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STUDY OF
INTER-LIVER FUNCTIONAL COMPETITION IN HETEROTOPIC AUXILIARY LIVER TRANSPLANTATION USING A RAT MODEL

Faculty of Medicine

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CHAPTER 1

GENERAL INTRODUCTION

Liver Transplantation: successes and challenges

On the first of March 1963, Dr. Starzl and associates performed the first **liver transplantation** in human at the University of Colorado.[1] Despite the extensive and highly successful four-year laboratory experience with dogs, Starzl's team failed this initial clinical attempt. It took another 4 years before the first recorded success in the history of human liver transplantation was realized. On July 23rd, 1967, a 1.5 years old girl with primary hepatocellular carcinoma underwent a liver transplantation and lived for 13 months before dying from diffuse metastases. Three decades later, more than 60,000 liver transplantations have been performed worldwide,[2] which has indeed revolutionized the care for patients with end-stage liver disease: patients, who otherwise were doomed to die within months to years, now have the opportunity for extended survival with excellent quality of life after transplantation.[3] Around 80 to 90% of the patients who undergo liver transplantation today are expected to be able to survive for more than a year, and most centres report a five-year survival rate above 70%.[4-6] The drastically improved survival is mainly attributed to the development of effective immunosuppression agents, especially the introduction of cyclosporine and tacrolimus,[7] to the use of better solutions for graft preservation[8, 9] and to advances in anaesthetic and surgical techniques. Nowadays, liver transplantation is indicated for acute or chronic liver failure from more than sixty different diseases, amongst which cholestatic disorders, chronic hepatitis, alcoholic liver disease, metabolic diseases, fulminant hepatic failure and hepatocellular carcinoma are the most frequent indications.[10] The age of the patients ranges from the first week of life to adults over 75 years of age.[11, 12]

Despite the recorded successes of liver transplantation, the procedure still faces potential **complications**. ***Poor immediate graft function*** and ***infection*** are the major causes of graft and patients loss early after transplantation.[13, 14] Although immunosuppression can safely be withdrawn in about 20% of the recipients,[15] the majority of the long-term survivors requires life-long use of those drugs and are confronted with the chronic exposure to the ***toxic effects of immunosuppressive agents.*** Besides metabolic abnormalities such as diabetes mellitus, hyperlipidemia, hypertension and nephrotoxicity, even at the lower therapeutic doses of the calcineurin inhibitors (cyclosporine and tacrolimus), 80% of recipients have some degree of impaired renal function and 4% may develop renal failure with half of them requiring renal transplantation.[16] In addition, the growing susceptibility to infection and the rising risk of malignancy add an increasing toll to the outcome. While more promising medications will become available, efforts have been made to induce recipient tolerance specific to the transplanted donor organ. Future

applications of the donor-specific-tolerance induction in human may eventually lead to complete elimination of toxic immunosuppressive drugs. Another major problem facing transplant clinicians is the ***recurrence of the original diseases,*** which may occur in the patients with autoimmune diseases (such as primary biliary cirrhosis, primary sclerosing cholangitis and autoimmune hepatitis) and viral hepatitis (such as hepatitis B and C). It has been found that there is a direct correlation between overall immunosuppression, particularly steroids and antilymphocyte preparation, and viral replication. Although with regular administration of anti-hepatitis B specific immunoglobulins, hepatitis B relapse has become rare, almost all patients transplanted for hepatitis C will relapse despite the use of ribavirin. In some of them, fluctuating transaminases, cholestasis and fibrosis will eventually develop and progress to liver failure.[13]

The issue of utmost importance, however, is the widening gap between the rising number of potential recipients and the limited number of donors. Till 1999, the number of patients waiting for liver transplantation has reached over 11,000 in the United Stats, and, tragically, many of them would have lost their lives before a proper donor is available, as figure shows that more than 1,000 patients on the waiting list died in 1997 alone.[17] In the Euro-Transplant countries as well as in the United Kingdom, the mortality rate of patients on the waiting list is about 15 to 18%.[4] To address the problem of **donor shortage**, transplant surgeons are looking at new techniques to make better use of those organs that are offered. With ***marginal donor***, such as older donors (> 60 years), history of alcoholism, elevated transaminase, steatosis, and prolonged reamination, the uncertainty stays at whether there will be early graft function after transplantation. These donors who were turned down in the past are now being routinely utilized with success. In rare situations, even a liver with a metabolic defect, such as familial amyloidotic polyneuropathy, has been implanted into patients who would otherwise die. Further efforts are required to better define the donor criteria, so that the usable marginal organs can be distinguished from those that are unlikely to function. Other targets remain to make better match of donors and recipients, and to have greater understanding of the mechanism of ischemia-reperfusion injury.[11, 18, 19] The technique of ***splitting livers*** allows two patients to benefit from one donor, one with the right and the other with the left liver lobe. Besides that two transplant teams are needed to complete the recipients' operations, expertise and skill to divide a liver properly are essential for successful use of this technique.[20, 21]. With the advantage of short cold ischemia time, the use of a ***living related donation*** is another way to expand the donor pool. The elective nature of this procedure allows it to be performed at the best time for the recipient.[22-25] As the donor operation is not done for the physical benefit of the healthy donor and faces a complication

rate of 13-20% and even mortality,[26] moral and ethical considerations are raised on this approach. These innovative techniques have predominantly been employed in children, and up to 40% of the paediatric patients may be candidates for one of these modified procedures.[27, 28] The increasing shortage of donors has also encouraged the attempt to use animals as donors of transplant organs. Up to date, eight whole-organ liver transplants have been carried out using livers from chimpanzee, baboon and pig.[29-31] Only one recipient was able to survive for more than 70 hours.[32] Hyperacute rejection is the major cause of death in the immediate post-transplant period after a ***xeno-transplantation***.[33] Using transgenic animals bearing human complement regulatory proteins or modulating the antigenic target on the donor organ will be the first step to successful xeno-transplantation.[34] Further problems still remain to be solved on acute and chronic rejection; uncertainty of the physiological effects of the animal proteins produced by xenograft; and the risk of contamination of humans by animal viruses. Although recent clinical studies suggest that ***hepatocyte transplantation*** may be useful for providing metabolic support during liver failure to bridge patients to whole organ transplantation, and for replacing whole organ transplantation in metabolic liver diseases of certain type, shortage of human livers available for hepatocyte isolation and the necessitate for long-term immunosuppression are two major hurdles to widespread application of this procedure.[35] Current researches in experimental models are aimed especially at developing a tightly regulated human hepatocyte cell line that grows economically in culture and exhibits differentiated liver functions.[36] Similarly, a ***bio-artificial liver support system*** capable of sustaining the life of the patients with acute liver failure could lead to improved survival and an effective device may even be able to support the recovery of the native liver without liver transplantation. However, systems as such are still under investigation.[37, 38]

Auxiliary Liver Transplantation: specificities and difficulties

In comparison to **orthotopic liver transplantation** (OLTx), in which the diseased native liver is totally removed and replaced by a liver graft, **auxiliary liver transplantation** (ALTx) is characterised by leaving a whole or a part of the native liver *in situ* in parallel to an implanted hepatic graft. This concept is originated from an experimental work of Welch in 1955.[39, 40] Despite the fact that dramatically improved survival rate has made OLTx the standard treatment for the patients with end-stage liver diseases, theoretically, ALTx has several ***advantages*** over OLTx. Firstly, in case the graft is damaged by severe post-transplant rejection or vascular complications, which would otherwise lead to a fatal consequence of hepatic failure in OLTx, the remnant native liver in ALTx may provide some degree of synthetic and

clearing liver function. The imminent death of the recipient or the necessity for immediate re-transplantation may thus be obviated.[28, 41-43] Secondly, liver grafts obtained by innovative surgical techniques such as split and living related donor are often relatively small in size and may not always provide sufficient function for OLTx. These grafts can still be useful for ALTx, where matching of the donor and the recipient body size is not required.[44-46] Thirdly, an auxiliary liver may enable patients with terminal hepatic failure to be "bridged" for a few days or possibly weeks until a proper liver donor becomes available, and in this case a heterotopic procedure could preserve the orthotopic site for subsequent transplantation. An efficient auxiliary graft may even "bridge" the patient till the native liver recovers. For this purpose, concordant species could be potential donors of auxiliary "bridging" livers for selected patients when the need for transplantation is extremely urgent.[34, 47, 48]

The first auxiliary liver transplantation in human was performed by Absolon in 1964,[49] and it was till 1972 when an auxiliary transplantation truly prolonged a human life.[50] During the following two decades, ALTx was done solely in a heterotopic manner - **heterotopic auxiliary liver transplantation** (HALTx), where a graft (usually partial) is placed below the un-resected native liver. The initial clinical results of HALTx were rather disappointing. Amongst the reported fifty HALTx during the period 1964-1984, only two patients survived longer than a year, and one of them lived fourteen and half years after transplantation with a well functioning graft.[51-53] One of the major problems associated with HALTx is the limited space in the abdominal cavity for an additional large liver graft and the gravity gradient of the venous outflow caused by implanting the engrafted liver at a non-physiological position. This may lead to insufficient venous drainage of the engrafted liver and inadequate portal perfusion of the engrafted and even the native liver.[54] An insufficient reperfusion of the liver graft could cause primary non-function immediately after transplantation, and, at the long-term, graft atrophy may occur due to inter-liver competition.[55-59] Though the portal "banding" technique is a common practice to secure a sufficient amount of portal flow to the graft,[56-59] the difficulty often remains on the proportional distribution of portal blood to the two livers according to the purpose of the transplantation.[60] Vascular complications especially thrombosis of the portal vein are also more likely to develop after HALTx than OLTx. The high morbidity and mortality caused by these disadvantages have led to temporary abandonment of HALTx in the early 70s. Many efforts have been made ever since to improve post-transplant survival. Most notably, based upon the experiences in animal studies,[53, 61-66] the contributions of Terpstra's group have improved the surgical techniques of HALTx with markedly increased post-HALTx survival rate.[54, 58, 66-71] Since 1980s', the concept of ALTx has further been extended by the introduction of a new approach - **auxiliary**

partial orthotopic liver transplantation (APOLTx), where the left or the right lobe of the native liver is resected and replaced by an auxiliary graft.[28, 41, 42, 72-78] The physiological position of the hepatic graft by this approach results in an optimal outflow pressure. Accumulating clinical results have shown a reduced incidence of post-transplant portal thrombosis,[41, 77, 79] and banding of the portal vein to the native liver is also less required except in cases of non-cirrhotic metabolic liver diseases.[42, 78, 80, 81] Since the use of only segments 2 and 3 of the graft usually provides sufficient function, this technique is commonly performed with reduced-size, split or living related donor livers.[28, 43, 81, 81]

Up to date, ALTx, particularly APOLTx, has been considered as a successful alternative to OLTx in specific clinical situations. HALTx, which avoids the surgical trauma of more extensive liver dissection, has been used to treat selected patients with end-stage ***chronic liver diseases*** at high risk for OLTx.[52, 67-69, 82-84] Currently, most of the alleged difficulties of native hepatectomy are no longer relevant for experienced surgeons.[54] The major concern for using HALTx to treat patients in this category lies on the original disease in the remaining native liver, which may further affect the engrafted liver, and may promote secondary tumour development.

Children suffering from liver diseases represent about 15% to 20% of the liver transplant registrants.[85] They have a specific spectrum of indications with mainly hepatic-based ***inborn errors of metabolism***.[86] Since many of these diseases cause structural hepatic damage and eventually lead to cirrhosis, whole-liver replacement by OLTx is the preferred procedure. For certain types of non-cirrhotic metabolic disorders, such as type 1 Crigler-Najjar syndrome, urea cycle enzyme deficiencies, disorders of fatty acid metabolism, familial hypercholesterolemia, hemophilia and ornithine transcarbamylase deficiency, an auxiliary liver may correct the partial enzymatic deficiency responsible for the disease without the need to remove the otherwise normal native liver.[41, 42, 78, 81, 86-89] ALTx also preserves the patient's native liver, which remains accessible for future gene transfer therapy.[27, 90, 91] Difficulties stay on how to determine the optimal graft volume required to correct the metabolic deficiency, to appreciate the necessary portal supply to the two livers and to avoid the possible damaging effects of the retaining diseased native liver. The long-term prospect of this procedure needs more time to adjudge.

