[28]. A correlation between the persistent absence of plasma arginine and the rapid rise in ammonia may be entertained. However, the observation that arginase is released from the necrotic liver cells into the plasma compartment[29,30], and the observation that it induces conversion of plasma arginine into ornithine and urea (a rise in plasma ornithine levels was indeed observed, fig. 10), emphasizes the need for measurements of intracellular concentration of arginine before inferences about the activity of the urea cycle can be made.

In conclusion we believe that our animal model of acute hepatic failure is comparable to the human condition of acute hepatic failure. Since the model is reversible as well as reproducible and does not constitute a biological hazard, it can be used for studies of the pathogenesis and complications of acute liver failure. Moreover, since our model utilizes a large animal with a life expectancy of about 48 hours, testing of hepatic support systems or assessment of auxiliary liver transplantation is possible.

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CHAPTER 5

Fourth experiment

AUXILIARY TRANSPLANTATION OF PART OF THE LIVER IMPROVES SURVIVAL AND PROVIDES METABOLIC SUPPORT IN PIGS WITH ACUTE LIVER FAILURE.

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AUXILIARY TRANSPLANTATION OF PART OF THE LIVER IMPROVES SURVIVAL AND PROVIDES METABOLIC SUPPORT IN PIGS WITH ACUTE LIVER FAILURE.[1]

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Abstract

In pigs subtotal ischemic liver cell necrosis was induced four days after auxiliary transplantation of 60% of the liver of a MLC-compatible donor (ATPL group, n=13). In control animals (n=14) temporary liver ischemia was preceded by division of the hepatic ligaments and creation of an end-to-side portacaval shunt.

In the ATPL group six animals died from gastric hemorrhage, intestinal strangulation, or sepsis. The remaining seven animals survived in excellent condition until sacrifice 26 days after the induction of liver ischemia.

Excellent graft function was demonstrated by uptake and excretion of ^{99m}Tc-HIDA at cholescintigraphy, ammonia detoxification, synthesis of clotting factors and glucohomeostasis. Electroencephalographic recordings in the animals that underwent transplantation, did not change from preischemic levels. Evidence of hepatic regeneration was found in the transplanted livers but could not be demonstrated in the damaged host livers. The control animals died in coma within 72 hours.

These results indicate that auxiliary transplantation of a partial liver provides metabolic support and improves survival in animals with induced acute liver failure.

Introduction

The mortality rate in patients with acute hepatic failure is 80-90%[1]. Death is usually caused by cerebral edema, brain stem dysfunction with respiratory or circulatory failure, or bleeding, resulting from the inadequacy of hepatic metabolism and protein synthesis. Regeneration is often mentioned, but rarely documented[2]. A variety of modalities of artificial liver support systems have been used in an attempt to prolong the life of these patients until the diseased liver has recovered from the insult, but none of these has been proved to be effective[3]. If the concept is valid that the liver will regenerate if time permits, better support systems should be developed.

Transplantation of a liver allograft in the heterotopic auxiliary position is a potential candidate for such a hepatic support system since it leaves the host liver *in-situ* and surgically it is a less extensive procedure than orthotopic liver transplantation. If the patient`s own liver has recovered, the graft can be removed.

However, results of clinical and experimental studies in auxiliary liver transplantation have been disappointing, with a few exceptions[4-8].

To prove that a heterotopic liver transplant can support life during acute hepatic failure a reproducible animal model of acute hepatic failure was developed (Chapter 4). In this article we report the beneficial effect of auxiliary transplantation of 60% of the liver on animal survival and hepatic function in pigs with induced acute hepatic failure.

Methods.

Pigs. Thirty-seven female Yorkshire pigs, weighing 26.3±0.8 kg (mean±SEM), were used. Donor and recipient were of similar size and body weight. The animals were randomly allocated to two groups: animals in group A (n=17) underwent an auxiliary partial liver transplantation while control animals (group B, n=20) received an end-to-side portacaval shunt. Four days after the first operation the native liver of the animals in both groups was rendered ischemic by occlusion of the hepatic artery for six hours.

Surgical technique.

Group A. Heterotopic liver transplantation was performed as described previously[9]. After removal of the donor liver organ, perfusion was started *ex-vivo* through a cannula in the portal vein using Euro-Collins solution (4° C). A cholecystectomy was performed and a polyethylene tube was inserted through the cystic duct into the common bile duct for cholangiography studies. The two left lateral lobes of the donor liver were resected, reducing the liver graft weight to 62.9±1.1% (mean±SEM). A continuous atraumatic 2/0 polydiaxonone (Ethicon^{R}) suture was placed at the cut surface after resection.

In the recipient animal all the liver ligaments were transected. A silicone tube was inserted into the common bile duct and two 2/0 silk ligatures were tied around this tube to prevent collateral circulation through the wall of the duct to the host liver. A specially designed vessel occluder and an electromagnetic blood flow sensor (Skalar Instruments, Inc., Delft, the Netherlands) were placed around the isolated hepatic artery of the recipient and the leads were guided through the abdominal wall.

The transplant was placed in the right subhepatic space. Revascularization of the graft was obtained by end-to-side anastomosis between the suprahepatic vena cava of the graft and the infrahepatic vena cava of the recipient. Inflow of portal blood was achieved by end-to-side anastomosis between the donor portal vein to the recipient portal vein followed by an end-to-side anastomosis of the graft hepatic artery to the recipient`s infrarenal aorta. The host portal vein was ligated and divided in the liver hilum. Restoration of the bile flow was achieved by an end-to-side choledochoduodenostomy (Fig.1). Four silver electrodes were placed on the dura through burr holes for electroencephalographic monitoring.

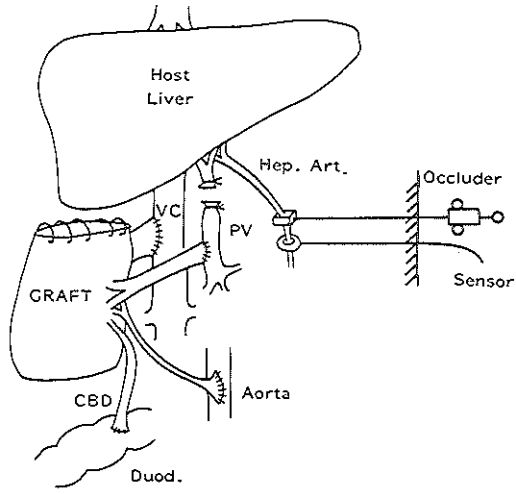

Fig. 1. Schematic drawing of the auxiliary partial livertransplantation VC = vena cava, Hep.Art. = hepatic artery, PV = portal vein, Duod. = duodenum, CBD = common bile duct.

Group B. In the control animals all the liver ligaments were transected and an end-to-side portacaval shunt was constructed. A silicone tube was inserted into the common bile duct; a flow sensor and vessel occluder were placed around the hepatic artery and cerebral electrodes were positioned as well.

Throughout the surgical procedures Ringer's solution was administered. In group A all animals received a blood transfusion of 400 ml obtained from the donor animal to correct blood loss during the transplantation procedure. Just before recirculation of the transplant or construction of the portacaval anastomosis an additional 500 ml of Haemaccel^{R} was administered. All animals received ampicillin (0.5 g) and kanamycin (0.5 g) intravenously at the beginning of surgery and immediately afterwards.

Immunosuppression. No immunosuppressive drugs were given. Donor and recipient combinations were matched for the mixed lymphocyte reaction test as described previously[10].

Induction of acute hepatic failure. Four days after the operation ischemic hepatic necrosis was induced in both groups by tightening the

externally driven occluder around the hepatic artery without anesthesia. Total arrest of the hepatic blood flow was confirmed by electromagnetic flow monitoring. The arterial occlusion lasted for six hours. Just before induction of liver ischemia and immediately afterwards 0.5 g ampicillin and 0.5 g kanamycin were administered intravenously. During hepatic artery occlusion all animals received a continuous infusion of glucose (12g/kg/24 hr) to which cimetidine (800 mg/24 hr), potassium and phosphate (37 mmol/24 hr and 45 mmol/24 hr respectively) were added. Sodium bicarbonate (30 ml, 8.4%) was given directly after restoration of hepatic artery blood flow to correct metabolic acidosis.

Follow-up studies. Blood samples were taken before surgery, the first postoperative day, before induction of ischemic hepatic necrosis, 24 and 48 hours after liver ischemia and weekly thereafter. Hemoglobin, WBC, platelets, serum levels of glucose, bilirubin, SGOT, venous ammonia and fibrinogen, the Normotest^{R} and the activated partial thromboplastin time (APTT) were determined by standard laboratory techniques. Electroencephalograms (EEGs) were made before induction of hepatic necrosis and repeated at 24, 48, and 72 hours and just before sacrifice at 26 days. The EEG recordings were read independently by an electroneurologist and graded 0-5 as described by Opolon[11]. Intravenous angiography, as described previously[12], was performed one and three weeks after host liver ischemia. During this procedure a cholangiography of the liver transplant was also done.

Cholescintigraphy of the transplant with ^{99m}Tc-HIDA as described in detail elsewere[9], was carried out weekly after induction of ischemia.

DNA and RNA contents of the transplant and the recipient liver at surgery and at sacrifice were calculated by multiplying the amount of nucleic acids per gram wet weight of liver biopsy specimens with the total weight of the liver[13]. The wet weight of the recipient liver at the time of surgery was estimated by taking the weight of the liver of pigs of the same sex, age and body weight.

Histology. Wedge liver biopsies were taken at operation and at autopsy. Percutaneous needle biopsies of the transplant and the host liver were carried out in the first week after acute host liver failure. Specimens were stained with hematoxylin, azophloxin and saffron and then examined on a blind observer basis. An assessment was made of the presence and degree of cholangitis, cholestasis, hepatocellular necrosis, and cellular infiltration. Rejection was estimated by the degree of periportal cellular infiltration and vasculitis. Postmortem examinations were performed in all cases.

Surviving animals were sacrificed 26 days after inducing host liver ischemia.

Statistical analysis. Data are expressed as mean values ± SEM. Statistical analysis was performed using the Wilcoxon rank-sum test.

Results.

Survival. No deaths occurred intraoperatively. In group A (n=17) four animals died before the induction of host liver ischemia. Two pigs died because of technical failure (portal vein thrombosis; air embolism) and two pigs died of pneumonia and bleeding from a gastric ulcer. The remaining thirteen animals provide the data for further evaluation of group A.

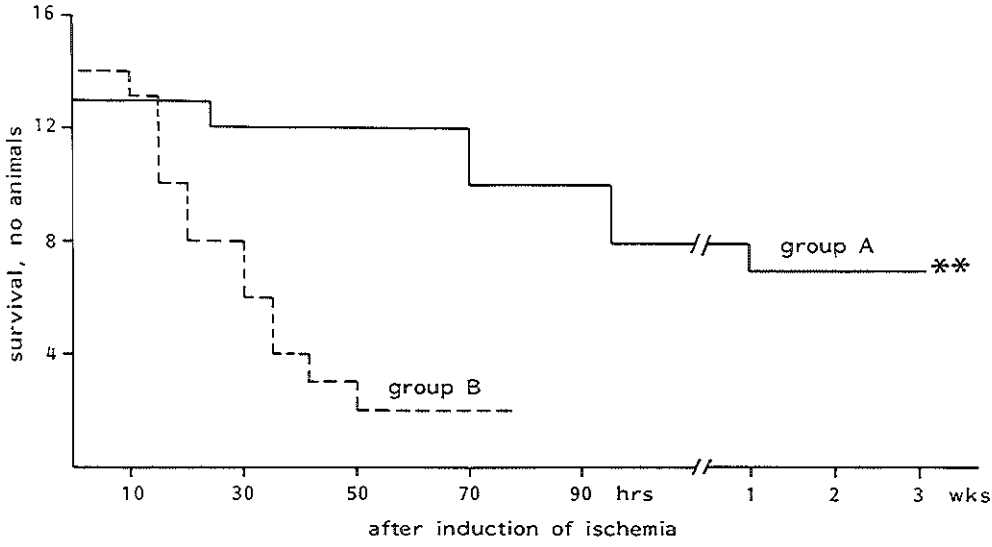

Fig. 2. Animal survival after the induction of ischemia. Solid line represents animals that received an auxiliary partial liver transplant. Interrupted line represents control animals. ** $p < 0.001$, compared with controls.

In the control group (n=20) six animals were excluded from the study. Four pigs died before induction of hepatic failure (kinking of the hepatic artery by the vessel occluder resulting in hepatic failure before the fourth postoperative day, transfusion of incompatible blood and bleeding from the central venous line). Two animals had to be excluded because the occluder around the hepatic artery did not function.