It has been shown that even with optimal intensive care, less than 20% of the patients with ***acute liver failure*** and ***sub-acute liver failure*** may survive without a liver transplantation.[92, 93] Even though the failing liver may have the potential to regenerate with time, most patients will suffer fatal neurologic damage before the liver recovery actually occurs. To date, liver

transplantation is still the best life saving procedure for many patients with acute liver failure. Although ALTx was initially proposed as a "bridge" to "tide the patient over" the period of acute hepatic insufficiency,[40] currently, most of the lives of the patients with acute liver failure are saved by OLTx with a one-year survival rate of over 60%.[94-96] Evidently, the potential recovery of the failing liver is completely negated by the total removal of the native liver in the OLTx procedure, and the majority of the patients who survive the OLTx surgery have to depend on life-long use of immunosuppressive drugs. In contrast, an auxiliary liver graft may provide temporary functional support for the patient to allow the native liver to recover. Following a sufficient recuperation of the diseased native liver, the auxiliary liver graft can then be either removed or left to atrophy. The toxic immunosuppressive agents can be tapered or eventually withdrawn, and its consequent complications will thus be avoided.[57-59, 72, 75-77, 97-109] Recently, Erhard et al. have demonstrated the feasibility of using arterialised portal supply to sustain the liver graft in ALTx for acute liver failure patients. The main advantage of this modified approach is to leave the hilum and the portal vein of the native liver untouched, which may reduce the incidence of possible complications such as arterial vascular stenosis or portal vein thrombosis caused by performing intrahilar vascular dissection and anastomosis. It also avoids disturbance of the native liver recovery due to "portal flow steal phenomenon".[110, 111] Current difficulties of using ALTx to treat patients with acute liver failure are mainly twofold: (1) There is still a lack of reliable pre-operative measurements to assess regenerating potential of the native liver for selecting appropriate candidates for the procedure. Indeed, histological evaluation of the presence of viable hepatocytes, fibrosis or regeneration in the diseased liver does not appear to have clear predictive value;[99, 112, 113] (2) The remaining, severely diseased native liver may put the newly engrafted liver at risk and lead to functional failure, or may also cause development of the so-called "toxic liver syndrome" with subsequent multi-organ failure.[104, 114, 115]

Rat Model of Auxiliary Liver Transplantation: evolution and questions

The first animal liver transplantation was performed in the dog by Welch in 1955[39] and later studies described this attempt in other animal species including the baboon,[116] the monkey,[117] the pig,[118] the mouse[119] and the rat.[120] The use of genetically well-defined inbred strains of rats allows indeed a variety of experiments to be set up with controlled immunological features. This, in addition to other advantages, has made the rat the most commonly used experimental animal for liver transplantation studies, since large animals such as the pig or the dog are of limited use for immunological studies due to

the difficulty of obtaining inbred strains.

In 1966, Lee and Edgington introduced the first rat model of ALTx. In this initial model, 32% of the donor liver was implanted at a heterotopic position; the blood supply of the engrafted liver was exclusively arterial from the recipient's coeliac axis; and the venous return of the graft went through the suprahepatic inferior donor vena cava anastomosed to the infrahepatic native cava. The portal vein, the infrahepatic inferior vena cava and the common bile duct of the liver graft were ligated. Apart from a liver mass reduction of approximate 68%, the portal vein and the hepatic arterial supply as well as the bile drainage of the recipient's native liver remained intact.[120] Further modification was added to this model by the same authors two years later. At this time, the engrafted liver was re-vascularized with both the portal venous and the hepatic arterial supply, while the native liver was deprived of portal blood. The bile drainage of the engrafted liver was not yet reconstructed. Serum aspartate aminotransferase activity and histology were used as indicators of allogeneic liver rejection. In addition to the description of histo-pathological changes during the rejection process, it was also observed that re-establishing portal supply to the engrafted liver would prompt graft regeneration. It was therefore concluded that the portal blood flow was the major factor influencing liver regenerative capacity.[121]

Kort et al. reported a technique, in which the infrahepatic donor vena cava was used to drain the engrafted liver in the rat HALTx model. It appeared that this modification could drastically reduce the incidence of post-transplant graft congestion, which was frequently seen in the original model. Consequently, the survival rate after HALTx was improved. However, neither the hepatic artery nor the bile drainage of the engrafted liver was re-constructed.[122]

Further improvement of survival was obtained by performing the procedure of re-connecting the graft common bile duct to the recipient's duodenum.[123] A delicate method to share portal blood supply between the two livers was later described by Hess et al. The engrafted liver was perfused with mesentericosplenic venous blood, while the native liver retained the pancreaticoduodenal portal flow along with hepatic arterial supply. Due to technical difficulties, hepatic arterial supply to the engrafted liver was not re-established.[124] By examining the effects of different surgical manipulations of the native liver on the graft regeneration, their studies showed that the fate of the auxiliary liver graft was directly influenced by the functional state of the recipient's native liver independent of the amount of portal blood flow.[124, 125]

Müller reported his version of rat HALTx in 1983, in which the liver graft was implanted in the left loin following a left nephrectomy. The auxiliary liver was supplied with sole arterial blood through an anastomosis between

the recipient's left renal artery and the graft's portal vein, while the hepatic artery of the graft was ligated. This made it the first rat model of ALTx with an arterialised portal supply. Venous outflow of the engrafted liver was drained into the recipient's vena cava through an anastomosis between the inferior vena cava of the graft and the left renal vein of the recipient. The common bile duct of the engrafted liver was connected to the recipient's left ureter. No surgical manipulation was done on the native liver. Instead of conventional hand-sutured technique, an intra-vascular polyethylene "stent" was used to connect the graft's portal vein to the recipient's renal artery. This simplified technique shortened the total ischemic time of the graft and led to an improved two-week survival rate of 77% after transplantation. In this specific setting of a portal-arterialised liver graft functioning in parallel with a normal native liver, the isogenic auxiliary liver graft showed gradual atrophy, but its histological microstructure was preserved even six months after transplantation.[126]

The "cuff" technique, or extra-vascular stent, is another approach to simplify the method of micro-vascular anastomosis. It was applied in rat HALTx surgery by Marni et al. to perform both portal vein and vena cava anastomoses. With this method, occlusion time of the recipient's portal vein needed to perform the portal anastomosis and the total ischemic time of the liver graft were considerably decreased, which contributed to a two-week survival rate as high as 90%. To favour the competitive position of the engrafted liver, the recipient's native liver was handicapped with both a partial hepatectomy and a common bile duct ligation, while no re-arterialisation of the graft was attempted. Two months after the transplantation, the engrafted livers enlarged to a size twofold that of the original ones, whereas the recipient's native livers appeared atrophic. The authors mentioned the interest of using this model for transplantation immunology studies.[127]

Lee et al. described two specific variations of rat HALTx in 1986. The first one was called the "double-liver" model, in which the suprahepatic inferior vena cava of the engrafted liver was anastomosed to the infrahepatic portion of the recipient inferior vena cava; the graft's portal vein was anastomosed more distally to the recipient's inferior vena cava at the level of the renal vein. The inferior vena cava between the two anastomoses was then ligated, which led to the perfusion of the engrafted liver with systemic venous blood. The graft's hepatic artery was anastomosed to the recipient's infrarenal aorta. No manipulation was done on the native liver. Along with its later arterial-venous fistula version,[128-130] this model was designed to study liver regeneration following transplantation. The second variation introduced by Lee was the so-called "reversed circulation" model. The recipient's portal vein was connected to the suprahepatic vena cava of the engrafted liver, and

the engrafted liver was drained through an anastomosis between the graft's portal vein and the recipient's infrahepatic vena cava. The native liver was thus deprived of its portal supply. This model was developed to study the effects of a reversed circulation on the hepatic physiology.[131]

Schleimer et al. recently reported a new approach in the rat HALTx, in which the portal vein of the engrafted liver was arterialised via the recipient's renal artery.[132] The difference with Müller's technique is that the engrafted liver's outflow was made possible through an anastomosis performed directly between the donor and the recipient vena cava, and the bile duct was re-connected to the recipient's duodenum. The recipient's native liver was subjected to an 85% partial hepatectomy in order to induce acute liver failure. With the support of a portal arterialised auxiliary liver, the failing native liver regenerated to about 2.5% of the body weight and 83% of the rats did survive an observation period of three months.[133]

To date, two different types of APOLTx models have been described in the rat. The partial liver graft was implanted at an orthotopic site either above - in a reversal fashion[134, 135] or at the left side of the partial hepatectomised native liver.[136]

The rat model of ALTx has been used for various studies such as to evaluate new surgical techniques,[120, 122, 123, 126, 127, 131, 135, 137, 138] and related physiological[128, 128, 139, 140] or morphological changes of the liver;[141, 142] to test its feasibility for correcting metabolic deficiency[143-146] or for maintaining hepatic function and supporting native liver regeneration at the time of acute liver failure;[133] to study transplantation immunology[126, 147-149, 149-152] and to investigate the mechanisms of inter-liver functional competition.[120, 121, 124, 125, 133, 153-160]

In addition to technical difficulties, there are other problems with the use of the rat model of HALTx. Comparing to OLTx, the rat model of HALTx is characterized by the coexistence of the engrafted liver and the recipient's native liver after the transplantation. The commonly used parameters such as animal survival and serum biochemical tests could thus not discriminate the functionality of each individual liver. The question remains on how to evaluate the post-transplant viability of an individual liver, especially the engrafted organ. A possible approach is to handicap the native liver to such a degree that it will be insufficient to guarantee survival. The post-transplant animal survival and liver biology will then necessarily reflect the viability of the newly engrafted liver. In the previous studies, different surgical manipulations of the native liver have been used for this purpose either with a 68% partial hepatectomy alone [121, 122] or with a 68% partial hepatectomy plus a common bile duct ligation.[123, 127] Up to date, there has been no report on the possible differential effects of these various approaches on post-transplant

animal and graft survival, as well as the animal serum biochemical values. This was investigated in the current work. Furthermore, as a non-invasive measurement, magnetic resonance imaging (MRI) was tested for its capability to monitor the post-transplant hepatic viability in this rat model.

After HALTx, the interaction of the two livers also influences transplantation immunology[126, 147-149, 149-152] and may play a role in the inter-liver competition. Starzl et al. were among the first to observe that an auxiliary liver graft would atrophy in the presence of a healthy recipient's native liver after HALTx.[161, 162] The term of "functional competition", which was originally used to describe the compensatory hyperplasia of one liver lobe after occlusion of the bile duct of the other lobe,[163] was applied to depict this phenomenon. This competitive behaviour of the two livers has also been noticed later in the APOLTx.[42, 78, 80] Despite many efforts made over the past three decades, the precise mechanisms of this phenomenon are still not fully understood. Some investigators claim that atrophy or hypertrophy of the liver graft is the result of the functional competition between the two livers governed by the body demand for liver function. Shalm[164] and Hess et al.[123] showed that atrophy of the auxiliary liver together with functional deterioration of the graft was due to the presence of the recipient's un-handicapped native liver. Van der Heyde et al. demonstrated in the dog that by increasing a certain degree of functional impairment on the native liver, for instance through performing a common bile duct ligation, the graft atrophy could be prevented.[165] Hess confirmed these results in a rat model and showed that the auxiliary engrafted liver would be replaced by connective tissue if no manipulations on the native liver were done.[123] Van der Heyde et al. also reported that a host portacaval shunt would enhance the function of the graft and prevent its atrophy.[166] In contrary, other investigators dispute this theory and emphasize the importance of portal blood itself for maintenance of hepatic graft integrity by showing that the graft atrophy can be prevented by diversion of all portal flow through the graft.[162, 167] Using a "split transposition" dog model, Marchioro et al. reported that the liver fragments receiving splanchnic blood were always hypertrophic compared with the hepatic lobes perfused by systemic venous blood. Their studies suggested that only the splanchnic portal inflow, which carried certain substances, was essential for the preservation of hepatocytes integrity and could boost liver regeneration.[162, 168, 169] In a series of studies using this "double fragment model", in which the separated two liver fragments were provided with blood of various sources, Starzl et al. claimed that the main splanchnic venous "hepatotrophic factors" were endogenous hormones, amongst which the single most important was insulin.[170-173] While new substances with hepatotrophic effect continue to emerge, to date, it has generally been accepted that hormone hepatotrophic factors are the key elements for liver regeneration. Therefore, after HALTx, the liver that

receives the major part of portal supply will eventually regenerate, and the final outcome of the inter-liver competition actually depends upon the distribution of the "hepatotrophic factors" between the two livers.[42, 56-59, 80, 153, 154, 162, 170, 171, 174-178] No consensus exists so far on whether the physiological status of each individual liver may influence the inter-liver competition. This was investigated in the current work using a rat model of HALTx, in which the physiological balance was favourably slanted towards either the engrafted liver or the recipient's native liver through surgical manipulations. In addition, an effort was also made to re-examine the controversy on whether changing the content of the portal supply would alter hepatic regeneration capacity following a standard partial hepatectomy.

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CHAPTER 2

POST-TRANSPLANT EVALUATION OF HEPATIC VIABILITY IN THE RAT MODEL OF HETEROTOPIC AUXILIARY LIVER TRANSPLANTATION

2.1. Evaluation of Graft Viability in Heterotopic Auxiliary Liver Transplantation in the Rat

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SUMMARY

In the rat model of heterotopic auxiliary liver transplantation, the coexistence of the engrafted liver and the recipient's native liver makes it difficult to evaluate the post-transplant graft viability. In this study, auxiliary liver transplantation was performed in Wistar rats, in which the recipient's native liver was handicapped with a 68% partial hepatectomy and a common bile duct ligation. Serum biochemistry of the liver was analysed and compared with that of the selected control group. The surgical handicap of the liver showed severe damaging effects: the handicapped native livers appeared atrophic at autopsy and no long-term animal survival could be achieved without an auxiliary liver transplantation. As the engrafted liver corrected the cholestasis of the handicapped native liver, significant differences of serum biochemistry were found between the transplanted group and the control group: for bilirubin concentration and gamma glutamyl transferase activity from post-operative day 3 to 28 ($p < 0.05$); alkaline phosphatase on day 3, 7, 14 and 28 ($p < 0.05$); alanine aminotransferase activity on day 3 and 14 ($p < 0.05$) and aspartate aminotransferase activity on day 14 ($p < 0.05$). The efficiency to induce hepatic failure and to hamper its regeneration capacity in the native liver makes animal survival and liver biology as reliable parameters to evaluate the post-transplant graft viability in this rat model.