Median animal survival in group A after six hours of acute host liver ischemia and auxiliary partial liver transplantation was 26 days (fig.2). Seven animals were sacrificed at the end of the experiment in excellent condition 26 days after the ischemic period. Death in the other animals was due to upper gastrointestinal bleeding (four cases), sepsis and intestinal strangulation. All animals in this group had vital grafts at autopsy examination. In the control group (B) median animal survival was 29.5 hours ($p < 0.001$ compared with group A). After liver ischemia twelve animals died spontaneously within 72 hours and two pigs had to be sacrificed after this period with a grade 4 encephalopathy. In all cases death was due to acute hepatic failure, although in two pigs death was precipitated by a bleeding gastric ulcer and one pig had concomitant intestinal strangulation.

Ischemic procedure. The procedure of inducing acute hepatic failure by host liver ischemia was initially well tolerated by all animals in both groups. Flattening of the blood flow curve on the oscilloscope indicated total hepatic artery occlusion in all pigs. The flow through the hepatic artery was restored immediately in all but two animals after release of the vessel occluder as demonstrated by flow measurements. Thrombosis of the hepatic artery at the site of the vessel occluder was seen in four animals at autopsy examination. In all the other animals in both groups the hepatic artery was patent. Histology of the liver in group B showed an estimated 85% necrosis at autopsy; only small zones of normal-appearing hepatocytes were seen around the vena cava, close to the liver capsule, and near the diaphragm. The same picture was observed in the host liver of the animals in group A that died shortly after induction of liver ischemia. In the animals with long-term survival in group A the host liver was transformed in a flat fibrotic structure adhaerent to the diaphragm. It should be mentioned that large fields of normal-appearing hepatocytes were always seen in the host liver at autopsy examination 26 days after liver ischemia.

Electroencephalography. Electroencephalography readings in group A just before induction of ischemia scored 0 to 2 (fig.3). No significant changes were noticed in the first three days after ischemia or just before sacrifice. In group B the EEG readings deteriorated in all animals in the

days after hepatic ischemia. All pigs that survived 48 hours had reached grade 3 or more.

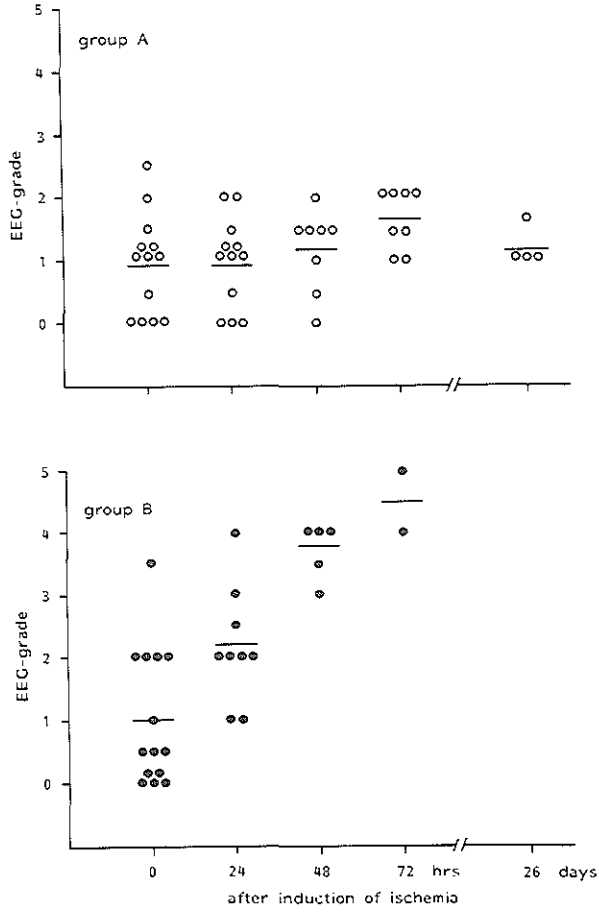

Fig. 3. EEG readings in animals that underwent ATPL prior to ischemia (group A) and in animals that did not receive an auxiliary partial liver transplant (group B).

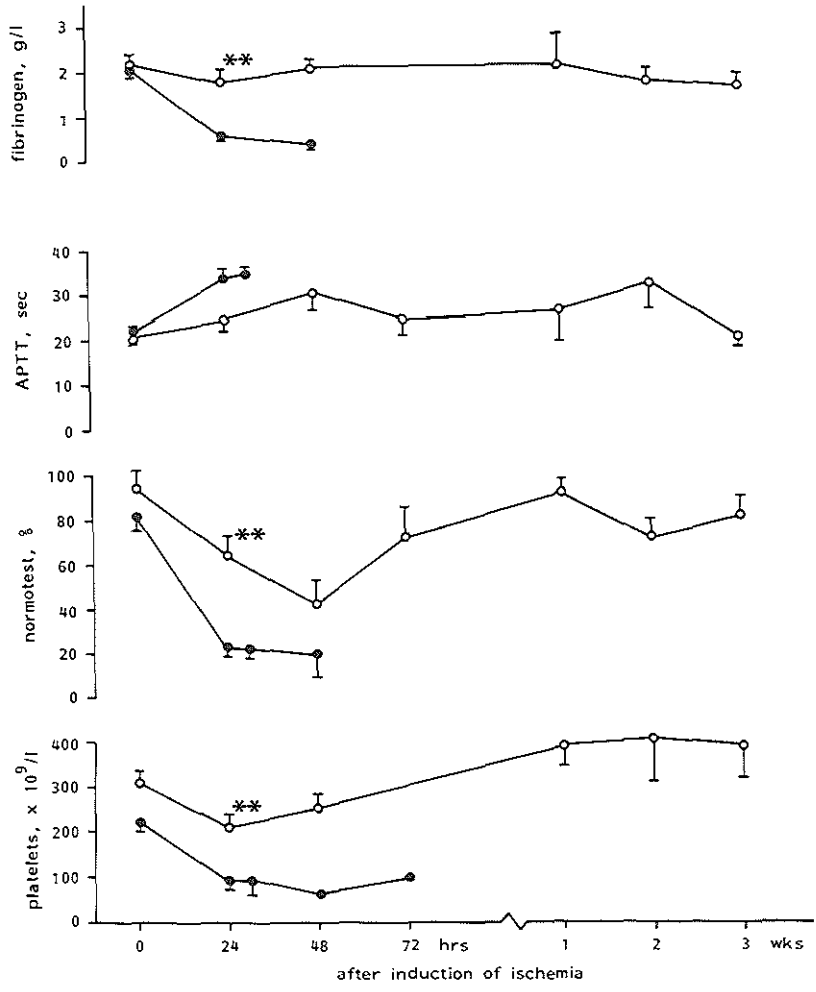

Fig. 4. Fibrinogen levels, APTT, normotest^{R} and platelet counts after six hours liver ischemia in animals that underwent ATPL (group A, o-----o) and in control animals (group B, ●-----●). * $p < 0.01$, compared with controls.

Biochemistry. In all the animals that received an auxiliary partial liver transplant before induction of host liver failure, transient minor changes were observed in fibrinogen level, activated partial thromboplastin time, Normotest^{R} and platelet counts, which rapidly returned to normal values (fig.4). In group B a decrease in coagulation factors was observed in all animals, which was clinically confirmed by oozing of blood from the surgical incisions indicating hemorrhagic diathesis.

In all pigs there was a significant rise in serum bilirubin and SGOT values after the ischemic period (fig.5). The levels in group A became normal within one week. The transplants in group A showed excellent ammonia detoxification as compared with the values in group B. Blood glucose levels were within normal range in both groups, but in the control animals glucose 20% was administered continuously to prevent death from hypoglycemia after liver ischemia whereas the pigs in group A showed glucohomeostasis without glucose infusion.

Graft survival. Excellent graft function in group A was demonstrated by synthesis of clotting factors, ammonia detoxification, glucohomeostasis, and animal survival after host liver ischemia. In addition, sequential HIDA-hepatobiliary scintigraphy was performed in seven animals surviving more than one week. All grafts showed normal uptake of the radiopharmacon in the liver and excretion into the duodenum. Scintigraphy showed slight uptake in the recipient liver in three cases ; the recipient liver of the other four animals could not be visualized. Patency of all hepatic artery and portal anastomoses in these seven animals was demonstrated by intravenous angiography. Cholangiography showed no bile leakage or stenosis at the choledochoduodenostomy.

At autopsy examination all vascular and bile duct anastomoses were patent in all pigs that received an auxiliary partial liver transplant. The grafts appeared to be virtually normal at macroscopic inspection. Histologic examination showed only mild or moderate signs of chronic rejection in the animals that survived more than one week. Acute rejection as indicated by vasculitis was not seen and hepatocellular necrosis was not prominent in the grafts. Cholangiolitis ranging from mild to moderate was seen in seven cases.

Determination of nucleic acid contents of the transplants in the animals of group A that survived one month suggested compensatory hyperplasia (fig.6). DNA and RNA contents of seven liver grafts at sacrifice increased with 31%$\pm$16% and 48%$\pm$16%, respectively, compared with values at the time of transplantation ($p=0.06$). DNA and RNA contents of recipient livers in the same animals decreased significantly with 43%$\pm$10% and 69%$\pm$2%, respectively.

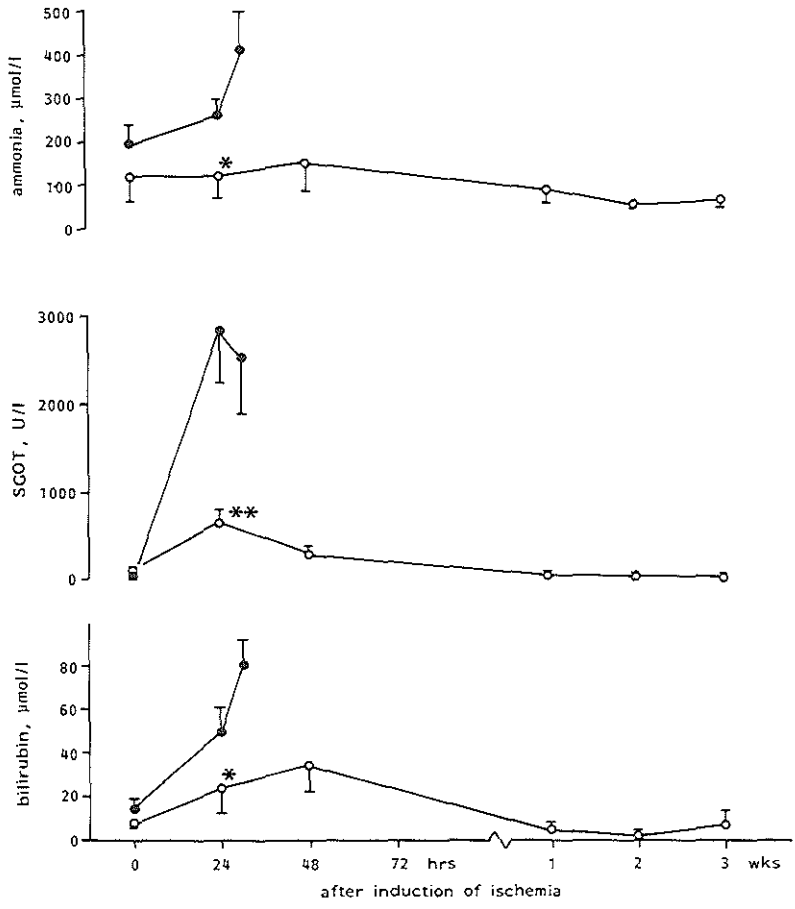

Fig. 5. Plasma ammonia concentrations, SGOT and bilirubin levels in animals that underwent ATPL (group A, o-----o) and in control animals (group B, ●-----●). * $p < 0.01$, ** $p < 0.001$, compared with controls.

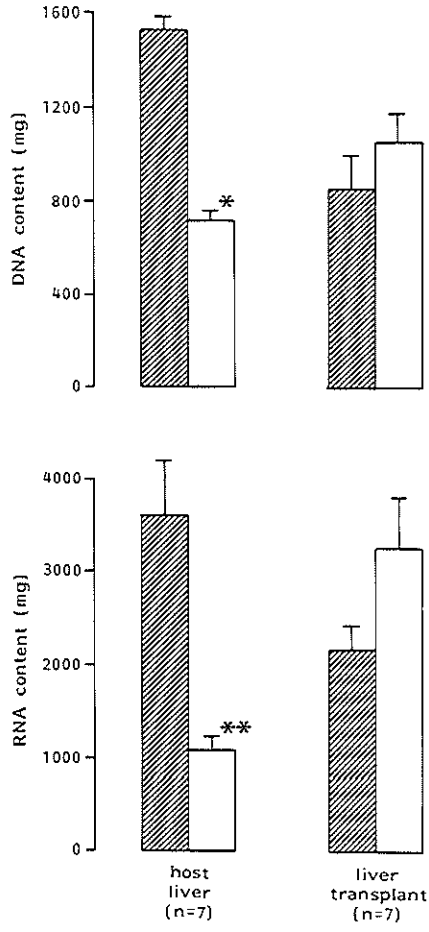

Fig. 6. Nucleic acids contents of recipient liver and liver graft at transplantation (shaded area) and at sacrifice (white area). * $p < 0.01$, ** $p < 0.001$, compared with operative values.