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INTRODUCTION

Although the first attempt of liver transplantation in human was a heterotopic auxiliary liver transplantation (HALTx) (*1*), orthotopic liver transplantation (OLTx) has long been accepted as the standard treatment for end-stage liver diseases. This is mainly due to the poor results of clinical HALTx. However, HALTx has always been theoretically attractive with advantages over OLTx in some specific cases (*2-4*). HALTx avoids the surgical trauma of more extensive liver dissection and the an-hepatic phase needed for the transplantation. For the patients with end-stage chronic liver disease at high risk for OLTx, HALTx certainly represents a life-saving alternative (*5*). In addition, a heterotopic auxiliary liver may correct some inborn errors of metabolism with enzymatic deficiency without removing the otherwise normal liver (*6*). Above all, HALTx is particularly useful for treating patients with fulminant and subfulminant hepatic failure in whom the function of the native liver may still recover. Following a successful recuperation of the native liver, the auxiliary liver can then be removed, thus avoiding the life-long use of immunosuppressive drugs and its potential complications. This has been proven by encouraging clinical results from different organ-transplantation centres in recent years (*7-10*).

However, the engrafted liver and the recipient's native liver may behave competitively to each other. As a consequence, the atrophy of the engrafted liver is the major cause of the failure in HALTx. Even though this "inter-liver competition" phenomenon was recognized as early as at the beginning of clinical and experimental HALTx (*11, 12*), its mechanisms still remain incompletely understood. Amongst other experimental protocols, the rat HALTx is an ideal model to perform studies related to this more exceptional but important clinical situation (*13, 14*).

As the engrafted liver coexists with the recipient's native liver in this rat model, how to evaluate the post-transplant graft viability was an open question. In the current study, the function of the native liver was surgically handicapped. The most commonly used serum biochemical parameters were tested as possible indicators to evaluate the post-transplant evolution of the engrafted liver.

MATERIALS AND METHODS

Experimental Animals

All animals of this study received care in accordance with institutional, national and European guidelines. Male out bred Wistar rats (B & K

Universal Limited, England) weighing 240-320g were used. The rats had free access to food and water before surgery.

Experimental Groups (*Table 1*)

HALTx group (n = 12): HALTx was performed in all rats of this group, in which the recipient's native liver was handicapped with both a 68% partial hepatectomy (68% PH) and a common bile duct ligation (CBDL).

In order to evaluate post-transplant liver biology, a proper control group with long-term animal survival was selected from four experimental models:

Group 1 (n = 10). The liver handicapped in the same way as for the recipient's native liver in the HALTx group: a portacaval shunt (PCS), a 68% PH and a CBDL.
Group 2 (n = 10). A PCS and a 68% PH.
Group 3 (n = 10). A PCS and a CBDL.
Group 4 (n = 10). A 68% PH and a CBDL.

Surgical Techniques

All operative procedures were carried out under ether anaesthesia with clean but non-sterile instruments, and x 12.5 magnification. During the operation, the body temperature of the rat was maintained at approximately 36°C by using heating pads.

(a) *HALTx*: The HALTx was performed according to the technique described elsewhere (*15*). Briefly, 32% of the donor liver was used for transplantation after perfusion with cold (4° C) heparinized physiological saline solution via the portal vein. The native liver of the recipient rat was handicapped with both a 68% PH and a CBDL, while the hepatic artery remained intact. Following a right nephrectomy, the engrafted liver was implanted in the right paravertebral gutter under the native liver of the recipient. The cuff technique was used for the anastomoses of both the vena cava and the portal vein. The engrafted liver received its blood supply from the portal vein of the recipient. The venous drainage was made through the right renal vein of the recipient to the vena cava. Restoration of the bile drainage was done by insertion of the intubated common bile duct of the donor liver into the recipient duodenum. No re-arterialisation of the engrafted liver was attempted in this study. The graft fixation was performed to avoid twisting and kinking of the donor vena cava as previously reported (*15*) (See photo illustration at the end of this section).

During the operation, cold ischemia time of the engrafted livers (starting from saline perfusion via the portal vein and ending at portal re-vascularization of the engrafted liver) was kept between 72 and 79 minutes.

(b) *PCS*: The method described by Lee (*16*) was modified with the cuff technique to perform a PCS. After a left nephrectomy, the left renal vein was freed up to the vena cava, which was then cross-clamped. An incision was made into the anterior wall of the stump of the left renal vein. The portal vein was divided from the pyloric vein to the liver hilus, tied, transsected and cuffed with a 0.4 cm length polyethylene tube. The cuffed portal vein was then inserted into the left renal vein and secured with a 5-0 ligature.

(c) *68%PH*: The median lobe and the left lobe of the liver were ligated and then excised according to the standard method introduced by Higgins and Anderson (*17*).

(d) *CBDL*: The common bile duct was double ligated with non-absorbable sutures and the segment in between was then removed.

Post-operative Care and Follow-up

All rats were given intra-muscular injection of 10 mg Na Cefazolin immediately after surgery and this was repeated daily for three days. No immunosuppressive agent was administered to the rats. During the following twenty-four hours, rats were given 5% glucose to drink, after which the pre-operative diet of food pellets and water was resumed.

All rats were observed daily and survival time of each rat was recorded.

For the serum biochemical analysis, a blood sample of 0.4 - 0.5 ml was collected from the rat's tail vein pre-operatively, on the post-operative day 3, 7, 14, 21, 28, 56 and 84. Serum bilirubin concentration (TBil), gamma glutamyl transferase activity (GGT), alkaline phosphatase (ALP), alanine aminotransferase activity (ALAT), aspartate aminotransferase activity (ASAT), lactate dehydrogenase (LDH) and total protein (TP) were determined by using BM / Hitachi System 747 (Boehringer Mannheim GmbH, Diagnostica). The reference intervals (range) used in this laboratory were for serum TBil 0.00 – 0.20 ml/dl, GGT 0 U/l, ALP 58.18 – 328.14 U/l, ALAT 17.38 – 59.55 U/l and ASAT 51.73 – 107.89 U/l.

An autopsy was performed whenever a rat died. The long-term survivors were sacrificed at the end of the 3-month observation period. The livers were excised, cleaned of gross adhesions, and fixed in 10% formalin. Four-micron thick sections were stained with hematoxylin and eosin (HE) or trichrome

and examined under light microscopy.

Table 1. Experimental groups and post-operative animal survival

Experimental groups	Survival time (days, mean ± SD)			
Control groups				
G1 (n = 10): PCS + 68% PH + CBDL	0.5 ± 0.3			
G2 (n = 10): PCS + 68% PH	0.8 ± 0.6			
	Survival rates			
	1 week	1 month	2 months	3 months
Control groups				
G3 (n = 10): PCS + CBDL	100%	80%	20%	20%
G4 (n = 10): 68% PH + CBDL	100%	100%	80%	50%
HALTx group (n = 12)	100%	66%	50%	50%

Note: PCS, portacaval shunt; 68% PH, 68% partial hepatectomy; CBDL, common bile duct ligation; HALTx, heterotopic auxiliary liver transplantation.

Statistical Analysis

The data were expressed as the mean ± standard deviation (SD). The distribution of the data was checked by Kolmogorov test. The Student's *t*-test was applied for the normally distributed data, while the not-normally-distributed data were evaluated by the Mann-Whitney rank sum test. *P* values equal to or less than 0.05 were considered statistically significant.

RESULTS

Post-operative Animal Survival (*Table 1*)

HALTx group. All 12 rats survived the first post-transplant week. The 1-month and the 3-month survival rate were 66% and 50%, respectively. The causes of post-operative deaths were mainly due to liver abscess (3/6), cholangitis (1/6), peritonitis (1/6) and rejection (1/6). The biochemical data of the six long-term survival rats were compared with that of the control group.

Control groups. Despite the comparable vascular cross-clamping time, all rats in the group 1 died of liver failure within 24 hours after surgery and in the group 2 reduced liver handicap could not significantly improve animal survival. In the group 3, two out of ten rats died of infectious complications (cholangitis and peritonitis) during the 1st post-operative month and six rats that died between the 1st and the 2nd month showed signs of biliary congestion with ascites. The 2-month survival rate was only 20% in this group. All 10 rats of the group 4 survived the first post-operative month. One rat died of pneumonia later on and cholangitis caused the other 4 animal losses. The 3-month survival rate of this group was 50%.

Serum Biochemical Analysis

Since none of the rats in the group 1 and 2 survived longer than two days and the 2-month survival rate of the group 3 was as low as 20%, only the data of the group 4 were available to compare with those of the HALTx group.

The most commonly used serum biochemical parameters (TBil, GGT, ALP, ALAT, ASAT, LDH and TP) were evaluated as possible indicators of liver viability for this rat model. No significant differences between the two groups were found for LDH and TP.

Serum TBil, GGT, ALP, ALAT and ASAT levels were elevated in the rats of two groups after surgery. However, the rats in the HALTx group had significantly lower values of TBil than that in the group 4 on day 3 ($p < 0.001$), 7 ($p < 0.001$), 14 ($p < 0.001$), 21 ($p < 0.001$) and 28 ($p < 0.005$) (*Figure 1a*). The same profile appeared for GGT on day 3 ($p < 0.001$), 7 ($p < 0.01$), 14 ($p < 0.05$), 21 ($p < 0.005$) and 28 ($p < 0.05$) (*Figure 1b*). Serum ALP also showed a pronounced difference between the two groups on day 3 ($p < 0.01$), 7 ($p < 0.05$), 14 ($p < 0.05$) and 28 ($p < 0.01$) (*Figure 1c*). In the HALTx group, serum ALAT was significantly lower than those of the group 4 on day 3 ($p < 0.05$) and 14 ($p < 0.001$) (*Figure 1d*) while ASAT differed significantly between the two groups on day 14 ($p < 0.05$) (*Figure 1e*).

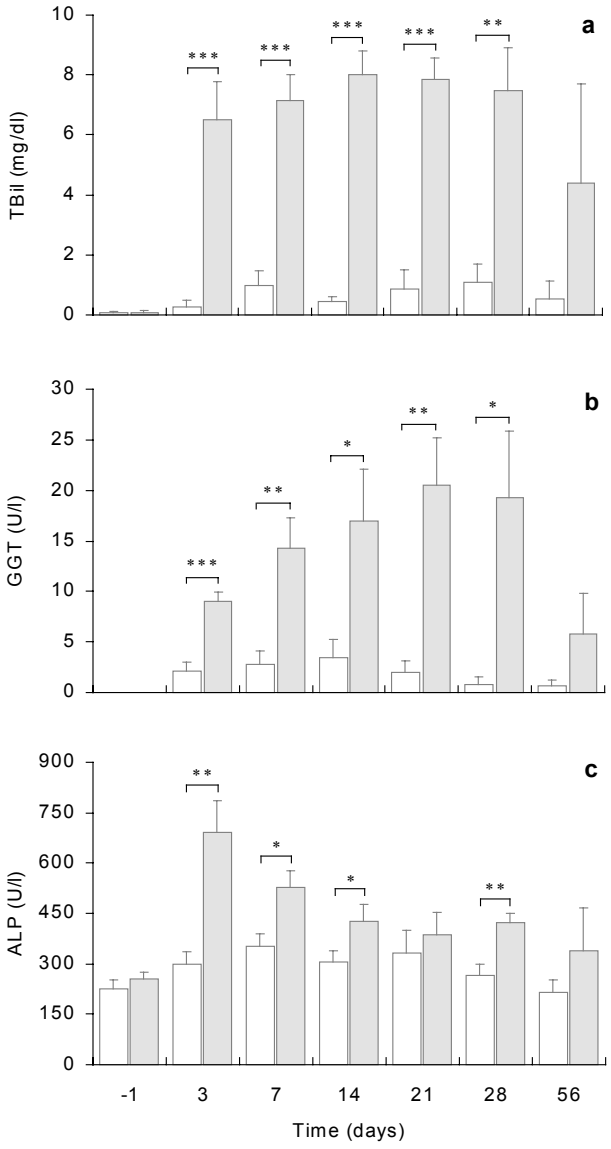


Figure 1. Continued

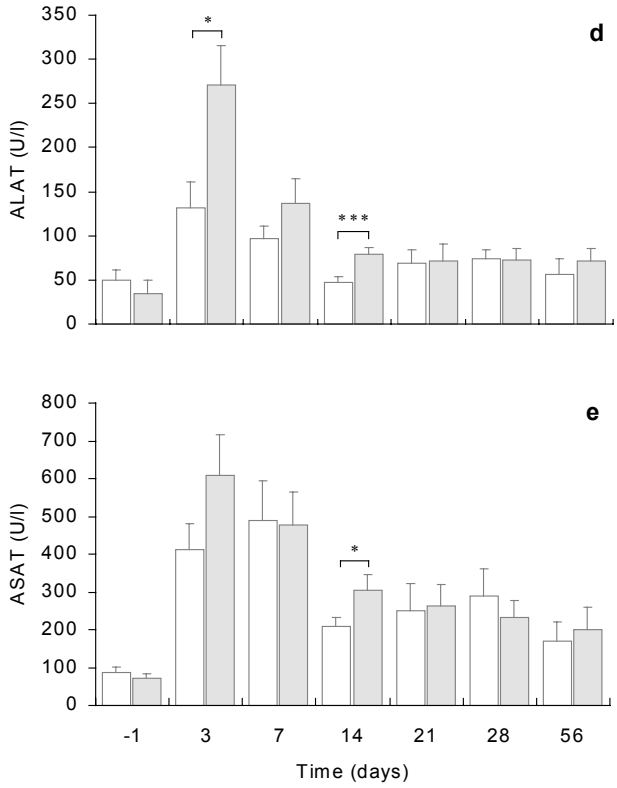
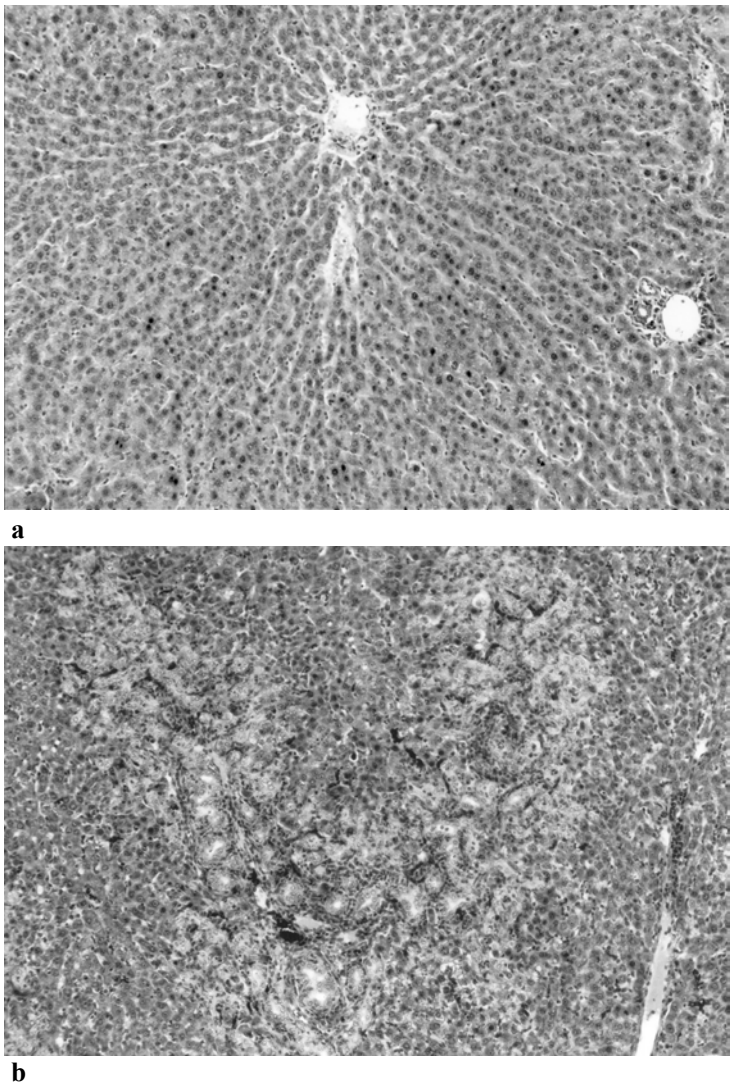


Figure 1. Comparison of serum biochemical tests (mean ± SD) between the HALTx group (☐) and the control group 4 (■): (**a**) bilirubin concentration (TBil), (**b**) gamma-glutamyl transferase activity (GGT), (**c**) alkaline phosphatase (ALP), (**d**) alanine aminotransferase activity (ALAT) and (**e**) aspartate aminotransferase activity (ASAT). Asterisk indicates statistically significant differences between the two groups. * $p \leq 0.05$, ** $p < 0.01$ and *** $p < 0.001$.



a

b

Figure 2. Histological findings of 3-month survival rats: (**a**) HALTx group: The engrafted liver preserved its normal lobular architecture without major histopathological alterations (original magnification x 125). (**b**) Control group 4: The liver completely lost its general lobular architecture with the proliferation of the cholangioles lined by swollen cuboidal epithelium (original magnification x 125)

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Autopsy and Histology

Three months after the transplantation, the 6 long-term survivors of the HALTx group were sacrificed for histological analysis. At autopsy, the engrafted livers of these rats appeared normal in size and colour and the cuffed portal vein and vena cava anastomoses remained patent. These engrafted livers revealed a normal lobular architecture without major histopathological alterations (*Figure 2a*), whereas the general lobular architecture of the handicapped native livers was lost and replaced by ductular metaplasia in the portal tracts accompanied by fibrosis.

In the group 4, the 5 long-term survival rats were also sacrificed 3 months after the surgery. A bile duct cyst of 1-3 cm diameter was found in the hepatic hilus of all animals. The livers were macronodular with disappearance of the general lobular architecture characterised by the proliferation of the cholangioles lined by swollen cuboidal epithelium (*Figure 2b*). There was no substantial difference with the native liver of the recipient in HALTx group.

DISCUSSION

Since the technique of HALTx in rat was first described in 1966 (*13*), this rat model has been used in experimental researches of various subjects such as liver regeneration, hepatotrophic factors, allogenic microchimerism and graft rejection (*18-20*). As the two-liver state in this model makes it difficult to evaluate the viability of the engrafted liver after the transplantation, a possible approach is to handicap the recipient's native liver to such a degree that it will eventually cease its function. In this study, liver failure was induced surgically by means of handicapping the native liver with both a 68% PH and a CBDL. Corresponding to a previous observation (*14*), it appeared that this handicap could damage the organ so severely that not only the liver failure was assured causing animal death, but also the hepatic regeneration capacity was completely impaired leading to atrophy in long-term survivors. Therefore, the post-transplant animal survival relays primarily upon the function of the engrafted liver, and the serum biochemistry of the liver reflects the viability of that engrafted organ despite the presence of the recipient's native liver.

In evaluating the post-transplant graft viability, the combination of a PCS, a 68% PH and a CBDL should be the most appropriate control since the native liver in the HALTx group was handicapped in the same way. However, without an auxiliary liver, this approach was always lethal. A reduced handicap with a PCS and a 68% PH also failed to significantly improve

animal survival. Although the rats subjected to a PCS plus a CBDL did survive longer, a 2-month survival rate of 20% could not provide sufficient data for a long-term comparative study. Most rats of this group died between the 1st and 2nd post-operative month with signs of cholestasis and ascites, which indicated that this handicap could induce a subacute liver failure. In the absence of long-term survival, no real control or comparison could be done and even less traumatic surgical procedures had to be tested. A 68% PH plus a CBDL without a PCS eventually appeared to be the only viable control of this model, which facilitated a long-term comparison of serum biochemistry with the HALTx group.

Along with autopsy and pathological examination, post-operative animal survival is indeed a commonly used parameter to evaluate the viability of the engrafted liver after HALTx. However, survival rate does not allow to evaluate, for instance, the degree of the liver injury caused by preservation and re-perfusion, and to appreciate the post-operative complications of the engrafted liver. Serum biochemistry of the liver is an un-invasive measurement, which can be used for systematic evaluation of graft viability by simple means. In contrary to previous publications (*18, 21*), in this study, LDH was not significantly different between the HALTx group and the control group. This could be due to the fact that LDH is an enzyme present in a wide variety of tissues including heart, skeletal muscle, kidney and brain in addition to the liver. Thus muscle trauma induced by the operative procedures can interfere and influence LDH value mainly in the early observation phase. Since TP measures different sorts of proteins, which may be affected positively or negatively by the experimental interventions, it lacks specificity for the liver function as shown in this study.

Serum TBil, GGT and ALP are the parameters directly linked to hepatobilirary cholestasis. In the present study, the serum levels of TBil, GGT and ALP increased drastically in the rats of the control group after a 68% PH and a CBDL, but not in the HALTx group despite the fact that the recipient's native liver was subjected to the same handicap. This demonstrated that the function of bile secretion was mainly taken over by the engrafted liver from the recipient's native liver, which was consequently less cholestatic. During the first post-operative month, the significantly lower levels of serum TBil, GGT and ALP in the transplanted rats were thus a sign of proper function of the engrafted liver. The elevation of serum ALAT and ASAT immediate after the operation indicated hepatocellular damage in both the transplanted and the control groups. The lower peak values and faster normalisation of both parameters in the rats of the HALTx group suggested that the engrafted liver reduced the deleterious effect of cholestasis on the native liver.

As the high success rates of the HALTx procedure can be achieved with a sole portal blood supply, the re-arterialisation of the engrafted liver has not been considered as a necessity for this rat model (*14, 22, 23*). However, using OLTx model, the recent studies have demonstrated the beneficial effects of the re-arterialisation on improving micro-vascular perfusion (*24*), modifying the expression of class I and II MHC antigens (*25, 26*), maintaining an adequate oxygen supply and tissue ATP (*27*), reducing the post-transplant biliary complications (*28-30*) and increasing animal survival rate (*31, 32*). Whether equal mechanisms may be implicated in the HALTx has not yet been addressed. As the interaction between the engrafted and the native liver added an important element to this specific rat model, this question indeed warrants further attention.

In conclusion, the data of present study show that in this rat model of HALTx, handicap of the native liver with a 68% PH and a CBDL can efficiently induce liver failure and hamper its regeneration capacity. The post-transplant animal survival and normalization of the liver biology reflect well the viability of the engrafted liver.

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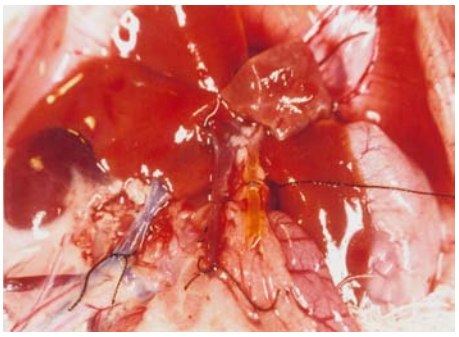
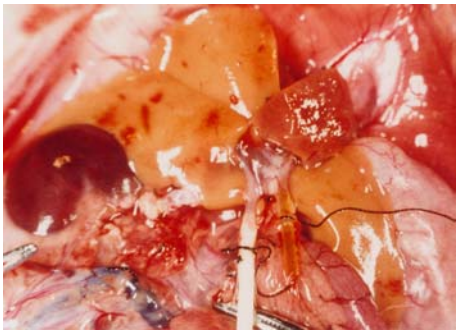


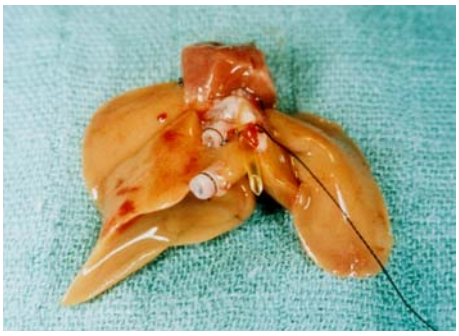
Donor liver preparation

After a 68% partial hepatectomy, the common bile duct was intubated; the portal vein and the vena cava were isolated.



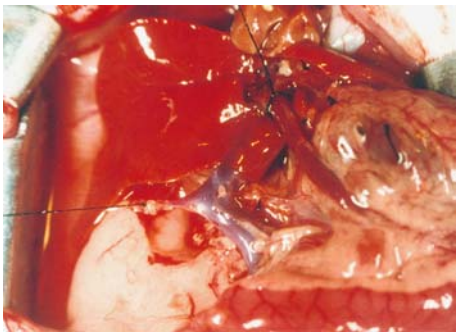
Donor liver perfusion

The donor liver was then perfused with cold saline solution through the portal vein.



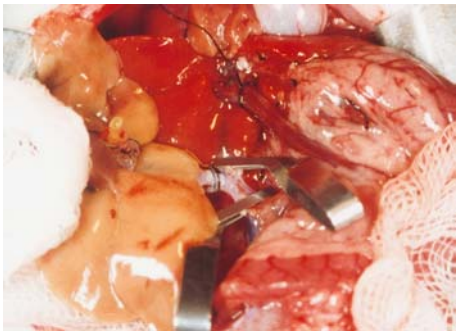
Cuff preparation

Both the portal vein and the vena cave of the graft were cuffed with a polyethylene tube.



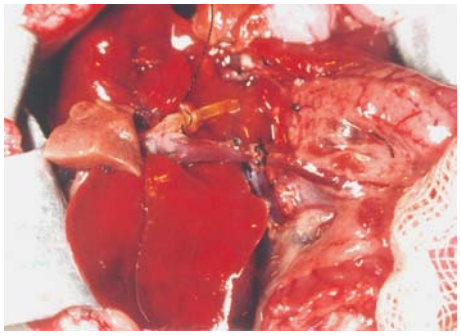
Recipient preparation

The native liver of the recipient was handicapped with a 68% PH and CBDL. The portal vein and the right renal vein were prepared for vascular anastomoses.



Graft venous outflow

The venous drainage of the engrafted liver was made through the right renal vein of the recipient to the vena cava.

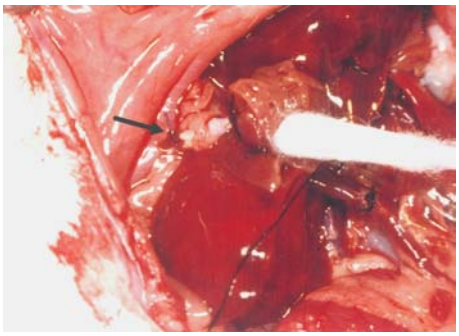


Graft blood supply

The engrafted liver received its blood supply from the portal vein of the recipient.

Graft bile drainage

The intubated common bile duct of the graft was inserted into the recipient duodenum.



Graft fixation

The remained donor diaphragm was fixed to the recipient's lateral abdominal wall.