Discussion

In our experimental model acute host liver failure was induced by ischemia of the liver for six hours. In another study we demonstrated that ischemia for four hours resulted in a 50% survival rate but that six hours of hepatic ischemia was followed by hepatic coma and death in all animals (Chapter 4). It is important that all hepatic ligaments are meticulously divided and that collateral circulation through the wall of the common bile duct is interrupted. Without this additional procedure survival after hepatic artery occlusion and portacaval shunting is still possible. All requirements for a satisfactory animal model of acute hepatic failure as stated by Terblanche et al. are met in this experimental model[14] (i.e., potential reversibility, reproducibility, death caused by coma after a period of time sufficiently long to allow studies on hepatic support procedures, the use of a large animal, and induction of liver failure with minimal hazards to personnel). Death from liver failure occurred in all animals in group B after induction of acute liver failure. The severity of liver insufficiency was reflected by the degree of encephalopathy, clotting disorders, and increase in liver enzymes. The auxiliary transplants consisting of 60% of a donor liver were able to sustain life in the pigs in group A. Excellent metabolic support by the graft was demonstrated by synthesis of clotting factors and ammonia detoxification.

The EEG readings before induction of ischemia were abnormal in some animals in both groups. Portacaval shunting might explain the abnormal EEG readings in group B, while in group A suboptimal graft function might be responsible. It has been demonstrated by others that portacaval shunts in pigs result in increased levels of ammonia[15]. The EEG readings in group A remained slightly abnormal but in group B severe encephalopathy was reached within 48 hours.

Hyperplasia in the transplanted livers in the pigs with long-term survival was demonstrated by the increase in both DNA and RNA content. However, in all recipient livers, few signs of regeneration were seen after the ischemic period, although areas with normal-appearing hepatocytes were always present. Data on liver regeneration after partial hepatectomy have been reported extensively[16], but reports on regeneration after toxic, viral or ischemic liver damage are less numerous[2,17,18].

Results of previous experiments in which the ability of an auxiliary hepatic graft to prolong life and to induce regeneration in the recipient liver in the presence of acute host liver failure have been controversial. Auxiliary liver transplantation in dogs with chemically induced liver cell

necrosis resulted in an 80% animal survival rate, while all control animals died within six days after administration of a hepatotoxic agent[4]. However, death in the control animals occurred without evidence of severe hepatic failure. Heterotopic liver transplantation was carried out by Lilly et al. in pigs 24 hours after hepatic ischemia induced by hepatic artery ligation and mesenteric-caval shunt[5]. A good rate of survival was obtained in the pigs that underwent transplantation, while all control animals died within 72 hours. However, after removal of the liver grafts within ten days all grafts appeared to be totally infarcted with necrosis and abscess formation. Nevertheless it was stated that total host liver recovery occurred within this short period of time. Others have been unable to reproduce this experiment[7]. Diaz et all transplanted dogs whose hepatic lesions were produced by peroperative clamping of the porta hepatis after construction of a portacaval shunt[6]. After removal of the graft only one dog survived for 25 days. Using an identical experimental model, Szekely et al. found that the first signs of regeneration in the host liver appeared only several days after the end of hepatic support[19]. It should be noted that in none of the described experiments was splanchnic blood directed into the graft. It has been shown that efferent pancreatic blood is essential for the integrity and optimal function of the liver. Without portal blood liver atrophy will most likely ensue[20-22]. In our experiment the liver graft was provided with portal blood while the portal vein of the recipient was transected in the liver hilum to ensure optimal graft perfusion. Therefore regeneration of the host liver in our study may have been impaired by the lack of hepatotropic factors. Furthermore the follow-up period may have been too short to detect host liver regeneration.

It still remains questionable whether sufficient regeneration in the liver after extensive toxic or ischemic injury does occur. Longer follow-up periods and further studies on host liver regeneration with intact portal inflow are needed. If induction of liver failure predates the transplantation procedure, which will be the case in man, the host portal vein may not need to be ligated as an intrahepatic block present in patients with acute liver failure, will probably direct sufficient portal blood flow through the graft[23].

The problem of space after an auxiliary liver transplantation in the abdominal cavity was circumvented in our study by reducing the size of the graft to 60% of its original weight. This avoided compression on blood vessels impairing graft function and diminished the possibility of cardiopulmonary dysfunction by elevation of the diaphragm. Futhermore the partial hepatectomized liver transplant may release a regeneration-stimulating factor that might enhance repair mechanisms in

the diseased host liver[24].

The concept of auxiliary transplantation of the liver in the presence of host liver failure is to remove the graft after the transplantation procedure once the patient's own liver has recovered. However, the graft can be left *in-situ* if the host liver fails to regenerate.

The results of our study indicate that an auxiliary partial liver transplant is capable of providing metabolic support during and after fulminant hepatic failure even if host liver regeneration does not occur. We would therefore recommend that auxiliary heterotopic liver transplantation is reconsidered in patients with fulminant acute liver failure or in patients with chronic non malignant liver disease in whom the procedure of orthotopic liver transplantation carries too much risk.

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CHAPTER 6

Fifth experiment

HEMODYNAMICS AND COAGULATION DISORDERS IN EXPERIMENTAL AUXILIARY LIVER TRANSPLANTATION FOR FULMINANT HEPATIC FAILURE

This chapter has been submitted for publication

HEMODYNAMICS AND COAGULATION IN EXPERIMENTAL AUXILIARY LIVER TRANSPLANTATION DURING FULMINANT HEPATIC FAILURE[1]

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Abstract

In pigs ischemic liver cell necrosis was induced by 6 hours occlusion of the hepatic artery and the portal vein 3 days after construction of a side-to-side portacaval shunt and division of the hepatic ligaments. Two-third of the liver of a MLC-compatible donor was heterotopically transplanted 13 hr (group I), and 3 hr (group II) after induction of liver failure.

In group I (n=11) 3 animals died of liver failure before or shortly after induction of anesthesia. Of the remaining pigs, 2 animals survived more than 2 weeks. In group II (n=10) intraoperative hypotension was prevented by the reduction of the interval between liver failure and transplantation, and by fluid replacement that was monitored by a thermodilution catheter. Significant decrease in cardiac output and increase in pulmonary and systemic vascular resistance were observed during auxiliary partial liver transplantation (APLT). In the immediate postoperative period 6 pigs died of deficiencies in hemostasis that were caused by consumptive coagulopathy related to severe host liver damage rather than fibrinolysis. Two pigs in group II survived in good condition 12 and 42 days after APLT. In the longer surviving pigs of both groups either the graft or the host liver recovered.

Processes that might be responsible for the observed hemodynamic changes and coagulation disorders are discussed. These results indicate that APLT is technically feasible in severely ill pigs with acute hepatic failure.

Introduction

In patients with fulminant hepatic failure caused by massive hepatocellular necrosis orthotopic liver transplantation is currently not considered with a few exceptions[1]. Operative mortality rate in end-stage cirrhotic patients who receive an orthotopic liver transplant in the period of acute hepatic decompensation may be as high as 80%, mostly due to severe bleeding and hypotension[2]. Removal of the host liver in an area of extensive venous collaterals accounts for most of the blood loss[3]. In auxiliary heterotopic transplantation for non-malignant liver disease, the basic surgery consists of three vascular anastomoses and restoration of a bile outflow tract after limited dissection; this technique may improve the discouraging results so far obtained in patients with acute hepatic decompensation. The beneficial effect of auxiliary transplantation of 60% of a donor liver on host survival and hepatic metabolism in experimental animals that received a transplant before induction of liver failure, has been demonstrated previously[4].

In the present study we investigated the perioperative effects of auxiliary partial liver transplantation (APLT) on hemodynamics and coagulation status in pigs with fulminant hepatic failure, induced before the transplantation procedure.

Methods

Pigs. In female Yorkshire pigs (28.0 ± 0.6 kg, mean ± SEM) a side-to-side portacaval shunt, division of the hepatic ligaments and cholecystectomy was carried out. External vessel occluders were applied in the liver hilum around the hepatic artery and the portal vein (Fig.1A). Three days after this operation acute liver necrosis was induced by 6 hr occlusion of the hepatic artery and portal vein in the liver hilum, as described previously[4].

Two consecutive series of transplantations were performed. Our surgical technique of APLT has been described elsewhere[5]. At the end of the operation the vessel occluders were removed and the side-to-side portacaval shunt was abolished by placing two large hemostatic clips at the site of the anastomosis (Fig.1B). Truncal vagotomy and pyloroplasty were added to the surgical procedure. Transplantation in both groups was performed in donor-recipient combinations matched for the mixed lymphocyte reaction test; body weights of donor and recipient animals were similar. In the first group (n=11), animals received an auxiliary partial liver transplant 13 hr after induction of acute hepatic failure. In the second group (n=10), APLT was performed 3 hr after induction of acute liver failure.

Anesthesia. After induction of anesthesia with small intravenous doses of ketamine chloride or thiopental, endotracheal intubation was performed. Anesthesia was maintained with nitrous oxide and oxygen (2:1) and minimal amounts of enflurane. The animals were paralysed with pancuroniumbromide and were ventilated using a Siemens 900B Servo ventilator. End-expiratory carbon dioxide was maintained between 4 to 5 volume %. Analgesia was supplemented with small doses of Fentanyl^{R}. During the operation Ringer's solution, 0.9% NaCl, and Haemaccel^{R} were given. Metabolic acidosis was corrected by administration of sodium bicarbonate. All recipient animals received 800 ml donor blood during surgery. Fresh frozen plasma to supply clotting factors was administered to all pigs during APLT. All animals received ampicillin (0.5g) and kanamycin (0.5g) intravenously at the beginning of surgery and immediately afterwards. No immunosuppressive drugs were given.

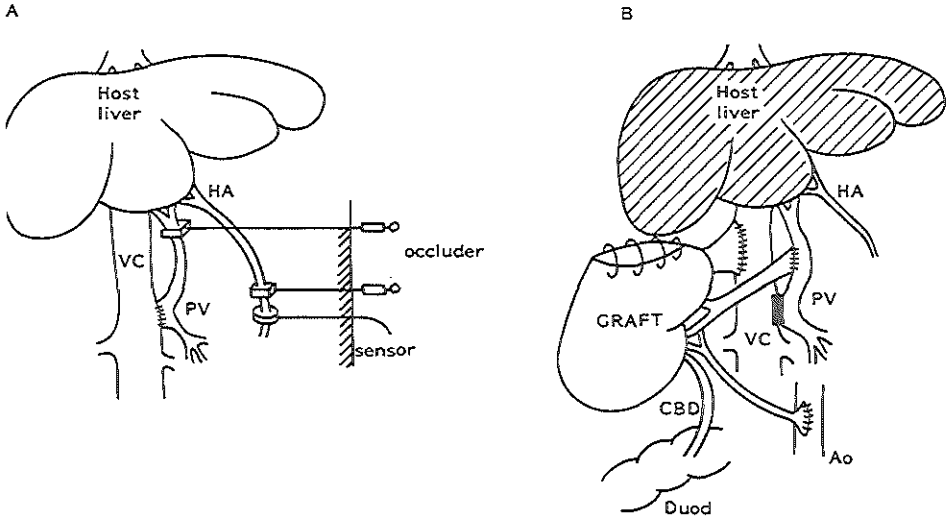

Fig. 1. A. Schematic drawing of anatomy after portacaval shunt and occluders around hepatic artery and portal vein.
B. Situation after APLT, and induction of host liver failure
VC = vena cava, HA = hepatic artery, PV = portal vein, Ao = aorta, Duod = duodenum, CBD = common bile duct.

Hemodynamic monitoring. In group I the arterial blood pressure was monitored by a catheter introduced into the carotid artery. In group II monitoring of the pulmonary arterial pressure, and the pulmonary capillary wedge pressure (PCWP) was added with the use of a Swan-Ganz catheter ; the cardiac output (CO) was measured by the thermodilution method[6]. The systemic vascular resistance (SVR) was calculated with a computer program from the formula SVR = (mean arterial pressure - central venous pressure) x 80/CO and pulmonary vascular resistance (PVR) from PVR = (mean pulmonary pressure - PCWP) x 80/CO. The urine production was monitored during surgery and in the immediate postoperative period.

Coagulation monitoring. In group II Normotest^{R}, activated partial thromboplastin time (APTT), and fibrinogen level were measured. The coagulation profile was studied during host liver ischemia, during surgery, and in the immediate postoperative period using thromboelastography with whole blood during minimal two hours[7,8] (Fig.2).

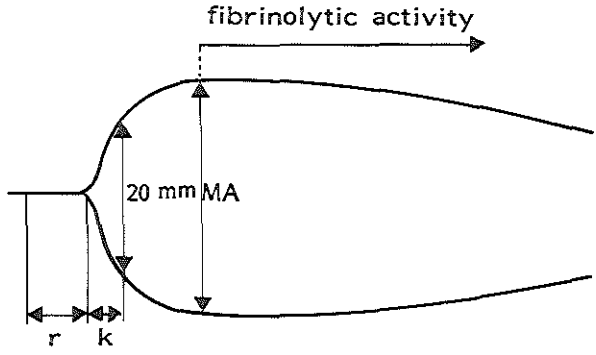

Fig. 2. Scheme of thromboelastogram. The time necessary for the initiation of clotting is referred to as the r-value (reaction time). After initiation of clotting the clot should reach a total amplitude of 20 mm in 2 to 8 minutes; this time is called the k-value (clot formation time). Total width of the clot is expressed as MA (maximum amplitude) which should reach a minimum of 50 mm. Fibrinolytic activity is measured by the decrease of MA in minimal two hours.

Follow-up studies. Blood samples were taken before the first operation, before and after induction of ischemic hepatic necrosis, during the second operation, on the first day after liver transplantation and weekly thereafter. Hemoglobin, leukocytes, platelets, serum aspertate aminotransferase and gamma glutamyl transaminase were determined by standard laboratory techniques. Intravenous angiography and ^{99m}Tc-HIDA scanning as described previously[5], were performed under general anesthesia in the second postoperative week. Liver biopsies of the host liver and the graft were taken at the end of the transplantation procedure and at autopsy.

Statistical analysis. All data are expressed as mean values $\pm$ SEM. Statistical analysis was performed using the Student's t-test for paired and unpaired data; values for $p < 0.05$ are considered to be significant.

Results

In group I (n=11) one animal died of liver insufficiency before liver transplantation could be performed 7 hr after induction of host liver ischemia. Two pigs died during the transplantation procedure of hypotension and ventricular fibrillation 6 and 9 hr after induction of acute liver failure. APLT was carried out in 8 pigs in group I. All animals in group II (n=10) received a heterotopically placed partial liver graft.

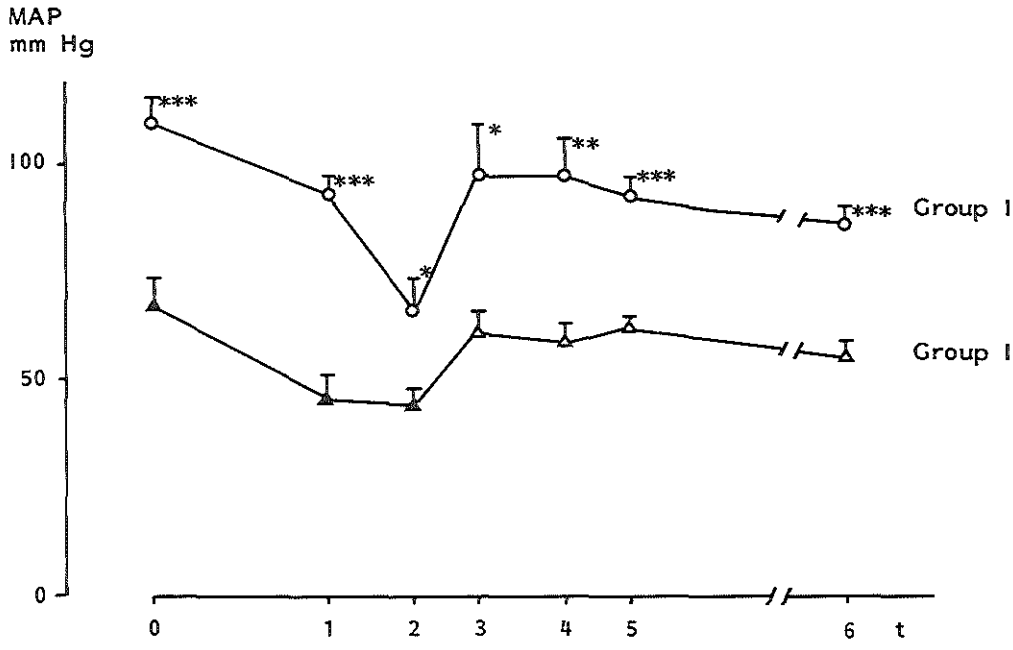

Fig. 3. Mean arterial pressure in 8 animals of group I and in 10 animals of group II that underwent APLT (mean ± SEM). 0 = pre-APLT; 1 = vena cava occlusion; 2 = vena cava and portal vein occlusion; 3 = graft reciculation; 4 = clamp on aorta; 5 = clamp off aorta; 6 = end operation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with group I.

Hemodynamics. In group I mean arterial pressure was significantly decreased at the beginning and during the second operation, compared to the pigs in group II that received large amounts of fluids (Fig. 3). Fluid substitution during APLT was 1.7 ± 0.2 L in group I and 4.5 ± 0.3 L in group II. There was no difference in blood loss during surgery in group I and II (606.3 ± 81.0 ml and 735.0 ± 115 ml respectively). In all pigs in group II CO and PCWP decreased with 66% and 43% respectively during occlusion of the recipient vena cava and the portal vein (Fig.4).

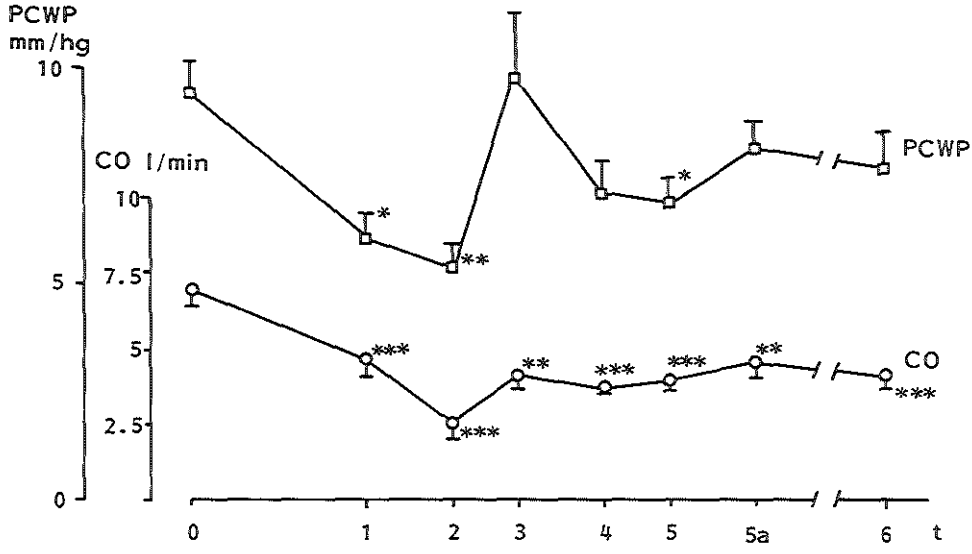

Fig. 4. Pulmonary capillary wedge pressure (PCWP) and cardiac output (CO) in 10 animals of group II that underwent APLT (mean ± SEM). 0 = pre-APLT; 1 = vena cava occlusion; 2 = vena cava and portal vein occlusion; 3 = graft recirculation; 4 = clamp on aorta; 5 = clamp off aorta; 5^a = end host liver ischema; 6 = end operation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with preoperative values.

CO remained decreased thereafter while the PCWP returned to normal. The SVR and the PVR increased with maximal 103% and 190% during APLT and

remained elevated in the immediate post operative period (Fig.5). The pressure in the recipient portal vein in group I and II animals was 17.1 ± 1.3 mmHg before transplantation and decreased after recirculation of the transplant to 12.4 ± 2.2 mmHg (28% decrease).

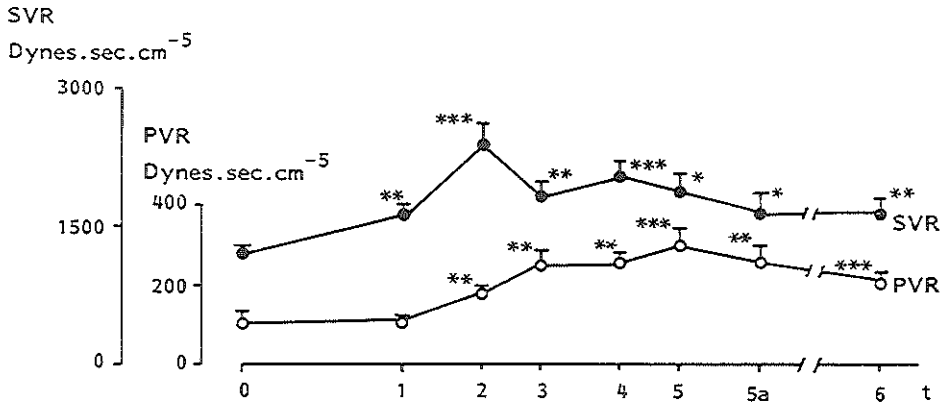

Fig. 5. Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) in 10 animals of group II that underwent APLT (mean ± SEM). See fig. 4 for explanation of time points. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with preoperative values.

Coagulation profile. In group I platelets decreased after recirculation of the recipient liver from 268.8 ± 16.8 to 140.4 ± 22.2 x 10^9/L ($p < 0.01$). In group II the platelets decreased from 224.6 ± 19.2 to 83.8 ± 10.9 x 10^9/L ($p < 0.01$) after the end of host liver ischemia. The reaction time (r) and k-value of the thromboelastograms that are associated with clot formation increased with 53% and 174% respectively while the maximum amplitude, indicating clot stiffness, decreased with 31% after recirculation of the recipient liver, compared to preoperative levels (Fig.6). Hemoglobin content and hematocrit did not change significantly during the same period due to transfusion of blood. A further decrease in amplitude of the thromboelastogram, indicating enhanced fibrinolytic activity, was not noted after the end of host liver

ischemia. The Normotest^{R} in group II decreased from 45.9 ± 5.6% before the transplantation to values below 5% in the four animals surviving 24 hours. Fibrinogen level decreased from 1.7 ± 0.2 to 0.5 ± 0.2 g/L while APTT increased from 27.1 ± 1.9 to 39.4 ± 2.5 sec during the same period. In the 2 animals of this group that survived more than one week after APLT the thromboelastogram and values of the Normotest^{R}, APTT, and fibrinogen became normal.

Laboratory tests. In both groups there was a sharp rise in aspertate animotransferase level uptill hundred times normal values after 6 hours host liver ischemia. In the pigs that survived the first 2 days the level normalized almost completely within two weeks. A rise of bilirubin was also seen after induction of liver failure and the level remained slightly elevated in the animals surviving for longer periods after APLT.

Survival. Three animals of group I died within 48 hr after the operation of technical problems (portal vein thrombosis, air embolism) and one pig died of intraabdominal bleeding; in 2 other animals the cause of death could not be detected at autopsy. The remaining 2 pigs of this group both died of bleeding from a gastric ulcer 17 and 19 days after APLT. In group II, 2 pigs died within 2 days after APLT of intestinal strangulation or air embolism. Postoperative intraabdominal bleeding related to deficiencies in hemostasis caused the death of 6 other animals. Two pigs in this group survived in good health untill death at 12 and 42 days after the operation because of host liver suppuration.

In the 4 animals that survived the immediate postoperative period, histological examination showed more than 75% hepatocellular necrosis in recipient livers at the end of the transplantation procedure. In group I the grafts at autopsy in the 2 longer surviving pigs appeared to be almost completely necrotic with severe inflammatory infiltrates. In one of these pigs the portal vein was occluded but the other vascular and bile duct anastomoses were patent. The host liver in these animals appeared to be recovered almost completely from the previous ischemic period. The transplants of the 2 pigs in group II at autopsy examination were virtually normal at macroscopic inspection and all anastomoses were patent. Histological examination showed mild cholangitis and inflammatory infiltration of round cells and polymorphonuclear leukocytes. Slight hepatocellular necrosis of less than 10% was seen in these grafts. In contrast to the findings in the 2 long survivors from the first group, subtotal liver necrosis was demonstrated in the host liver of these 2 pigs.

In both groups the results of hepatobiliary scintigraphy and of I.V.-angiography in the second postoperative week were consistent with the findings at autopsy examination.