2.2. T_1 Relaxation Times for Viability Evaluation of the Engrafted and the Native Liver in a Rat Model of Heterotopic Auxiliary Liver Transplantation: a pilot study

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SUMMARY

Following a heterotopic auxiliary liver transplantation, commonly used measurements are either invasive or non-indicative of individual viability of the coexisting engrafted and native livers. Magnetic resonance imaging (MRI) was therefore tested for its potential to monitor the post-transplant hepatic viability in a rat model. Thirteen Wistar rats were systematically evaluated with MRI and serum biochemical liver parameters. Post-transplant complications and the causes of animal death were identified by autopsy and histo-pathological examinations. The data of the healthy survivors were compared with those of the rats that developed complications. On MRI, the hepatic complications could be depicted in the individual livers. A specific pattern of signal evolution was found in the livers of the healthy survivors: the mean T_1 relaxation times of the engrafted livers increased immediately after transplantation (476 ± 64 ms, mean ± standard deviation, pre-operative; 730 ± 48 ms, week 1) and then declined steadily to a three month value of 489 ± 246 ms; while, following a transient first rise (476 ± 64 ms, pre-operative; 589 ± 28 ms, week 1), the mean T_1 value of the native livers increased again four weeks after surgery and reached a three month value of 859 ± 43 ms. However, in the rats with various complications, the mean T_1 relaxation times of the engrafted livers continued to increase throughout the first post-operative month (760 ± 48 ms, week 1; 922 ± 76 ms, week 4), while that of the native liver only varied mildly (546 ± 25 ms, week 1; 473 ± 25 ms, week 4). After the first post-transplant week, the healthy engrafted livers could already be distinguished from those with complications by a significant decrease in T_1 relaxation times. These data suggest that, besides demonstrating major complications, MRI may allow one to monitor the viability of each liver by analysing the relative signal intensity and T_1 relaxation times after a heterotopic auxiliary liver transplantation.

NMR in Biomedicine 2001; 14:350-359

INTRODUCTION

In heterotopic auxiliary liver transplantation (HALTx), a donor liver graft is implanted into the recipient without complete removal of the diseased native liver. This operative procedure avoids the surgical trauma of more extensive liver dissection and the an-hepatic phase needed in case of orthotopic liver transplantation (OLTx). For the patients with end-stage chronic liver disease at high risk for OLTx, HALTx can indeed be a life saving alternative [1]. In addition, without removing the otherwise normal liver, an auxiliary liver may correct some inborn errors of metabolism with enzymatic deficiency [2,3]. Above all, HALTx has the advantage over OLTx in treating patients with fulminant and sub-fulminant hepatic failure in whom the function of the native liver may still recover. Following a successful recuperation of the native liver, the auxiliary liver can then be removed or left to atrophy, thus avoiding the life-long use of immunosuppressive drugs and its potential complications [4-9].

Despite these advantages, HALTx has not widely been used. Comparing to OLTx, HALTx is technically more demanding with a higher incidence of post-transplant complications such as arterial and venous thrombosis. Moreover, as the remaining native liver is functionally competing with the newly engrafted liver, graft atrophy is another major cause of failure [10,11]. Since the so-called "hepatotrophic factors" are generally accepted as the prime factor for the preservation of hepatic integrity, a proper sharing of the portal blood between the two livers is important for the success of the procedure [12]. Up to date, a reliable method of dividing the portal blood flow proportionally between the two livers is still lacking. Careful monitoring of the post-transplant evolution of the two livers is therefore crucial, since an early identification of complications can lead to a possible surgical correction or other interventions that may reverse the otherwise failing procedure. In case of a HALTx for fulminant or sub-fulminant hepatic failure, an accurate assessment of the regeneration of the native liver is also important to define the exact time to taper and eventually to stop immunosuppression, and possibly to remove the engrafted liver [13]. Amongst the most commonly used measurements for the post-transplant evaluation, biological tests of the liver can only show the global hepatic function rather than the respective contributions of each individual liver. Although the individual function of the engrafted liver or the native liver is assessable by Hida scintigraphy [14,15], various post-transplant complications that may lead to graft dysfunction, such as acute rejection, vascular occlusions, biliary obstruction and abscess formation, cannot be distinguished by this technique [16]; besides, the procedure uses radioactive tracers. Histological examination, which does show the state of the examined organ accurately, requires invasive biopsy procedures and cannot be repeated frequently without risk.

As a non-invasive technique, magnetic resonance imaging (MRI) may have the advantages of providing reliable information of each individual liver after a HALTx. Moreover, the post-transplant evolution of the two livers recorded by a systematic MRI monitoring may be useful for studies on the mechanisms of "inter-liver competition" with important impact on liver transplantation and hepatological research. This potential had not yet been explored so far and was tested in the current study using a rat model of HALTx.

MATERIALS AND METHODS

Experimental Animals

Male outbred Wistar rats (B & K Universal Limited, England) weighing 280-350 g were used as donors and recipients. All the animals received humane care according to the European guidelines for animal care (Het Belgisch Staatsblad, Feb. 29th, 1992). The rats had free access to water and standard pellet food. Before surgery, food but not water was withdrawn from the recipient rats for 12 hours.

Experimental Design

The combination of a 68% partial hepatectomy (68% PH) and a common bile duct ligation (CBDL) does severely handicap the function of the native liver and cause atrophy. An engrafted liver in this condition can benefit from stimulating trophic factors circulating in the portal system to regenerate [17]. This operative procedure with drastic changes in the engrafted and the native liver was specifically chosen to test the potential of MRI in post-transplant monitoring.

MRI measurement and serum biochemical liver tests were carried out on a regular basis. An autopsy and subsequent histo-pathological examination were performed whenever a rat died or at the end of the 3-month follow-up period when all the long-term survivors were sacrificed.

Sixteen rats were subjected to HALTx. Only the rats that survived the surgical procedure longer than one week were used for systematic post-transplant evaluation, while those that died within the first post-operative week were regarded as surgical failure and excluded from the study. Based upon autopsy and histo-pathological evidence, the rats were subdivided into two groups: group 1 consisted of the rats free of any complications; the rats that developed post-transplant complications including infection, biliary obstruction, vascular thrombosis and rejection were included in group 2. The data of MRI, liver biology and histology of these two groups were compared.

Surgical Procedure

All operations were performed with ether anaesthesia, clean instruments and x 12.5 microscopic magnification. Body temperature of the rats was maintained at approximately 36°C by using heating pads during surgery.

HALTx was performed according to the technique described elsewhere [18]. Briefly, after a 68% PH, the donor liver was perfused with cold (± 4° C) heparinized Ringer's solution via the portal vein. Following a right nephrectomy, the liver graft was implanted in the right paravertebral gutter under the native liver of the recipient. The cuff technique was used for the anastomoses of both the vena cava and the portal vein. The engrafted liver received its portal blood supply from the portal vein of the recipient. The venous drainage was made by connecting the infrahepatic vena cava of the donor liver to the right renal vein of the recipient. No re-arterialisation of the engrafted liver was attempted in this study. Restoration of the bile drainage was done by insertion of the intubated common bile duct of the donor liver into the recipient's duodenum. As the portal flow of the recipient was completely shunted to the engrafted liver, the blood supply of the native liver remained solely from the hepatic artery. The native liver was further handicapped with both a 68% PH and a CBDL.

For all the operative procedure of HALTx in the rats of both groups, the cold ischemia time of the liver grafts was kept between 62 and 74 minutes. The duration of warm ischemia of the liver grafts (from the implantation to the blood re-circulation of the engrafted liver) was between 11 and 14 minutes.

At the end of the operation, the rats were transfused with 1-1.5 cc of Ringer's solution and an intramuscular injection of 10 mg Na Cefazolin was given. No immunosuppressive agent was administered. The pre-operative diet of food pellets was resumed 2 hours after the operation.

MR Evaluation

During MRI examination, the rat was anaesthetised with 1.2 vol % isoflurane through a gas mask. Examinations were performed on a 1.0 Tesla scanner (EXPERT, Siemens™ Germany) using a standard circular polarised knee-coil with a diameter of 25 cm. Each MR experiment lasted less than 30 minutes. To establish the reference value of T_1 relaxation time in the normal rat liver, thirteen rats of the same strain free of any surgical manipulation were scanned. MRI examinations of the transplanted rats were performed pre-operatively and weekly during the evaluation period of three months. An extra five rats subjected only to a 68% PH and a CBDL without HALTx were also evaluated weekly during the first post-operative month to identify the effects of these surgical interventions on the status of the native liver.

For visual evaluation, the rats were scanned with 4 mm thick axial slices with T_1-weighted contrast (T_1W) (TR/TE/NEX = 400 ms/12 ms/4), and 2 mm thick coronal slices with T_1W contrast (400 ms/12 ms/4).

For quantitative evaluation, proton relaxometry with calculation of T_1 relaxation times in the engrafted and the native livers was carried out. For this, a spin-echo sequence was used and measurements were repeated with different repetition times (TR/TE = 2000,1000,500,250/12 ms). A T_1 relaxation curve was obtained by plotting the signal intensities in a region of interest in the engrafted and the native livers as a function of the repetition time. The T_1 relaxation time was extracted from the plots by fitting the data points against the theoretical T_1 relaxation functions given by the equation:

$$S_{(TR)} = S_0 \cdot (e^{-TR/T1} - 2 \cdot e^{-(TR-TE/2)/T1} + 1)$$

with S_0 and T_1 as the unknown variables. Fitting was performed by use of a home-written computer program based on the Levenberg-Marquardt algorithm, as this is proven to be preferable above other fitting methods with regard to signal-to-noise consideration [19].

Liver Biology

A blood sample of 0.4 - 0.5 ml was collected from the rat's tail vein for serum biochemical tests. Using routine photometric tests on a Hitachi 747 analyser, serum alanine aminotransferase activity (ALAT) and bilirubin concentration (TBil) were determined pre-operatively and further on the post-operative day 3, 7, 14, 21, 28, 56 and 84.

Autopsy and Histology

During each autopsy, the examination was focused on the macroscopic appearance of the engrafted and the native liver, the patency of the vascular anastomoses and the common bile duct, the complications and the possible causes of animal death where premature death occurred.

The livers were excised, cleaned of gross adhesions, and fixed in a 10% formalin solution. Four-micron-thick sections were stained with hematoxylin and eosin (HE), and examined light-microscopically.

Statistics

The data were expressed as the mean ± standard deviation (SD). The distribution of the data was checked by Kolmogorov test. The Student's *t*-test or the Mann-Whitney rank sum test was applied for normally or not-normally distributed data, respectively. *P* values equal to or less than 0.05 were considered statistically significant.

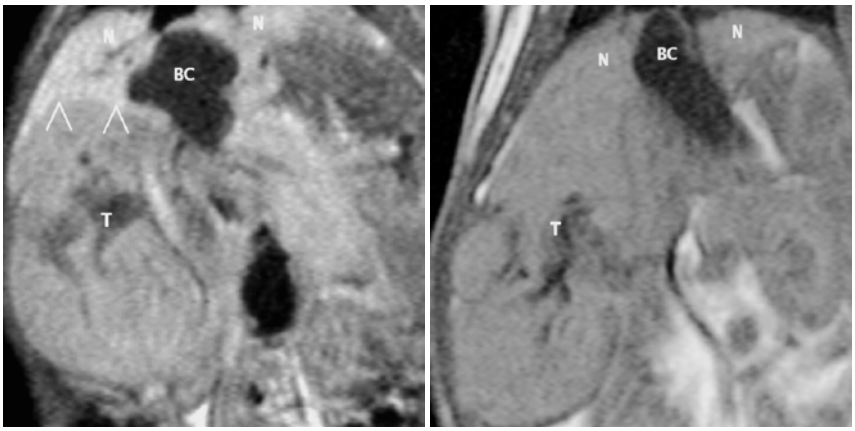
RESULTS

Post-operative Animal Survival

Three out of the sixteen rats were excluded from the study due to early post-operative death. One rat died of bleeding on the second day, while peritonitis secondary to bile leakage caused another two animal losses on the third and fourth day. Amongst the thirteen rats that survived longer than one week after HALTx, four appeared normal during the observation period of three-month and no signs of infection, biliary obstruction, vascular thrombosis and rejection were found at sacrifice. These four rats were included in group 1. Among the nine rats of group 2, eight developed various complications and died during the first seven weeks after transplantation (1 in week 2, 2 in week 3, 1 in week 4, 1 in week 5, 1 in week 6 and 2 in week 7). Cholangitis (n = 4), liver abscess (n = 2), vena cava thrombosis (n = 1) and rejection (n = 1) were found to be the major causes of animal death. Only one out of nine rats in group 2 survived longer than seven weeks, which was sacrificed at the end of the eighth week due to abnormal MRI data. Portal vein thrombosis of the engrafted liver was identified.