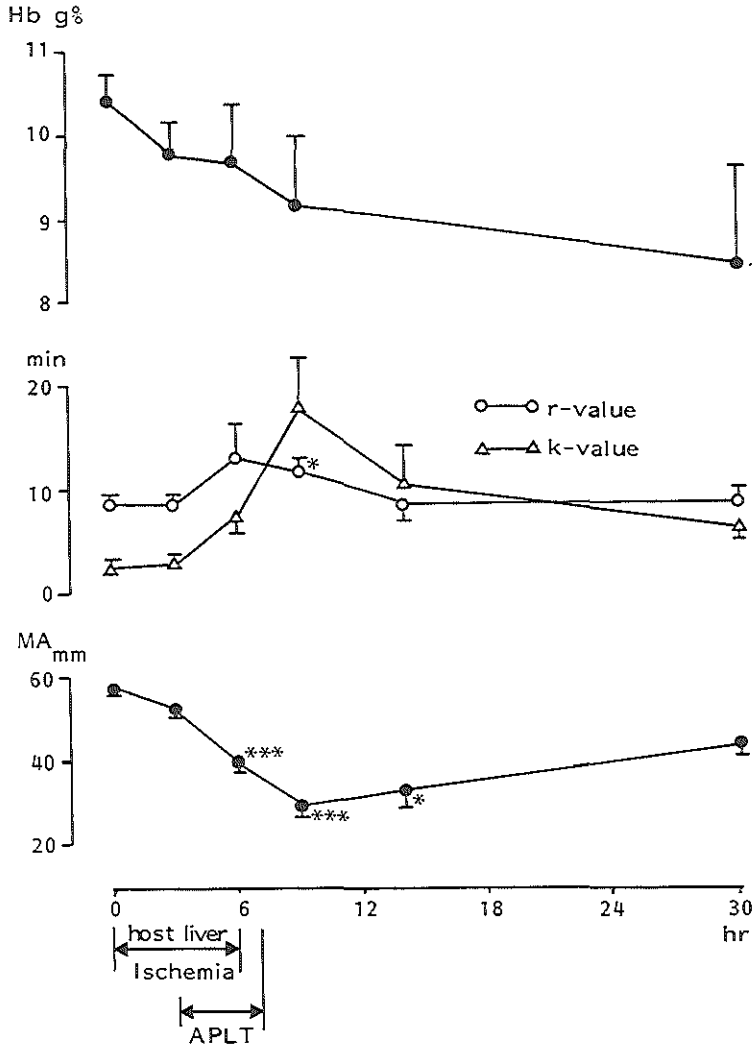

Fig. 6. Hemoglobin level, r-value, k-value, and maximum amplitude of thromboelastogram in 10 animals that underwent host liver ischemia and APLT (mean ± SEM). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with preoperative values.

Discussion

In previous APLT experiments we used recipient animals that were healthy at the time of operation[4,5,9]. Although sufficient metabolic support of the transplant in the event of host liver failure could be demonstrated[4], problems resulting from end-stage liver disease at the time of the transplantation procedure had not been studied. In the present study a different experimental design was used to imitate the clinical situation in man, in which problems concerning hemodynamics and hemostasis could be investigated. The severity of fulminant hepatic failure in our model was reflected by the poor condition of the animals at the time of transplantation as evidenced by hypotension and the death of 3 animals before or shortly after induction of anesthesia. Fulminant hepatic failure in man is invariably associated with severe coagulopathy. Coagulopathy of hepatic failure is explained by thrombocytopenia, failure of synthesis of clotting factors and consumption coagulopathy as direct consequence of liver cell necrosis[10].

Reported models so far used in liver transplantation experiments do not reflect the problems encountered in patients with fulminant hepatic failure[11]. Liver transplantation has been evaluated in pigs with fulminant hepatic failure induced by Amanita phalloides toxin[12]. In that study hemorrhagic diathesis was only encountered in 3 out of 21 animals which is in contrast to findings in patients with fulminant hepatic failure[13]. Auxiliary liver transplantation for dimethylnitrosamine-induced acute hepatic failure has been performed by Kuster without hemorrhagic complications[14]. Drug induced hepatic insufficiency, however, has the disadvantage of low reproducibility and therefore may explain the reported favorable results[15]. Temporary auxiliary liver transplantation in acute liver failure, induced by one hour host liver ischemia has been, described in dogs[16]. The transplant was positioned in the thorax and removed on the 5th postoperative day. Although the time needed for host liver recuperation in the latter study seems to be very short, 2 dogs survived 10 and 15 days. Severe hemorrhagic diathesis was absent in this experiment and the period of hepatic support very short; nevertheless it was suggested that the temporary auxiliary liver transplant is capable of supporting life during acute hepatic injury in the dog. Huguet, however, did not observe reversal of encephalopathy by an auxiliary liver transplant in pigs with induced host liver ischemia[17]. In his study severe disseminated intravascular coagulation was noted and no animals survived more than 9 hours.

In our study acute hepatic failure was also produced by means of temporary

ischemia. Hemodynamic changes and coagulation disorders developed that resembled the syndrome of fulminant hepatic failure in man. In the pigs in which acute liver failure was induced 13 hr before surgery (group I), the condition of the animals became very poor. Most animals were hypotensive at the start of surgery and perioperative mortality was high. Fluid administration of a mean 1.7 L per animal did not improve the blood pressure in the animals of group I. Fresh frozen plasma was also administered routinely during transplantation and prevented major bleeding problems during surgery in this group, although low blood pressure probably obscured manifestation of clotting disorders.

In an attempt to improve results of APLT in these severely ill pigs the time interval between liver ischemia and the transplantation was reduced to three hours (group II), and anticipation of hemodynamic changes was attained by the use of a flow-directed baloon-tip pulmonary artery catheter. As a result of this approach the condition of the animals in group II improved as was reflected by the higher arterial blood pressure at the start of surgery and throughout the second operation. A significant decrease in arterial blood pressure, CO and PCWP was noted during total obstruction of recipient hepatic flow and occlusion of inferior vena cava. Decrease in systemic blood pressure is well known after interruption of hepatic bloodflow[18,19,20], and has been observed previously by our group[21]. In the previously reported experiments of auxiliary liver transplantation in the presence of liver failure[14,16,17,22], no study has been performed to evaluate the hemodynamic changes during the transplantation procedure.

The occurrence of hypotension in fulminant hepatic failure is well recognised, although pathogenesis remains obscure[23,24]. It has been suggested that central vasomotor depression is more important than primary heart failure[25]. Our study has demonstrated that large amounts of fluid were necessary to maintain PCWP at near normal levels following graft recirculation. In spite of this, CO was markedly lower than normal while at the same time SVR was increased. This indicates a primarily myocardial depression following reinstatement of blood flow through the donor liver, with subsequent release of toxic substances like oxygen free radicals from the ischemic liver or splanchnic region as has also been suggested by others[26,27,28]. Administration of serum, obtained immediately after recirculation of the ischemic host liver, to an in-vitro beating rat heart model caused cessation of cardiac contractions (unpublished observations). Consequent hypotension was partially compensated by massive increase of SVR. Recirculation of the host liver after 6 hours ischemia did not result in further decrease of CO. However, at this point large quantities of sodium bicarbonate were administered to counteract the ensuing

metabolic acidosis and the heart action was stimulated with calcium chloride, thus obscuring possible negative effects of toxic substances on myocard function. In future experiments it may be possible to increase CO by the judicious use of vasodilators and positive inotropic agents. Increase of PVR in this study after APLT is an interesting observation. It might be partly due to intravascular coagulation after host liver recirculation. Furthermore, reperfusion of the host liver after an ischemic period stimulates thromboxane A_2 release which in association with thromboemboli results in constriction of smooth muscle around the pulmonary vasculature[27]. Fluid overload was not the cause of increased PVR, as PCWP remained mainly whithin normal limits.

In this study we found an elevation of the portal venous pressure after the temporary ischemia to 17.1 $\pm$ 1.3 mm Hg, normal values in the anesthetised pig being 3-7 mm Hg. This finding is in accordance with observations in patients with acute liver failure[29]. Portal venous pressure decreased with 28% after recirculation of the graft. This indicates that the graft functions as a portacaval shunt. Ligation of the recipient's portal vein after APLT as we performed in previous experiments[4], therefore, is not indicated in the presence of a diseased host liver.

Thromboelastography was used to evaluate the coagulation profile. Zuckerman and co-workers found a striking correlation between thromboelastographic parameters and standard laboratory coagulation tests[30]. Severe clotting disorders were seen immediately after the end of host liver ischemia in the second series of experiments. Prolonged clot formation time was demonstrated by increased r-values and k-values of the thromboelastograms. Decrease in maximum amplitude is explained by the sharp reduction in platelets count observed at the end of host liver ischemia. The hemoglobin level and hematocrit did not alter during the changes in thromboelastographic parameters: hemodilution as an explanation for the observed findings is thus less likely. Abnormalities in coagulation profile after recirculation of the ischemic liver parallel clinical findings. Major blood loss from the plane of resection of the graft nor from vascular anastomoses was noticed prior to host liver recirculation. However, as soon as occluders on the portal vein and hepatic artery of the host liver were removed and the host liver was reperfused, oozing of blood occurred. This phenomenon was not observed after permanent occlusion of the blood inflow to the host liver in another series of experiments (unpublished observations). Intravascular coagulation in the ischemic damaged host liver likely explains coagulation disorders. Other investigators suggest the release of oxygen-derived free radicals after organ ischemia[31]. Subsequent tissue damage and

intravascular coagulation with depletion of clotting factors could be an explanation of the observed bleeding at anastomotic sites. The substitution of fresh blood from the donor animal and fresh frozen plasma could not prevent postoperative mortality from hemorrhagic complications in group II. Platelets to correct low levels were not available. In the animals that survived the immediate postoperative period with severe clotting abnormalities, synthesis of clotting factors by the transplant and the production of platelets by the bone marrow resulted in a normal coagulation profile.

The pigs in group I surviving more than 2 weeks after transplantation had a necrotic transplant at the time of death. As biopsies of the host liver in these pigs showed subtotal necrosis at the time of operation, the liver grafts supported the animals only during the period of host liver recovery. The reason for graft failure in these pigs is not clear; failure caused by a technical problem is less probable in view of the findings at autopsy examination. In group II the host liver in the two longer surviving animals was transformed in a flat fibrotic structure, while the graft had increased in size considerably. The grafts supported these pigs completely as the host liver did not recover function. As time interval between induction of liver failure and APLT was reduced from 13 hr (group I) to 3 hr (group II), difference in graft survival between the animals of both groups as result of this measure can not be ruled out. The observations in the longer surviving animals of both groups tend to support the concept of 'functional competition' between host liver and graft as has been suggested by Van der Heyde and co-workers[32].

In summary, we found that hypotension in these very ill animals could be partly prevented by reduction of the time interval between liver failure and APLT and by massive fluid replacement. Problems in hemostasis are caused by consumptive coagulopathy rather than fibrinolysis. We demonstrated that APLT is technically feasible in these severely ill pigs with acute hepatic failure. Interestingly enough, in the longer surviving pigs either the graft or the host liver recovered. The underlying biochemical processes that are responsible for survival of one of the two livers need to be elucidated. It seems justified to further explore the possibilities of APLT as support system in liver failure in experimental and clinical studies.

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CHAPTER 7

General discussion and conclusions

7.1 Rationale of the study

As an adequate artificial system to support a patient with end-stage liver disease does not exist[1], the solution for these patients could be transplantation of a new liver either orthotopically, i.e. by complete replacement of the original organ, or heterotopically without removal of the host liver. Hepatocellular transplantation where liver cells are transplanted as free grafts has been used in the treatment of acute hepatic failure in rats[2,3,4]. This technique is theoretically attractive to support the diseased liver, because it is relatively simple. However, favourable results so far have only been obtained in rats, where liver failure was induced by dimethylnitrosamine, galactosamine or by a surgical procedure that was preceeded or accompanied by hepatocellular transplantation. Instead of being used for support in acute hepatic failure or end-stage chronic liver disease, liver cells transplanted as free grafts might supply an enzyme that is congenitally deficient. Matas and others suggested that isolated liver cells or small pieces of normal liver tissue transplanted as free grafts could supply the enzyme bilirubin uridine diphosphate glucuronyl transferase to Gunn rats[5,6]. These rats exhibit unconjugated hyperbilirubinemia due to a heriditary absolute deficiency of bilirubin uridine diphosphate glucuronyl transferase activity. Others, however were unable to confirm these reports and demonstrated disappearance of the transplanted tissue in a short period of time[7]. There are no reports of successful liver cell transplantation in large animal models or patients. Therefore liver transplantation using vascularized grafts is, at the present, the only therapy for liver failure, that can offer good results both clinically and experimentally.
In the case of end-stage chronic liver disease in man orthotopic liver transplantation is the technique performed by all liver transplantation groups and one-year survival now approaches 70% in selected patients[8,9,10].For malignant disease of the liver removal of the host liver is obviously essential. Long-term results in liver transplantation patients in whom a malignancy of the liver formed the indication for

transplantation are still poor with a tumor recurrence rate of more than 70%[9,11]. Advanced stage of the tumor at the time of transplantation or growth of residual tumor expedited by immunosuppressive therapy might be an explanation for the high recurrence rate. For non-malignant parenchymatous liver disease there are advantages of an orthotopic liver transplant over an auxiliary liver graft. There is room to accomodate the transplant and the vascular and bile duct anastomoses lie in correct anatomical position to each other. However, resection of the patient's own liver, often in an area with venous collateral blood vessels, is a major procedure that induces substantial blood loss during operation[10,12,13]. At Pittsburgh university hospital a mean of 42 units of red blood cells, 39 units of fresh frozen plasma, 19 units of platelets, and 8 units of cryoprecipitate were required *per patient* between 1981 and 1983[12]. If serious coagulation disorders are present, as is the case in advanced stages of chronic liver failure or in acute liver insufficiency, requirements will be even higher and orthotopic liver transplantation is not a realistic solution. In such patients where severe problems with hepatectomy can be anticipated auxiliary liver transplantation offers an alternative. However, experience with clinical auxiliary liver transplantation so far has not been encouraging as outlined in Chapter 1.3. The aim of our experiments was to further evaluate the surgical technique of auxiliary liver transplantation and its feasibility in the presence of acute host liver failure.