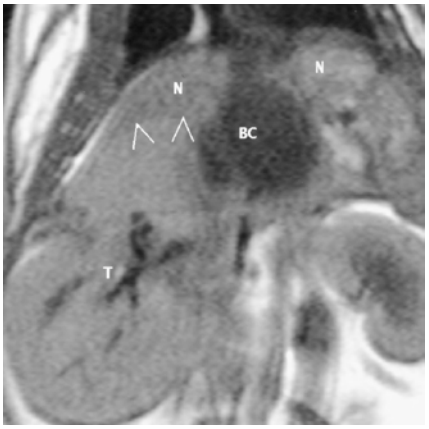
MRI

Using anatomical landmarks, the engrafted and the native liver could clearly be distinguished from each other on MR images. In the rats without complications (group 1), both livers remained homogeneous throughout the observation period. Notably, the signal intensity of the two livers showed a transition on the T_1W images during the observation period: the signal intensity of the engrafted livers evolved from hypo-intense to hyper-intense, while that of the native livers changed from hyper-intense to hypo-intense (*Figure 1a*). In comparison, in the rats with post-transplant complications (group 2), the signal of the engrafted livers was in-homogeneous and showed low signal areas on T_1W images at various times. These hypo-intense areas were later identified as complications such as abscess formation. Moreover, no transition of signal intensity was observed: the signal intensity of the engrafted liver remained hypo-intense, while that of the native liver was constantly hyper-intense even in the rat that was sacrificed two months after HALTx (*Figure 2a*).



a1

a2



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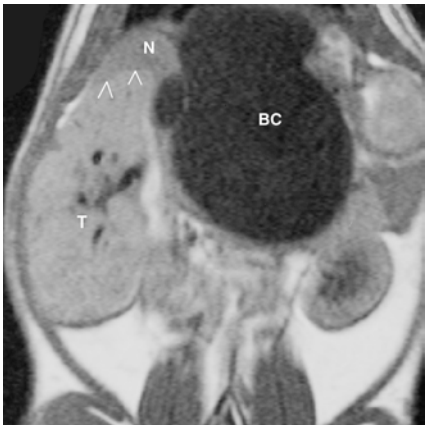

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b

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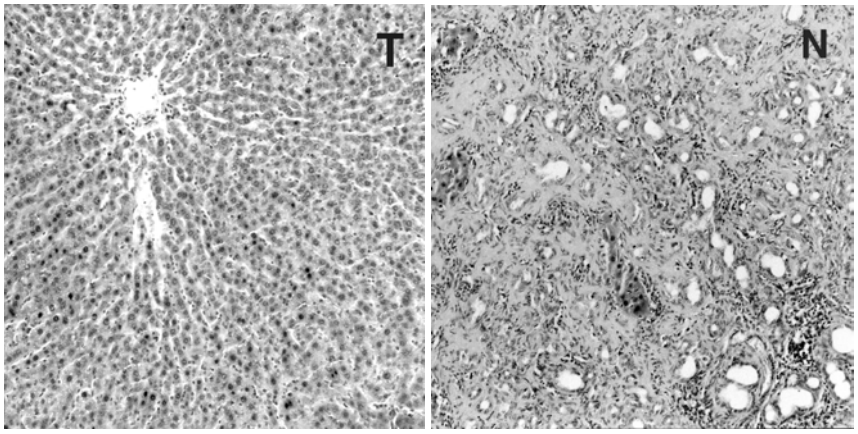
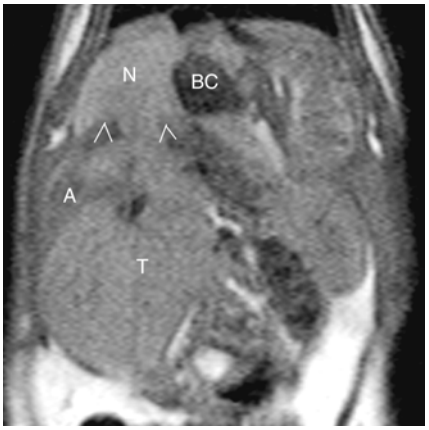
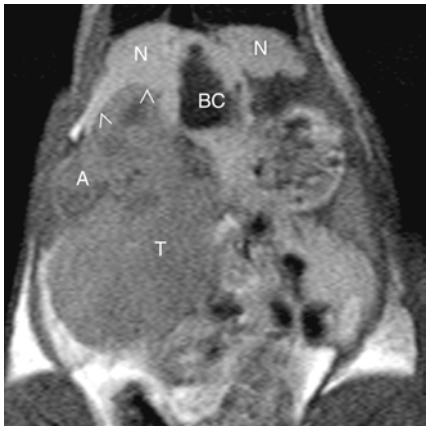


c

Figure 1. (a1-4) Post-HALTx evolution of signal intensity of a rat in group 1 (in a series of 2, 4, 6 and 12 weeks). Relative to that of the other liver, the signal intensity of the engrafted liver (T) evolved from hypo-intense to hyper-intense, while that of the native liver (N) changed in opposite direction during the 3 months observation period. '∧' shows the interface between the two livers. BC indicates a bile cyst. **(b)** Autopsy observation (3 months post-HALTx). The engrafted liver revealed a normal appearance with patent portal vein and vena cava anastomoses (indicated by '↑'); **(c)** Histological findings (3 months post-HALTx). The engrafted liver (T) preserved its normal lobular architecture without major histo-pathological alterations, while that of the native liver (N) was completely destroyed and replaced by ductular metaplasia in the portal tracts accompanied by fibrosis (original magnification x 125, HE staining).



a1



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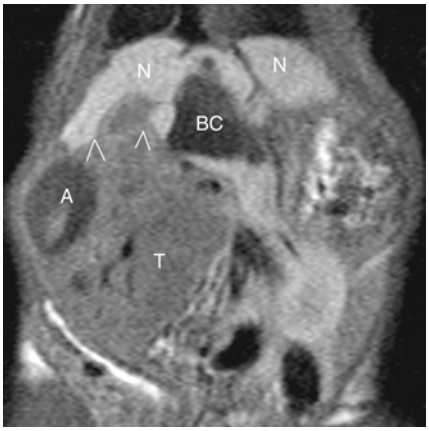

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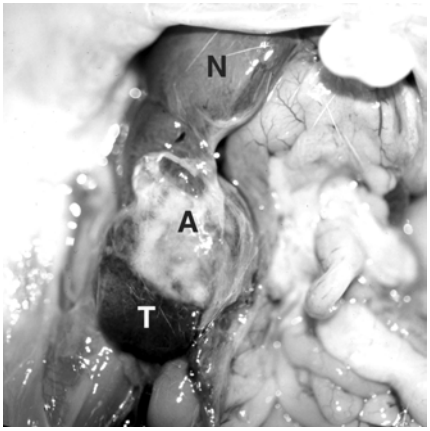

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Figure 2. Continued

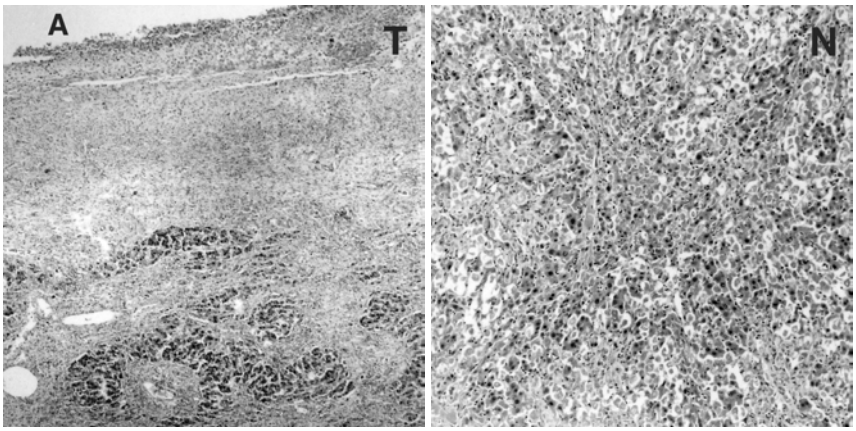

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Figure 2. (a1-3) Post-HALTx evolution of signal intensity of a rat in group 2 (in a series of 1, 2 and 4 weeks). Relative to that of the other liver, the signal intensity of the engrafted liver (T) remained hypo-intense, while that of the native liver (N) became more hyper-intense. The hypo-intensity in the engrafted liver (A) was identified as an abscess at autopsy. '∧' shows the interface between the two livers. BC indicates a bile cyst. **(b)** Autopsy observation (7 weeks post-HALTx). The engrafted liver (T) appeared atrophic and an abscess (A) was observed, while the size of the native liver (N) was less changed. **(c)** Histological findings (7 weeks post-HALTx). Besides remnants of hepatocytes, the general lobular architecture was hardly visible in the engrafted liver (T), which contained an abscess (A) (HE, original magnification x 100). The native liver (N) revealed ischemic necrosis of the hepatocytes (HE, original magnification x 250).

The evolution of the signal intensity was the visual reflection of changing T_1 relaxation times (*Figure 3*). The reference T_1 value of the liver established from thirteen normal rats was 476 ± 64 ms. In group 1, the mean value of the T_1 relaxation time of the engrafted livers showed a transient increase immediately after HALTx with a peak value in the first post-operative week (476 ± 64 ms, pre-operative; 730 ± 48 ms, week 1). Thereafter, the value fell back to the pre-transplant level at the one-month time point (464 ± 26 ms) and remained around this same range till the end of observation period of three months (489 ± 24 ms). Parallel to the evolution of the engrafted livers, the mean T_1 value of the native livers increased only slightly from a pre-operative value of 476 ± 64 ms to 589 ± 28 ms in the first week. The value increased again one month after the transplantation, kept on rising to another

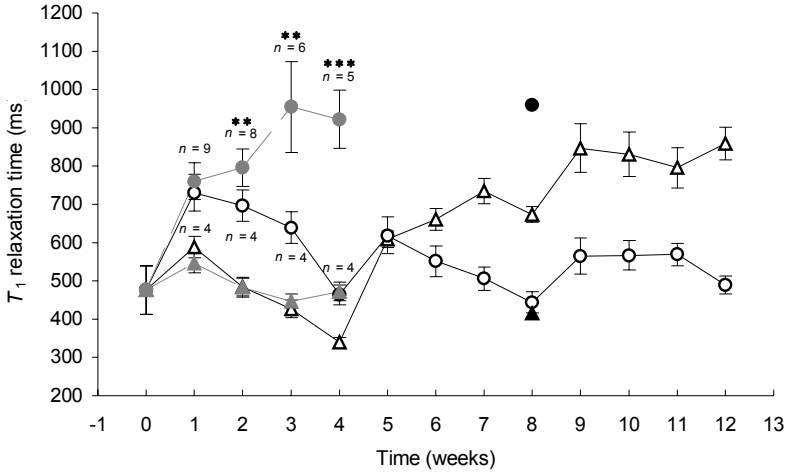

Figure 3. Post-HALTx evolution of mean T_1 relaxation time (ms) in group 1 (○ — ○, the engrafted livers; △ — △, the native livers) and in group 2 (● --- ●, the engrafted livers; ▲ --- ▲, the native livers; ●, the engrafted liver and ▲, the native liver of the rat that was sacrificed at the end of the second post-transplant month). Values are expressed as mean ± SD. The asterisk indicates statistically significant differences between the engrafted livers of group 1 (○ — ○) and the engrafted livers of group 2 (● --- ●). ** $p < 0.01$; *** $p < 0.001$.

peak in the seventh week (737 ± 33 ms), and remained high till the end of the third month (859 ± 43 ms).

In contrast to the pattern presented by group 1, group 2 showed a rather different profile (*Figure 3*). Instead of an initial increase followed by a decrease, the mean T_1 relaxation time of the engrafted livers in this group continued to increase throughout the first post-operative month (476 ± 64 ms, pre-operative; 922 ± 76 ms, week 4), while that of the native livers only varied mildly during the same period of time (476 ± 64 ms, pre-operative; 473 ± 25 ms, week 4). This high T_1 in the engrafted liver and low T_1 in the native liver was again recorded in the rat that was sacrificed at the end of the second post-transplant month (959 ms, the engrafted liver; 415 ms, the native liver). Compared with the engrafted livers in group 1, the mean T_1 relaxation times of the engrafted livers in group 2 were significantly higher in the second ($p = 0.005$), the third ($p = 0.001$) and the fourth post-transplant week ($p < 0.001$).

Table 1. Comparison of post-operative T_1 relaxation time (ms) between the native livers of experimental groups and the livers subjected only to 68% PH and CBDL

	68% PH/CBDL (n = 5)	NL-Group 1 (n = 4)	NL-Group 2
	Pre-operative reference value: 476 ± 64		
week 1	519 ± 54	589 ± 28	546 ± 25 (n = 9)
week 2	537 ± 81	485 ± 25	483 ± 24 (n = 8)
week 3	446 ± 52	427 ± 23	447 ± 18 (n = 6)
week 4	602 ± 50	340 ± 13***	473 ± 25 (n = 5)***
week 8		673 ± 21	
week 12		859 ± 43	

Notes: 68% PH: 68% partial hepatectomy; CBDL: common bile duct ligation; NL: native liver. Data are expressed as mean ± SD. The asterisk indicates statistically significant differences between the values of experimental group and that of the rats subjected only to 68% PH and CBDL. *** $p < 0.001$.

During the first three post-operative weeks, as shown in *Table 1*, the mean T_1 relaxation time of the native livers in both group 1 and 2 was similar to that of the livers subjected only to 68% PH and CBDL, all of which remained in or slightly above the normal range (476 ± 64 ms). This might indicate that the significantly higher T_1 value of the livers subjected only to 68% PH and CBDL in the fourth week ($p < 0.001$) as well as the constantly elevated T_1 of the native livers in the rats of group 1 four weeks after HALTx were caused by the factors beyond surgical trauma.