7.2 Surgical technique

In our experiments a technique of auxiliary liver transplantation was developed where all theoretical requirements for optimal graft function as outlined in Chapter 1.5 were met. In our series we used only partial liver grafts. The use of reduced size grafts offered a solution to space related problems as no difficulties were encountered in wound closure. Respiratory problems caused by the size of the graft as reported by others[14], were not seen in our experiments. Transplantation of reduced-size adult liver grafts has been successfully performed in patients as well in the orthotopic as in the heterotopic position[15]. However, the use of partial liver grafts raises several technical problems. The period necessary to reduce the size of the graft will increase ischemia time. If cooling is continued during the partial hepatectomy on the bench and the time required for the actual

transplantation of the host liver is short, this increased ischemia time probably will have no harmful effects[16]. Bleeding from the transected liver surface is another problem that might be expected with the use of partial liver grafts. Our solution has been meticulous ligation of all visible vessels and bile ducts in combination with compression by a continuous polydiaxanon (Ethicon^{R}) suture. Application of rapid polymerizing glue or cyanoacrylate on the transected liver surface as an additional procedure to ligation of all structures during transection of the donor liver has been used by others[15,17]. Ultra sonic dissection that has recently been introduced in liver surgery seems to facilitate hepatic transection[18]. It will be worthwhile to evaluate this technique during bench surgery in auxiliary partial liver transplantation.

In order to avoid atrophy of the transplant and problems with the biliary anastomosis it is important to have portal and arterial blood flow to the graft as previously discussed in Chapter 1.5. Low outflow pressure in the hepatic veins of the graft is also essential to avoid graft damage[19]. All these criteria were easily met by our technique where the graft is placed in the right upper part of the abdomen. The reduced size of the grafts we used, facilitated placement in this position. Reconstruction of the biliary outflow tract of the graft was performed in the dog by choledochoduodenostomy using a pullthrough technique. This method copied from liver transplantation experiments in rats, proved to be an adequate technique in the dog where the common bile duct is relatively small[20]. Anastomotic obstruction was only seen once in our study. In the pig choledochoduodenostomy was used as bile drainage procedure. De Jonge recently reported on three different biliodigestive anastomoses in the pig[21]. During a follow up period of three months he found no statistically significant differences in the incidence of cholangitis, leakage or stenosis between a direct choledochoduodenostomy, Roux-en-Y choledochojejunostomy, or choledochoduodenostomy with an anti-reflux procedure. Choledochoduodenostomy never resulted in bile leakage or stricture formation in our series. Of the various options, reconstruction of the biliary tract with the use of a jejunal Roux-en-Y loop should be considered in the case of auxiliary liver transplantation in man. This reconstruction will prevent or reduce the incidence of ascending cholangitis[22]. Furthermore, the Roux-en-Y loop has proven to be satisfactory in orthotopic liver transplantation in cases of biliary atresia and disorders in which the recipient common bile duct was inadequate[23].

Decompression of the portal vein and inferior vena cava during clamping at the time of construction of the venous vessel anastomoses was not used in our experiments. Although a drop in blood pressure was usually seen

during this period, the majority of animals recovered soon afterwards. Pump driven veno-venous bypasses to shunt blood from the lower to the upper half of the body, that are currently used in orthotopic liver transplantation[8], are unnecessary in auxiliary liver transplantation as the time of portal outflow occlusion is short and decompression will occur by the collateral pathways.

7.3 Regeneration

Resection of part of the transplant may result in release of factors which have been implicated as important promotors of regeneration[24,25]. Such factors may stimulate hepatocyte proliferation and help to restore liver mass during the regenerative process in a diseased host liver. Host liver regeneration following ischemic liver damage was not observed histologically in the pigs that received a transplant prior to liver failure (Chapter 5). DNA and RNA contents of recipient livers in that experiment decreased significantly whereas hyperplasia of the liver transplant was suggested at the same time. However, in the experiments performed on pigs that received a graft after induction of recipient liver failure, host liver regeneration was indicated in two animals (Chapter 6). In the latter experiment host liver regeneration, however, was not seen in two other long surviving animals. Therefore, one has to conclude that the supposed positive influence of an auxiliary partial liver graft on host liver regeneration was not a consistent finding in our experiments.
With our technique the recipient liver received no portal blood in all experiments where recipient livers were not damaged by ischemia at the time of transplantation. This ensured optimal portal blood flow through the graft. In the clinical situation there will usually exist a portal hypertension as a result of host liver disease[26], and ligation of the portal vein will probably not be necessary as the portal blood will flow mainly through the graft. In the experiments described in Chapter 6 a decrease in portal pressure was indeed found after transplantation in the presence of host liver failure. This suggests that the graft functioned as a portacaval shunt. If the host liver recovers, this will most likely result in shifting of the portal blood stream from the transplant to the host liver. Should the host liver not recover, than the graft could become "non-auxiliary" as time passes with atrophy of the recipient liver and hyperplasia of the graft. This was the case in the experiments described in Chapter 5 and has also been reported in the two long-term

survivors of heterotopic liver transplantation in man[14,27]. Liver cell regeneration evidenced by increased DNA synthesis and mitoses has been reported in patients dying from fulminant hepatitis[28]. The fact that patients with acute hepatic failure die despite demonstrable regeneration implies that the rate of liver cell necrosis is higher than of cell renewal. In the presence of an auxiliary partial liver transplant there will be more time available for hepatic regeneration. However, immunosuppressive medication will be essential in the case of liver allotransplantation and this might again impair proliferation activity in the host liver[29,30]. If total host liver regeneration nevertheless occurs, withdrawal of immunosuppression can be considered.

7.4 Functional competition

Van der Heyde and co-workers suggested that 'functional competition' exists between the host liver and a liver graft in the case of auxiliary liver transplantation[31]. He showed that auxiliary liver grafts could only survive if the recipient's liver was handicapped and that grafts would atrophy in the presence of a normal host liver. His study was carried out in dogs that received a transplant with arterial blood supply only. In that experiment atrophy of the graft could be prevented by construction of an end-to-side portacaval shunt to the recipient liver and ligation of the recipient hepatic duct. Portal venous blood inflow did not seem to be a prerequisite for transplant preservation in that study. The absence of severe rejection was remarkable as non-matched donor-recipient combinations were used. At variance with the results of van der Heyde and co-workers, we did not see in our experiments atrophy of the graft without histological signs of acute or chronic rejection. In the dog experiments (Chapter 2) the handicap to the host liver was the deprival of portal blood. Nevertheless, long-term graft survival after auxiliary liver transplantation untill 182 days was seen in the DLA-matched combinations. Rejection of the graft at the end of the experiment was more prominent than atrophy. Prolonged functioning of an auxiliary liver transplant in the presence of a normal host liver only deprived of portal blood has also been demonstrated several times in the experiments carried out in non-tissue-typed pigs (Chapter 3). In the study where both the liver transplant and the recipient liver received portal blood, complete host liver recovery after a functional handicap consisting of temporary ischemia, was seen twice (Chapter 6). The host liver in these pigs

recovered despite of the functional handicap and this would not be expected in view of the concept of 'functional competition'. Competition between host liver and graft is hard to define in biological terms. The presence or absence of portal blood with 'hepatotropic factors' essential for the integrity of the liver might be more important in respect to graft survival than the condition of the host liver[32]. Hormones and nutrients carried to the liver by the portal vein are important to prevent liver atrophy as has been emphasized by various studies[33,34,35]. Among the hormonal regulators affecting liver integrity and regeneration are insulin and glucagon[36]. It has recently been suggested that hormone receptors present on the liver cells play an important role after induction of hepatic regeneration[37]. The extensive and sometimes contradictory findings concerning hepatotropic factors so far reported in the literature indicate that the control of hepatic integrety and regeneration is a very complex and multifactorial process[33]. In human liver transplantation there usually exists end-stage host liver disease and competition for the hepatotropic factors will probably be less as the portal blood flow under those conditions will be directed mainly to the graft. If however auxiliary liver transplantation is to be used for correction of an inborn error of metabolism in a liver with viable hepatocytes, the liver that has first access to the beneficial hormones in the effluent pancreatic and splanchnic venous blood probably will be at an advantage over the other. In that case ligation of the portal vein to the host liver should be considered.

7.5 Rejection

Cell-surface antigens located on normal nucleated cells will stimulate a rejection response when presented to an allogeneic recipient. Nevertheless a transplanted liver appears to behave different in some species than for instance the kidney. Livers transplanted between pigs genetically dissimilar to one other were rejected less vigorously than expected as was demonstrated by different workers in orthotopic liver transplantation experiments[38,39]. It has been suggested that liver transplants in some species may provoke specific reduction of the immune response[40]. Recent observations in patients that received an orthotopic liver transplant show that arterial thickening typically indicating chronic rejection is seen in liver grafts[41]. So the liver transplanted in the orthotopic position generates a rejection reaction albeit perhaps

somewhat milder than encountered in other transplanted organs. Blood group compatibility is usually secured in clinical orthotopic liver transplantation. Successful transplants in the presence of blood group incompatibility, however, have been reported and very rapid rejection, within hours, as seen in kidney transplants is rare or non-existent for the liver[42]. This suggests that the transplanted liver induces an immunological reaction, that is less severe than encountered after transplantation of other organs. Alternatively, it is conceivable that the liver is less vulnerable to rejection than other organs (while evoking a similar immune response)[43]. Rejection in auxiliary liver transplantation has been studied less extensively. Given the wide variations in operative techniques used in the different studies and the problems with the assessment of rejection, evaluation is difficult. Rejection after human auxiliary liver transplantation seems to have played a minor role in the therapeutic failures reported[44]. We demonstrated that the auxiliary graft is subject to rejection. Severe acute rejection was seen in our experiments in non-tissue-typed dogs in spite of immunosuppressive medication (Chapter 2). The transplanted liver appeared to behave differently in non-tissue-typed pigs where acute rejection could not be demonstrated although no immunosuppressive medication was used (Chapter 3). An accidental match between donor and recipient in that experiment could not be excluded as tissue-typing was not performed. Auxiliary liver grafts, however, seem to be less subject to acute rejection in the pig than in the dog, and this is in accordance with observations in experimental orthotopic liver transplantation[38].

It has been suggested that the reticuloendothelial system of the liver participates in graft rejection[45]. The presence of a healthy host liver with an intact reticuloendothelial system may lead to a stronger immunological attack on the graft than will be the case in the presence of host liver disease[46].If auxiliary liver transplantation is to be used in the presence of severe host liver disease the role of the recipient's liver in rejection might be less significant. In our study in dogs (Chapter 2) long-term graft survival was observed in DLA-matched dogs that received immunosuppression. The influence of the intact reticuloendothelial system of the host liver in that experiment was not specifically studied but appeared not to prevent graft survival.

In our experiments graft survival might have been improved by blood transfusion as all recipient animals received a blood transfusion on the day of transplantation with blood from the donor animal. In renal transplantation it has been documented that there is an association between blood transfusions given to patients with renal failure during pretransplantation waiting periods or on the day of transplantation, and

improved graft survival of cadaveric renal allografts[47,48]. In orthotopic liver transplantation or auxiliary liver transplantation information is to date scarcely available concerning the influence of blood transfusion(s) upon graft survival[43]. This should be further examined both experimentally and clinically.
In the dog matching for histocompatibility antigens appeared to improve auxiliary liver graft survival considerably (Chapter 2), as was also shown for orthotopic liver grafts in dogs[49,50]. Because of donor shortage, time limits of liver preservation, and urgent recipient need, matching for the antigens of the major histocompatibility complex will not be easy in the clinical situation. To date, experience in orthotopic liver transplantation has shown no relationship between the degree of matching and results. As most patients received a poorly matched liver[41,51], the potential advantages of histocompatibility antigen-matching is difficult to assess in clinical liver transplantation.