Liver Biology

The mean serum ALAT values elevated in both group 1 and group 2 after transplantation, reached peak levels on the third day and then returned to normal range in two-weeks time. The mean ALAT values of group 2 showed a more marked increase than that of group 1 on day 3 (199 ± 57 U/l vs. 91 ± 54 U/l; $p = 0.05$) (*Figure 4a*). Serum TBil revealed pronounced differences between the two groups as shown in *Figure 4b*. Compared with those of group 1, the mean TBil values of group 2 were significantly higher on the seventh (1.31 ± 1.49 mg/dl, group 1; 12.52 ± 4.94 mg/dl, group 2;

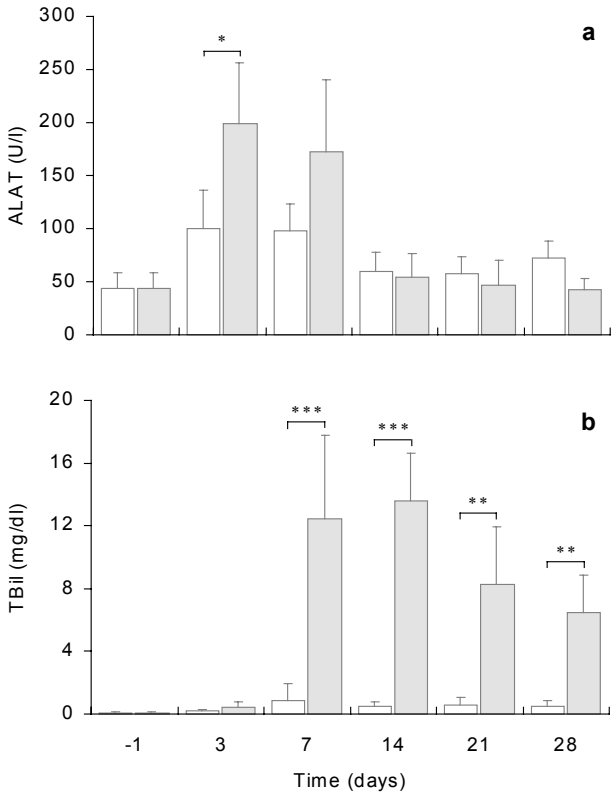

Figure 4. Post-HALTx evolution of **(*a*)** mean serum alanine aminotransferase activity (ALAT, U/l) and **(*b*)** mean bilirubin concentration (TBil, mg/dl) in group 1 (□) and group 2 (■). Values are expressed as mean ± SD. The asterisk indicates statistically significant differences between the two groups. * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$.

$p < 0.001$), the fourteenth (0.48 ± 0.34 mg/dl, 13.26 ± 2.81 mg/dl, respectively; $p < 0.001$), the twenty-first (0.63 ± 0.54 mg/dl, 8.26 ± 2.79 mg/dl, respectively; $p = 0.005$) and the twenty-eighth day (0.53 ± 0.34 mg/dl, 6.63 ± 2.01 mg/dl, respectively; $p = 0.007$).

Autopsy and Histology

At sacrifice, the four long-term survivors of group 1 showed enlarged liver grafts with normal appearance and patent anastomoses of the portal vein and the vena cava (*Figure 1b*), while their native livers were atrophic. The histological examination revealed that the general architecture of those

engrafted livers was well preserved, while that of the handicapped native livers was completely altered and replaced by ductular metaplasia in the portal tracts accompanied by fibrosis (*Figure 1c*).

In contrast, all the engrafted livers of the rats in group 2 appeared atrophic, while the size of the native livers was less changed (*Figure 2b*). As a result of common bile duct ligation, a bile cyst was observed at the hilus of the native livers in all the rats of group 1 and 2. Histological examination showed a drastically destructed lobular architecture of the engrafted livers due to cholangitis, rejection, portal vein thrombosis and liver abscess, while various degree of ischemic hepatocytes necrosis was observed in the native livers (*Figure 2c*).

DISCUSSION

MRI seems an excellent technique to monitor changes in both the engrafted and the native liver after a HALTx. As the anatomical border of each liver is identifiable, the post-transplant complications, such as intra- or inter-hepatic abscess and liver necrosis, can visually be depicted. Moreover, the data of this study show that, in the absence of steatosis [20], the better the hepatic viability, the higher the signal intensity on T_1W images. This finding corresponds well with a previous report by Siegelman and his colleagues, in which the similar signal pattern has been observed in a clinical case [21]. The signal intensity of the liver on each MR image indicates therefore the viability of the organ at that specific time point, especially in respect to its physiological position in the inter-liver competition, even when no obvious complications can yet be seen. MRI could thus have great clinical applications as an indirect and accurate measurement of hepatic viability. A technique as such is particularly needed in some clinical situations, for instance pre-surgical evaluation of transplant candidate in fulminant or sub-fulminant hepatic failure, post-transplant or post-hepatectomy assessment of liver viability.

Several experimental and clinical studies have demonstrated a close correlation between T_1 relaxation time and the water content of the liver tissue: the lower the T_1 value, the lower the water content [22-26]. In addition, a low T_1 may also be indicative of a normal bile production and energy metabolism [22]. As normal water content can only be preserved with integrity of cell membranes, an elevated T_1 could reflect the processes such as edema, cell death, disintegration and inflammation in the examined organ [23]. The transient post-transplant increase of T_1 value in the engrafted and the native livers observed in this study may reveal the degree of hepatic trauma caused by surgery. Following a peak value, the constantly decreasing T_1 relaxation

times of the healthy hepatic grafts demonstrate the recuperating process of the engrafted livers from the preservation and re-perfusion injury. Meanwhile, the functional deterioration of the handicapped native livers caused by biliary obstruction and partial hepatectomy is represented by the elevation of the T_1 relaxation time one month after HALTx. When the engrafted livers fully recover and eventually take over the hepatic function, the native livers enter a phase of progressive atrophy. MRI records this "take-over" as a cross-point around the fifth post-transplant week. As a functioning engrafted liver may reduce the toxic effects of the cholestasis of the handicapped native live, the discrepancy of the T_1 values between the native livers of the transplanted group and the handicapped livers without HALTx may be interpreted as another sign of this recovery. On the other hand, if a complication such as biliary obstruction, vascular thrombosis and abscess formation occur in the engrafted livers, this normal pattern is no longer seen. With increasing inflammation, the T_1 relaxation times of the engrafted livers, instead of declining, keep on rising after transplantation. Meanwhile, less altered T_1 relaxation times recorded in the native livers reveal that the handicapped organs remain relatively normal to compensate the functional deterioration of the engrafted livers.

In addition, as early as after the first post-transplant week, a dramatic difference of the T_1 relaxation times of the engrafted livers could be found between the healthy long-term survivors and the rats with complications. This observation may indicate that if no MRI sign of recovery from the operative trauma appears at this time, the engrafted liver is developing either an acute or a chronic complication. The eventual outcome of the engrafted liver and the native liver in the inter-liver competition can therefore be predicted at an early post-transplant stage through MRI analysis.

A previous study has shown that, in this specific rat model of HALTx, the functionality of the native liver is so severely damaged by surgical handicap that the rat will die unless a viable auxiliary liver is present. In case a post-operative complication occurs in the engrafted liver at a later stage, the animal is unable to survive, as the handicapped native liver is already atrophic at that time [17]. Despite a portal vein thrombosis developed in the engrafted liver, however, a rat of group 2 in this study survived longer than two months following transplantation. The T_1 relaxation time of its native liver prior to sacrifice was in the normal range. The reason why this handicapped native liver is still able to maintain certain function for such a long period is unknown. The progressive nature of portal vein thrombosis after transplantation may explain part of this observation.

Although definitive conclusions can only be drawn from a larger sample study, the data of this pilot experiment do show the beneficial potential of using MRI in post-HALTx monitoring. Prospective clinical investigations

are indeed worthy to test the findings of this study that major post-transplant complications can be depicted individually by this measurement, and that the hepatic function is also assessable by analysis of the signal intensity and T_1 relaxation times. These studies will demonstrate if the final outcome of each liver may be forecasted as early as after the first post-transplant week. As MRI allows documenting the evolution of the engrafted and the native liver systematically and non-invasively, this procedure can thus facilitate the studies on inter-liver functional competition.

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CHAPTER 3

STUDY OF INTER-LIVER FUNCTIONAL COMPETITION FOLLOWING HETEROTOPIC AUXILIARY LIVER TRANSPLANTATION

3.1. The Need to Handicap the Recipient's Native Liver in the Rat Model of Heterotopic Auxiliary Liver Transplantation

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SUMMARY

In the rat model of heterotopic auxiliary liver transplantation (HALTx), the opinion varies on whether and how the recipient's native liver should be handicapped. To avoid atrophy of the transplanted organ, in this study, two different handicaps were evaluated and their effects on post-operative animal survival and liver biology were described. With a sole portacaval shunt (group 1) all rats survived longer than 3 months. An additional handicap of the liver with either a 68% partial hepatectomy (68% PH) (group 2), or both a 68% PH and a common bile duct ligation (CBDL) (group 3) led to a 100% lethality within 2 days after surgery. When an auxiliary liver was transplanted to the rats handicapped with a 68% PH (group 4), serum TBil and ALAT values were significantly lower than those handicapped with both a 68% PH and a CBDL (group 5). Autopsy and histology of the long-term survivors revealed the atrophy of the engrafted livers and the regeneration of the native livers in group 4 whereas it showed the opposite in group 5. Thus the various manipulations of the native liver do influence differently the post-transplant animal survival, serum liver biochemistry and the outcome of the engrafted liver in this rat model of HALTx.

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INTRODUCTION

In recent years, more and more reports from different organ-transplantation centres have been published about encouraging results of using heterotopic auxiliary liver transplantation (HALTx) to treat patients with end-stage liver diseases especially fulminant and subfulminant hepatic failure [1-3]. This has raised new attention to study the potential problems of this operative procedure with animal models [4-5]. HALTx in the rat has long been used for experimental research, since it was first described by Lee and Edgington in 1966 [6]. The investigators have used this model to study amongst others, liver regeneration, hepatotrophic factors, allogeneic microchimerism and graft rejection [5,7,8]. Several technique modifications of this model have been introduced in that respect [9-12].

Although the observation of graft atrophy and the concept of "inter-liver competition" in HALTx were reported in the early 60's [13-15], opinion still varies on whether in this rat model the native liver of the recipient should be handicapped and in this case which approach should be used. Some authors handicapped the native liver either with a 68% partial hepatectomy (68% PH) [7, 10] or a 68% PH plus a common bile duct ligation (CBDL) [9, 11]. Others, on the contrary, left the native liver intact [5, 8, 12]. Up to date, there has been no report about how these different manipulations of the native liver do influence the post-HALTx animal survival and serum biochemical values. This was investigated in the present study.

MATERIALS AND METHODS

Experimental Design (*Table 1*)

To evaluate the effects of different handicaps on the native liver, three experimental groups were studied, in which the livers were put in functionally similar situations as the native livers in the HALTx model: group 1 (n = 10), an end-to-side portacaval shunt (PCS) (without further handicap of the liver); group 2 (n = 10), a PCS plus a 68% PH as additional handicap of the liver; and group 3 (n = 10), a PCS, a 68% PH and a CBDL to handicap the liver.

Two experimental groups were tested later on, since no long-term survivor and thus serum biochemical evolution could be obtained from the rats of group 2 and 3. In group 4 (n = 12), a HALTx was performed after the native liver was handicapped in the same way as in group 2 (PCS and 68% PH). In group 5 (n = 12), HALTx was performed after the handicap of the native liver as in group 3 (PCS, 68% PH and CBDL).

Experimental Animals

Male Wistar rats weighing 250-340g were purchased from Janssen Pharmaceutica N.V., Belgium. Donor and recipient rats were matched for size. Rats had free access to food and water prior to surgery. All animals received humane care as outlined in the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23, revised 1985).

Surgical Techniques

All operations were carried out under ether anaesthesia with clean but non-sterile instruments and x 12.5 magnification.

(a) *68% PH*: The median lobe and the left lobe of the liver were ligated and then excised according to the standard method introduced by Higgins and Anderson [16].

(b) *CBDL*: A segment of the common bile duct was removed after double ligation.

(c) *PCS*: The standard method described by Lee [17] was modified using the cuff technique to perform a PCS. After a left nephrectomy, the left renal vein was freed up to the vena cava, which was then cross-clamped. An incision was made into the anterior wall of the stump of the left renal vein. The portal vein was divided from the pyloric vein to the liver hilus, tied, transsected and cuffed with a 0.4 cm length polyethylene tube. The cuffed portal vein was then inserted into the left renal vein and secured with a 5-0 ligature.

(d) *HALTx*: The standard technique described by Marni [9] was used with a slight modification. After the donor liver was perfused with heparinized 4°C physiological saline solution via the portal vein, 32% of the liver was used for transplantation. The native liver of the recipient rat was handicapped with a 68% PH or both a 68% PH and a CBDL according to the study group, while the hepatic artery remained intact. The engrafted liver was then implanted in the right paravertebral gutter under the native liver of the recipient. The cuff technique described by Kamada [18] was used for the anastomoses of both the vena cava and the portal vein. The engrafted liver received its blood supply from the portal vein of the recipient. The venous drainage was made through the right renal vein of the recipient to the vena cava. Restoration of the bile drainage was done by insertion of the intubated common bile duct of the donor liver into the recipient duodenum. No re-arterialisation was performed. To this standard procedure, we

added a fixation of the remaining donor diaphragm to the recipient lateral abdominal wall in order to avoid twisting and kinking of the donor vena cava.

The duration of the different operative phases (mainly the portal vein and the vena cava cross-clamping time) was comparable among group 1, 2 and 3. The cold preservation time of the grafts (starting from the saline perfusion via the portal vein ending at the portal re-vascularization of the engrafted liver) was kept between 72 and 79 minutes in the rats of HALTx group 4 and 5.

Post-operative Care

All rats were given 10 mg Na Cefazolin intramuscularly after the operation and this was repeated daily for three days. No immunosuppressive agent was administered to the rats. During the following twenty-four hours, rats were allowed to drink 5% glucose, after which the pre-operative diet of food pellets and water was resumed.

Follow-up

Body weight was recorded daily and survival time was determined.