7.6 Immunosuppression

All methods to prevent or reverse rejection of liver transplants are based on experience in renal transplantation. Double-drug therapy with azathioprine and steroids was used most frequently in liver transplantation as we did in our dog experiments. With this regimen one-year survival rates exceeding 50% can be obtained after human orthotopic liver transplantation[10]. Anti lymphocyte globulin resulting in lymphoid depletion has also been given as an adjunct to azathioprine and prednisone during the first few weeks or months when the risk of rejection is the greatest[9,41]. Cyclosporin A, a fungus extract which depresses humoral and cellular immunity, appeared to be very effective in preventing or delaying rejection of kidney grafts[52]. Introduction of cyclosporin A in orthotopic liver transplantation did not lead to a significant improvement in survival in the patients treated by the Cambridge group[53]. This is in contrast to the report from Starzl and co-workers who claimed that the use of cyclosporin A and steroid therapy, starting a few hours preoperatively, leads to better results compared to conventional immunosuppression[8]. When patients transplanted in the major transplantation centers, who received cyclosporin A are compared with those who did not, no significant difference is observed in orthotopic liver graft survival[54]. This suggests that other factors as better patient selection and modification of technique contributed to the

improved graft survival rates after introduction of cyclosporin A. Nevertheless, cyclosporin A may prove to have special value in young patients. If the use of cyclosporin A leads to a concomitant reduction of steroid dosage then steroid induced growth retardation could be less as shown in pediatric liver transplantation[23,55]. Hepatotoxicity of cyclosporin A has been described. It is, however, rather infrequent and tends to be mild and reversible[56]. Cyclosporin A has to date not been used in clinical or experimental auxiliary liver transplantation and it may be a subject for further study to investigate if this drug is superior to conventional treatment in heterotopic liver transplantation.

7.7 Diagnosis of rejection

Clinically the diagnosis of rejection is difficult and is often made by the exclusion of other reasons for graft dysfunction. Discrepancies exist in the histologic description of rejection, particularly in reference to the appearance of acute and chronic forms[57]. Early reports based on experimental studies are confusing as technical complications played an important role and acute and chronic rejection are usually ill-defined. Biliary obstruction and/or cholangitis may be the result of rejection or be secondary to complications in surgical technique. In our study differentiation of hepatic rejection into an acute or chronic form on histological criteria is a rather artificial one. Predominance of vasculitis and polymorphonuclear infiltration in portal triads was defined as *acute rejection*. In dog experiments this has been reported as to represent the earliest manifestations of rejection[58]. Loss of small interlobular bile ductules associated with pseudo bile duct formation and round cellular portal infiltrates was thought to be present in the case of *chronic rejection*. Difference in morphology, however, between the acute and chronic lesions is sometimes not very clear and the pattern even becomes more complex in the case of biliary obstruction or ascending cholangitis. In the clinical situation viral liver infections, drug induced hepatitis, and recurrence of the primary liver disease may further lead to interpretational problems[57]. Final interpretation of the histologic specimen has to be correlated with other data available at the moment of the biopsy.

Biochemical and hematological changes that occurred in this study in the postoperative period in animals with a healthy recipient liver were not valid as index of graft viability or rejection.

Cholescintigraphy enabled us to assess graft function in a non-invasive way and in the presence of a healthy recipient liver. Hyperplasia of the transplanted liver with atrophy of the recipient liver was clearly demonstrated by hepatobiliary scanning in our study. In a patient that received an auxiliary liver transplant in the presence of end-stage cirrhosis hyperplasia of the graft and atrophy of the recipient liver could also be demonstrated by this technique[27]. There is no doubt that isotope scanning is a very valuable method to assess graft function after heterotopic liver transplantation.

7.8 Evaluation of metabolic support

To evaluate our technique of auxiliary liver transplantation in the presence of host liver insufficiency, a suitable animal model of hepatic failure was needed. Such a model was developed by temporary inducing hepatic ischemia in pigs (Chapter 4). Although technically somewhat complicated it proved to fulfil the criteria for a satisfactory animal model of acute hepatic failure as compiled by Terblanche[59]. These include: (1) potential reversibility; (2) reproducibility; (3) death due to hepatic coma after elapse of a time period sufficiently long to allow hepatic support procedures to be instituted; (4) the use of a large animal; (5) induction of liver necrosis without biohazard. All animals subjected to six hour liver ischemia developed severe encephalopathy, a rise in plasma ammonia levels, and increased plasma ratio's for tyrosine and phenylalanine. Hepatic coma and death was encountered in all these animals, and because of the reproducibility of this set of phenomena, it seemed appropriate to test auxiliary partial liver transplantation as a supportive measure in these pigs. It has been calculated that only 0.03-3.0% of the clearance capacity for putative toxins of the normal liver can be replaced by todays hemoperfusion and hemodialytic procedures[60]. At least 20% of the capacity of the normal liver to remove these toxins is estimated to be required for survival[60]. To date liver transplantation seems to be the only support system available that can theoretically take over this detoxification capacity. Auxiliary partial liver transplantation sustained life in pigs with acute liver failure in the fourth and fifth experiment, whereas animals without a transplant all died of hepatic failure. The synthetic function of the failing liver was adequately taken over by the graft, as demonstrated by synthesis of clotting factors. In the clinical situation it has been shown that the

auxiliary liver can provide sufficient metabolic support as two patients have survived seven and thirteen years after such a procedure[14,27]. After extensive liver resections in man, life can be sustained as long as about a fifth of the total liver remains functional[61]. We therefore assume that transplantation of 60% of a donor liver will be sufficient as support system in future clinical auxiliary liver transplantation.

7.9 Clinical prospects for auxiliary partial liver transplantation

Before proceeding to auxiliary liver transplantation in man one has to investigate whether the vascular anastomoses as performed in the experimental animal are feasible in man. An anatomical study in human cadavers was performed to find out if partial liver grafts could be placed in the subhepatic position as we used in our animal studies (unpublished observations). Vascular anastomoses could be established in all cases and were not more difficult than in the porcine experiments. Reduction of the graft by left hemihepatectomy facilitated the placement of the caval anastomosis just above or at the level of the renal veins. These observations are in accordance with conclusions from other human cadaver studies[62,63].

Candidates for experimental clinical partial liver transplantation are in the first place those patients where orthotopic liver transplantation is currently not considered. This is the case in most patients with fulminant hepatic failure[64]. An important aspect of the definition of fulminant hepatic failure as stated in Chapter 1.1, is the absence of preexisting liver disease which implies that, if the patient survives, hepatic structure and function might return. Such patients are in desperate need of an adequate hepatic support system. The patients that during intensive care treatment fail to respond within a 3-5 days, or appear to have a (sub)total liver necrosis confirmed by laparoscopy and histopathological studies, should be considered as candidates for auxiliary partial liver transplantation. The chance of spontaneous survival after intensive supportive care in these patients is minimal[65]. Early transferral to the transplantation centre is mandatory so that response to conventional therapy can be closely monitored and a consideration for transplantation can be made without delay.
Another group of patients that might benefit from auxiliary partial liver transplantation are the patients with chronic end-stage liver diseases

that are not accepted for orthotopic liver transplantation. There are important differences in patient selection among the main transplantation centers[54]. Patients that are currently not accepted in one center may have a chance in another. In The Netherlands selection criteria for orthotopic liver transplantation are rather stringent. Untill January 1985, 374 patients were referred as potential candidates for orthotopic liver transplantation but only 40 were actually transplanted[66]. Fourty seven patients with chronic active cirrhosis or primary biliary cirrhosis were not accepted because of badly deteriorated liver function and the change of a successful liver transplantation was thought to be not likely. Since there is no alternative therapy for these patients auxiliary partial liver transplantation might be justified from an ethical point of view in these otherwise hopeless situations. The risk of a coincidental primary hepatic malignancy in the host liver, not detected preoperatively, should be considered in these patients. However, that risk is small: only 13 patients (2.6%) who had liver replacement to treat end-stage liver disease were found to have a coincidental liver tumor out of 500 patients that received an orthotopic liver transplant in that same period[11].
Children with genetic liver disease are also potential candidates for auxiliary partial liver transplantation. Metabolic errors that do not primarily affect the liver such as type I Crigler-Najjar syndrome, protoporphyria, and familial hypercholesterolemia might be treated theoretically by a small auxiliary liver transplant[67]. On an individual and selective basis an auxiliary partial liver transplant could be performed in these patients, although problems related to 'functional competition' have so far not been elucidated sufficiently experimentally. According to the Dutch Central Bureau of Statistics the number of patients that die each year as a result of 'acute non-infectious-hepatitis' is about 25 patients. In addition 15 patients die yearly of 'non-infectious hepatitis not-specified'. Based on these mortality figures the number of patients that might be expected to require an auxiliary liver transplant in this group is estimated to be 40 yearly at the most. The number of patients expected to be in need of an auxiliary liver transplant in the group of patients with end-stage chronic liver disease is hard to define and will depend upon the indications used and the number of patients that is rejected for orthotopic liver transplantation. Because there is a well organized organ procurement program in The Netherlands, an adequate number of donor livers will probably be available to match the need[68], especially as the liver, heart, and both kidneys can be procured from a single donor without compromising the anatomy and preservation of any of the organs[69].
Auxiliary liver transplantation with partial grafts from living related donors is theoretically possible. Clinical auxiliary autotransplantation

of the left liver lobe has been reported once[70]. This attempt was not successful because the basic requirements for optimal graft function, as outlined in Capter 1.5, were not met. Clinical auxiliary partial liver transplantation using living related donors is currently not justified as no experimental data on this subject exist.

7.10 Conclusions

The objectives of this study as formulated in Chapter 1.6, have been achieved.

1. A method of auxiliary partial liver transplantation has been developed in the dog. All theoretical requirements for optimal graft function as stated in Chapter 1.5 were met. The same method could be applied to pigs without essential modifications.

2. Concerning the effects of tissue typing it is concluded that DLA-matching in the dog experiments improved graft survival after auxiliary partial liver transplantation. The transplants in non-tissue-typed donor recipient canine combinations were subject to acute rejection. In the porcine experiments, acute rejection of auxiliary partial liver grafts in non-tissue-typed combinations did not occur.

3. A model of transient acute hepatic failure was developed in the pig. Liver ischemia for four hours did not result in lethal hepatic failure in all cases. Six hours of hepatic ischemia, however, resulted in hepatic failure and severe encephalopathy, leading to death within three days. This period between hepatic failure and death was sufficiently long enough, to assess auxiliary transplantation of part of the liver as a support system.

4. It was demonstrated that an auxiliary partial liver transplant can provide sufficient metabolic support and can improve survival in pigs with acute liver failure, induced before or after the transplantation procedure. There was some evidence of hepatic regeneration in the auxiliary partial liver grafts. Regeneration in the ischemically damaged recipient liver was found twice in

pigs where liver failure was induced before the transplantation procedure.

5. Finally, it was feasible to perform auxiliary partial liver transplantation in severely ill pigs with acute liver failure, although postoperative mortality rate was high. Intensive perioperative monitoring was necessary to correct hemodynamic disturbances. Reduction of the time interval between induction of liver failure and transplantation from 13 to 3 hours improved the condition of the animals and may have influenced differences in graft survival. Severe coagulation disorders that suggested intravascular coagulation, were seen following recirculation of the ischemic host liver.

As a result of these experiments it seems justified to further explore the possibilities of auxiliary partial liver transplantation in man.

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SUMMARY

In this thesis studies on auxiliary partial liver transplantation in the dog and the pig are reported. The motive to perform this study was the fact that patients with acute hepatic failure or end-stage chronic liver disease are often considered to form too great a risk for successful orthotopic liver transplantation. Auxiliary partial liver transplantation may offer a solution for those patients.

In the introduction to this thesis in Chapter 1 the indication for liver transplantation is discussed. Potential advantages of auxiliary liver transplantation compared with the orthotopic technique are lined out. The results in the limited number of patients so far treated by auxiliary liver transplantation are reported. A review is given of the literature on experiments in laboratory animals in which the technique of auxiliary liver transplantation was tested. Attention is focussed on problems of space, position and blood supply to the graft. It appeared that optimal conditions in auxiliary liver transplantation demands small or partial donor livers and that the transplant should have a low outflow pressure, adequate hepatic arterial inflow as well as adequate inflow of portal venous blood. Problems related to biliary drainage and rejection are discussed.

The essential part of this thesis is our own experimental work, reported in the Chapters 2, 3, 4, 5, and 6. A surgical technique of auxiliary partial liver transplantation was developed and studied in the dog and the pig. In porcine experiments metabolic support of auxiliary partial liver transplants in the presence of acute host liver failure was investigated. All experiments were performed in the Laboratory for Experimental Surgery of the Erasmus University, Rotterdam. The five chapters are written in the form of scientific papers. Chapter 2, 3, 4 and 5 have been published (2, 5) or have been accepted (3, 4) for publication. Chapter 6 has been submitted for publication.

In Chapter 2 (first experiment) a technique for auxiliary liver transplantation of 60% of a donor liver is described in the dog in which all criteria for optimal graft function are met. The effect of matching for the major histocompatibility complex on liver allograft survival is reported. Long-term transplant survival was found in DLA-identical littermate beagles. In non-tissue-typed donor-recipient combinations most transplants were subject to acute rejection.

In Chapter 3 (second experiment) the feasibility of our technique of auxiliary partial liver transplantation and rejection phenomena were studied in pigs. Advantages of the use of the pig in liver transplantation

experiments include similarity of the pig liver to the human liver with regard to the macroscopic and microscopic structure. Transplantations were first performed in non-tissue-typed donor-recipient combinations without immunosuppressive medication. No problems were encountered from changing our laboratory animal and no essential technical modifications had to be made. In contrast to findings by others in orthotopic liver transplantation, the porcine graft was subject to immune attack albeit milder than encountered in the canine experiments.

In the experiments described in Chapter 4 (third experiment) a model of acute hepatic failure was developed in the pig. Six hours total liver ischemia resulted in grade 4 encephalopathy and death of subtotal liver necrosis within 50 hours. Encephalopathy, rise in ammonia levels and plasma ratios for putative toxins were comparable to the human condition of acute hepatic failure. And as such our large animal model fulfilled the accepted criteria of a satisfactory animal model of acute hepatic failure.

In Chapter 5 (fourth experiment) ischemic liver cell necrosis was induced four days after auxiliary partial liver transplantation. Excellent graft function and metabolic support was demonstrated by, ammonia detoxification, synthesis of clotting factors, and glucohomeostasis. Seven out of thirteen animals survived in excellent condition untill sacrifice at 26 days after induction of acute liver failure. Evidence of hepatic regeneration was found in the transplants but not in the damaged host liver.

The experiment described in Chapter 6 (fifth experiment) deals with hemodynamics and coagulation disorders in pigs where ischemic liver cell necrosis was induced before auxiliary partial liver transplantation. Hypotension and poor animal condition resulted in early death in 9 out of 11 pigs that received an auxiliary partial liver transplant thirteen hours after induction of liver failure. Reduction of the time interval between induction of liver failure and transplantation to three hours, improved the animal condition at the beginning and during the auxiliary partial liver transplantation, as evidenced by the higher blood pressure compared to the first group. Reduction of the time interval, however, did not improve the animal survival, as only two pigs out of ten survived more than two weeks after auxiliary partial liver transplantation. Decrease in cardiac output and increase of pulmonary and systemic vascular resistance was observed during auxiliary liver transplantation. Deficiencies in hemostasis explained by consumptive coagulopathy rather than fibrinolysis were noted that correlated with poor animal prognosis. In the four longer surviving pigs of both groups either the graft or the host liver recovered.

In Chapter 7 the foregoing experiments and clinical prospects for auxiliary partial liver transplantation are discussed. It is concluded that auxiliary partial liver transplantation is technically feasible in dogs and pigs.

Sufficient metabolic support was demonstrated in the presence of acute host liver failure. Efforts should be made to further evaluate the possibilities of auxiliary partial liver transplantations in man.

SAMENVATTING

In dit proefschrift worden experimenten beschreven, die betrekking hebben op auxiliaire partiële levertransplantatie bij de hond en het varken. Dit onderzoek werd verricht omdat het risico van een orthotope levertransplantatie bij patienten met een acute leverinsufficiëntie of terminale chronische leverinsufficiëntie vaak te groot wordt geacht. Voor deze patientengroep zou auxiliaire partiële levertransplantatie een oplossing kunnen bieden.

De indicatie voor levertransplantatie wordt besproken in de inleiding tot dit proefschrift in hoofdstuk 1. De potentiële voordelen van de auxiliaire levertransplantatie in vergelijking met de orthotope techniek worden vermeld. Van de resultaten in de kleine patientengroep die tot dusverre werd behandeld met auxiliaire levertransplantatie wordt verslag gedaan. Hierna volgt een overzicht van de literatuur betreffende experimenten in proefdieren, waarbij de techniek van auxiliaire levertransplantatie werd onderzocht. In het bijzonder wordt de aandacht gevestigd op het probleem van ruimtegebrek bij de ontvanger, en op de positie en de bloedvoorziening van het transplantaat. Het bleek dat auxiliaire levertransplantatie het beste kan worden verricht met kleine donorlevers of een deel van een donorlever. De druk in de afvoerende venen van het transplantaat moet laag zijn en het transplantaat moet worden voorzien van voldoende arterieel en portaal bloed. Problemen met betrekking tot de galafvloed en de afstoting worden besproken.

Het belangrijkste gedeelte van dit proefschrift wordt gevormd door ons eigen experimentele werk, dat wordt beschreven in de hoofdstukken 2, 3, 4, 5 en 6. Een methode van auxiliaire partiële levertransplantatie werd ontwikkeld en bestudeerd bij de hond en het varken. Bij varkens met acute leverinsufficiëntie werd onderzocht of het auxiliaire partiële levertransplantaat de metabole functies van de zieke lever kon ondersteunen. De experimenten werden alle verricht in het Laboratorium voor Experimentele Chirurgie van de Erasmus Universiteit te Rotterdam. De vijf hoofdstukken zijn geschreven als wetenschappelijke publicaties. Hoofdstuk 2, 3, 4 en 5 werden gepubliceerd (2, 5) of geaccepteerd voor publicatie (3, 4). Hoofdstuk 6 werd ter publicatie aangeboden.

In hoofdstuk 2 (eerste experiment) wordt een model van auxiliaire levertransplantatie bij de hond beschreven, waarbij 60% van een donorlever wordt gebruikt en waarbij aan alle voorwaarden wordt voldaan die noodzakelijk zijn om het transplantaat optimaal te kunnen laten functioneren. Het effect van de mate van overeenkomst van de belangrijkste histocompatibiliteits antigenen van donor en ontvanger op de overleving van het levertransplantaat wordt beschreven. Langdurige transplantaat

overleving werd gezien bij transplantaties tussen DLA identieke beagle "littermates". Transplantaties tussen donor en ontvanger waarbij géén weefseltypering was verricht resulteerden meestal in acute transplantaat afstoting.

In hoofdstuk 3 (tweede experiment) wordt verslag gedaan van een experiment bij varkens, waarbij de uitvoerbaarheid van onze methode van auxiliaire partiële levertransplantatie en de transplantaat afstoting bij dit proefdier werden bestudeerd. Een van de voordelen van het gebruik van varkens bij levertransplantatie experimenten is de overeenkomst in macroscopische en microscopische structuur tussen de varkenslever en de menselijke lever. Er werden transplantaties verricht tussen donor en ontvanger combinaties waarbij geen weefseltypering had plaatsgevonden en waarbij geen immunosuppressieve medicatie werd gegeven. Het veranderen van proefdier leverde geen problemen op en er waren geen essentiele wijzigingen in techniek noodzakelijk. Het transplantaat wekte bij het varken een afstotingsreactie op, die welliswaar minder heftig verliep dan wij bij de experimenten in de hond hadden gezien, doch die door andere onderzoekers bij orthotope levertransplantatie in het varken niet werd waargenomen.

In hoofdstuk 4 (derde experiment) worden experimenten beschreven waarbij een model van acute leverinsufficiëntie bij het varken werd ontwikkeld. Ischaemie van de lever gedurende 6 uur leidde tot ernstige encephalopathie (graad 4) en tot de dood binnen 50 uur tengevolge van subtotale levernecrose. De encephalopathie, de toename van het ammoniak en de stijging van de plasmaratio's van de verschillende toxinen waren vergelijkbaar met hetgeen, in geval van acute leverinsufficiëntie bij de mens wordt gezien. Dit model van acute leverinsufficiëntie bij een groot proefdier voldeed als zodanig aan de daarvoor geaccepteerde criteria.

In hoofdstuk 5 (vierde experiment) werd vier dagen na auxiliaire partiële levertransplantatie ischaemische levercelnecrose veroorzaakt bij varkens. Het transplantaat functioneerde voortreffelijk zoals bleek uit de daling van de plasma concentratie van ammoniak, de synthese van stollingsfactoren en een normale bloedsuiker spiegel. Zeven van de dertien proefdieren overleefden in goede conditie, totdat ze werden opgeofferd 26 dagen na de inductie van acute leverinsufficiëntie. In de transplantaten kon leverregeneratie worden aangetoond doch dit werd niet gezien in de beschadigde levers van de ontvangers.

In hoofdstuk 6 (vijfde experiment) wordt een experiment beschreven waarbij hemodynamische veranderingen en stollingsstoornissen worden onderzocht bij varkens, waarbij ischaemische levercelnecrose was geïnduceerd vóórdat auxiliaire partiële levertransplantatie had plaatsgevonden. De proefdieren die een auxiliair partieel levertransplantaat kregen 13 uur na de inductie van leverinsufficiëntie hadden een lage bloeddruk en verkeerden in slechte

algemene conditie. Negen van de 11 proefdieren stierven spoedig na de transplantatie. Bij een tweede groep proefdieren werd de periode tussen de inductie van leverinsufficiëntie en transplantatie teruggebracht tot 3 uur. Dit had tot gevolg dat de conditie en de bloeddruk van de proefdieren verbeterde in vergelijking tot de eerste groep, zowel aan het begin als tijdens de auxiliaire partiële levertransplantatie. De overleving van de proefdieren verbeterde echter niet nadat de periode tussen de inductie van leverinsufficiëntie en de transplantatie was teruggebracht. Van de tweede groep overleefden slechts 2 van de 10 proefdieren meer dan twee weken na de auxiliaire partiële levertransplantatie. Tijdens de auxiliaire levertransplantatie werd een vermindering gezien van de 'cardiac output' en een toename van de vaatweerstand in de longen en in de periferie. Stollingsstoornissen bleken eerder te worden veroorzaakt door verbruiks coagulopathie dan door fibrinolyse en correleerden met een geringe overleving van het proefdier. Er trad herstel op hetzij van de eigen lever hetzij van het transplantaat in de vier varkens van beide groepen die gedurende lange tijd overleefden.

In hoofdstuk 7 worden de voorafgaande experimenten en de mogelijkheden voor klinische toepasbaarheid van auxiliaire partiële levertransplantatie besproken. Er wordt geconcludeerd dat auxiliaire partiële levertransplantatie technisch mogelijk is in honden en varkens. Het transplantaat blijkt in staat bij een proefdier met acute leverinsufficiëntie de metabole functies van de zieke lever voldoende te kunnen ondersteunen. De mogelijkheid van auxiliaire partiële levertransplantatie bij de mens zou nader onderzocht moeten worden.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren in 1949 te Leiden. Hij behaalde zijn eindexamen Gymnasium B aan het Baarnsch Lyceum te Baarn in 1968. In dat zelfde jaar werd de medische studie aangevangen aan de Rijks Universiteit te Leiden. Na het behalen van het candidaats examen (cum laude) was hij gedurende een jaar werkzaam als student-assistent op de afdeling Fysiologie van de Leidse Universiteit. In 1973 werd het doctoraal examen afgelegd. Hierna was hij gedurende bijna een jaar als doctoraal-assistent verbonden aan het Laboratorium voor Pathologische Anatomie van het Academisch Ziekenhuis te Leiden, en was hij als student-assistent werkzaam op de afdeling Inwendige Geneeskunde van het Bronovo Ziekenhuis te Den Haag. In 1976 werd het arts examen afgelegd. Hierna werkte hij als arts-assistent op de afdeling Heelkunde van het Rode Kruis Ziekenhuis te Den Haag (Hoofd Dr. J.J. Hamming). Gedurende twee jaar volgde hij de opleiding tot internist in het Bronovo Ziekenhuis te Den Haag (Opleider destijds Dr. H. Schrijver). In 1979 begon hij de opleiding heelkunde in het Academisch Ziekenhuis Dijkzigt te Rotterdam onder leiding van Prof.Dr. H. van Houten en Prof.Dr. J. Jeekel. In het kader van deze opleiding werkte hij gedurende een half jaar in het Chirurgisch Laboratorium van de Erasmus Universiteit te Rotterdam (Hoofd Prof.Dr. D.L. Westbroek). In 1985 werd hij ingeschreven in het specialisten register.Sedertdien is hij als arts-specialist verbonden aan de afdeling Algemene Heelkunde van het Academisch Ziekenhuis Dijkzigt te Rotterdam.