For the biochemical analysis, a blood sample of 0.4 - 0.5 ml was collected from the tail vein. Serum total bilirubin concentration (TBil) and alanine aminotransferase activity (ALAT) were determined pre-operatively, on post-operative day 3, weekly for one month and monthly for 3 months.

An autopsy was performed whenever a rat died to identify possible complications. The livers were removed at sacrifice and fixed with 10% formalin. Four-micron sections were stained with hematoxylin and eosin (HE) or trichrome. Liver samples were studied by light microscopy.

Statistical Analysis

The data were expressed as the mean ± standard deviation (SD). The Student's *t*-test was applied for the data analysis. *P* values equal to or less than 0.05 were considered statistically significant.

RESULTS

Body Weight and Animal Survival

The body weight of all rats declined 24 hours after the operation with average 10.00 ± 6.47 grams per rat in group 4 and 18.33 ± 14.03 grams in group 5 (NS). Three weeks later, the rats of group 4 regained their pre-

operative weight and even exceeded up to average 24.07 ± 3.40 grams compared with 1.67 ± 11.16 grams in group 5 (NS).

Table 1. Experimental groups and post-operative survival

Experimental groups	Survival time				
G1 (n = 10): PCS	> 3 months (10/10)				
G2 (n = 10): PCS + 68% PH	0.81 ± 0.56 days (mean ± *SD*)				
G3 (n = 10): PCS + 68% PH + CBDL	0.51 ± 0.30 days (mean ± *SD*)				
	Survival rates				
	1 week	2 weeks	3 weeks	1 month	2 months
G4 (n = 12): HALTx + 68% PH	100%	100%	92%	92%	83%
	100%	83%	58%	50%	50%
G5 (n = 12): HALTx + 68% PH + CBDL					

Notes: PCS, portacaval shunt; 68% PH, 68% partial hepatectomy; CBDL, common bile duct ligation; HALTx, heterotopic auxiliary liver transplantation.

As shown in the *Table 1*, the rats of group 1 could well tolerate a PCS whereas all rats in group 2 and 3 died of liver failure within two days after the surgery.

In group 4, the 1-month survival rate was as high as 92%. Only two rats died of abscess in the native liver or in both the native and the engrafted livers on the 17th and 34th post-operative day. In group 5, only 50% of the animal survived longer than 3 months. Except a rat that died of rejection on day 23, all others died of infectious complications (cholestasis in 1, abscess in 3 and peritonitis in 1).

Serum Biochemical Parameters

Serum TBil of the rats in group 5 was significantly higher than those of group 4 on post-operative day 7 (p = 0.025) and 28 (p = 0.02) (*Figure 1a*). The rats (2$^{\#}$, 3$^{\#}$, 8$^{\#}$ and 10$^{\#}$) of group 5, that had the highest values of TBil on day 7, developed infectious complications and died within the following weeks. By disregarding the values of those rats that died of complications, the differences between the two groups were still recorded but they were no longer statistically significant (*Figure 1b*). The high TBil peak of group 5 on

day 28 was this time due to the two rats that developed secondary biliary cirrhosis of the engrafted livers found later at autopsy.

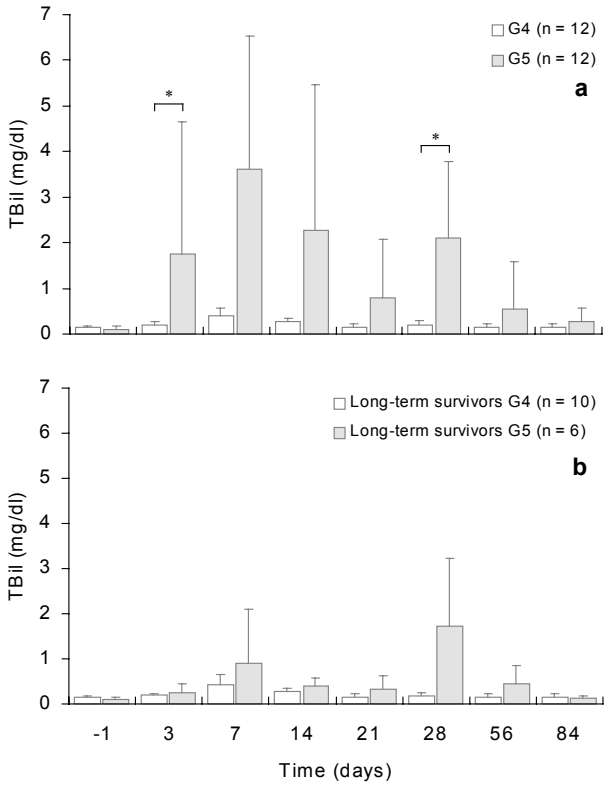


Figure 1. Comparison of the mean serum total bilirubin concentration (TBil) between group 4 and 5 post-HALTx: **(a)** Data of the whole group. Statistically significant differences were found on day 7 and day 28 ($p < 0.05$); **(b)** Data of the long-term survivors. No significant differences between the two groups could be seen.

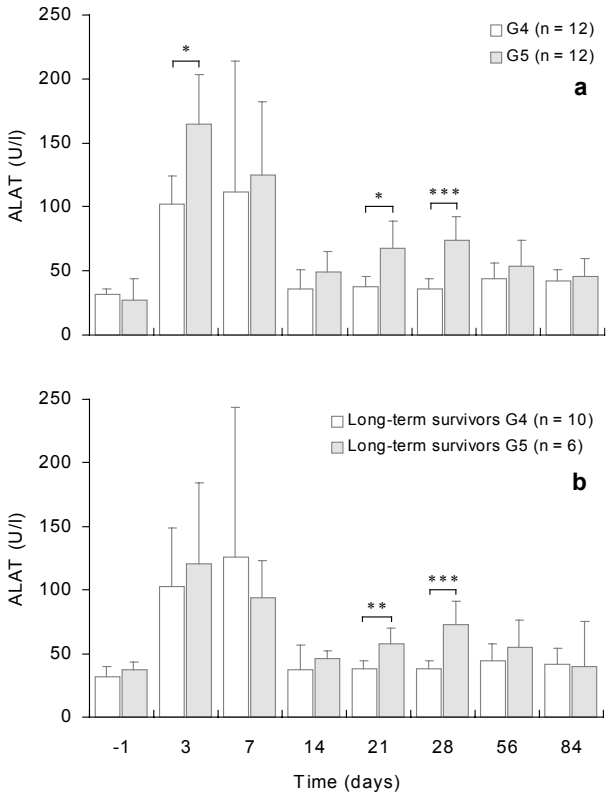
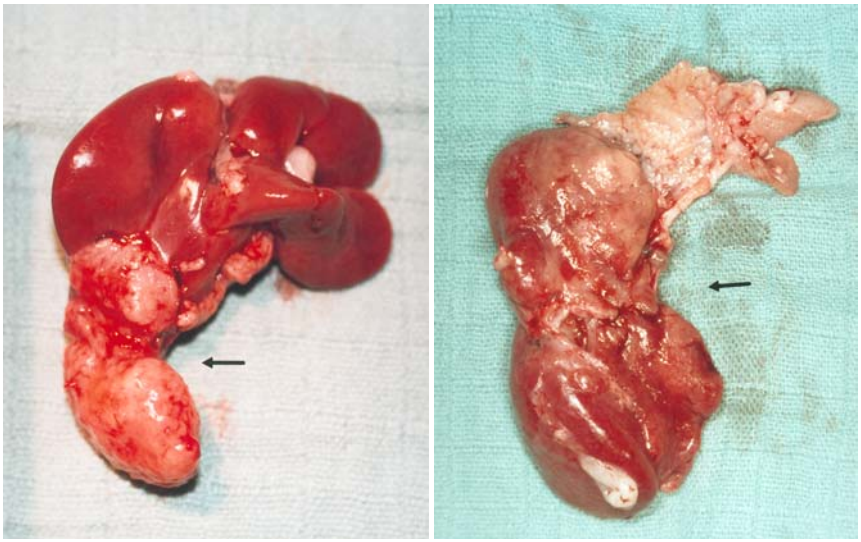


Figure 2. Comparison of the mean serum alanine aminotransferase (ALAT) between group 4 and 5 post-HALTx: **(a)** Data of the whole group. The statistically significant differences were found on day 3 ($p < 0.05$), day 21 ($p < 0.05$) and day 28 ($p < 0.001$); **(b)** Data of the long-term survivors. The differences between the two groups were not significant on day 3 and remained significant on day 21 ($p < 0.01$) and 28 ($p < 0.001$).

As showed in *Figure 2a*, significant differences of mean serum ALAT between the two groups were found on day 3 ($p = 0.014$), 21 ($p = 0.014$) and 28 ($p < 0.001$). Similarly to serum TBil, the highest ALAT values of group 5 on day 3 were again due to the rats that later died of infectious complications. When those values were excluded, the differences were no longer significant on day 3 but remained significant on day 21 ($p = 0.008$) and 28 ($p < 0.001$).



a b

Figure 3: Autopsy. **(a)** a rat of group 4 (HALTx with a 68% PH to handicap the native liver) sacrificed 7 months after the operation: the engrafted liver (↗) atrophied whereas the native liver had normal appearance; **(b)** a rat of group 5 (HALTx with a 68% PH and a CBDL to handicap the native liver) sacrificed 12 months after the operation: the size of the engrafted liver (↗) enlarged and the native liver underwent complete atrophy.

Autopsy and Histology

After the end of three months observation period, the long-term survival rats were killed at different time intervals. It was found that all the engrafted livers of the 10 survivors in group 4 underwent atrophy at sacrifice while their native livers showed normal appearance (*Figure 3a*). The general architecture of those engrafted livers was altered by fibrosis and ductular metaplasia. Only a few groups of hepatocytes were present (*Figure 4a*).

On the contrary, in group 5, the engrafted livers of all but 2 long term survivors appeared normal macroscopically whereas their native livers showed clear atrophy (*Figure 3b*). The general architecture of those engrafted livers was well preserved without major histological alterations (*Figure 4b*). The 2 remaining rats ($1^{\#}$ and $6^{\#}$) presented cirrhotic appearance of their engrafted livers and the histology revealed secondary biliary cirrhosis due to sub-obstruction of their hepaticoduodenostomy.

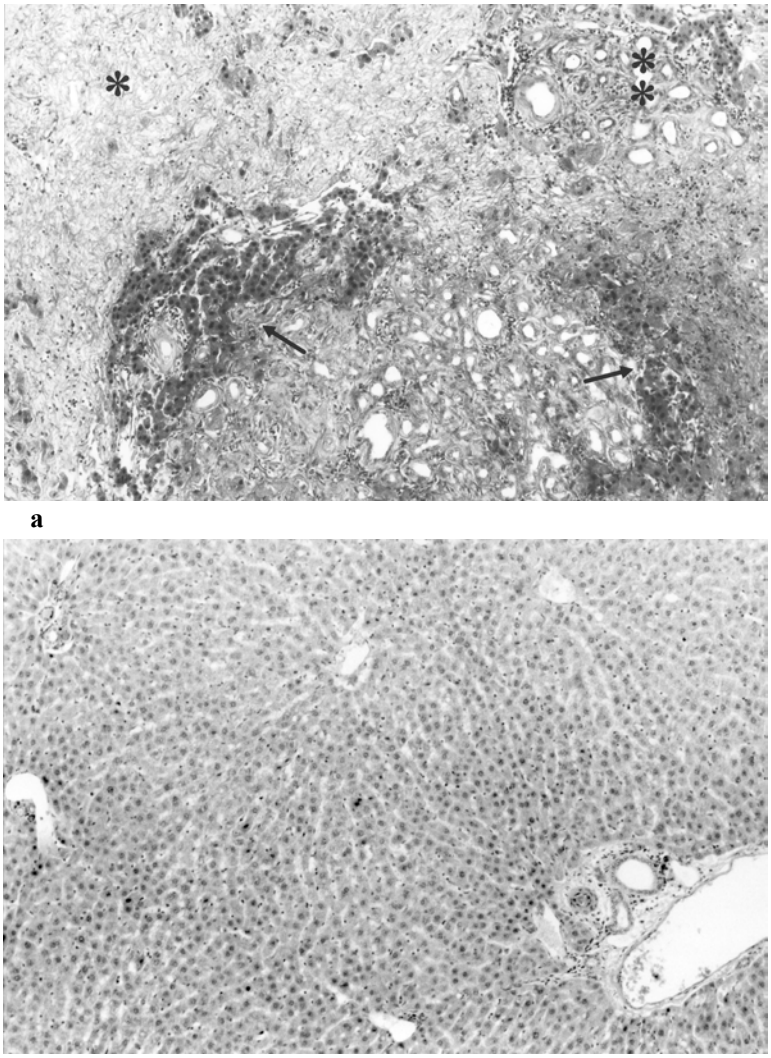


Figure 4: Histological observations. **(a)** a rat of group 4 (HALTx with a 68% PH to handicap the native liver) sacrificed 7 months after the operation: the general architecture of the engrafted liver was altered by fibrosis (*) and ductular metaplasia (**). Only a few groups of hepatocytes were present (↗) (Trichrome, x 10); **(b)** a rat of group 5 (HALTx with a 68% PH and a CBDL to handicap the native liver) sacrificed 12 months after the operation: the general architecture of the engrafted liver was well preserved and no major alterations were present (HE, x 10).

DISCUSSION

In the previous studies of rat HALTx, the post-operative animal survival and serum biochemistry were most commonly analysed without taking into account the role of the handicap of the native liver. In this study, we show for the first time how these handicaps can differently influence the post-transplant biochemical parameters.

The classical rat model of HALTx includes a complete shunting of the portal blood away from the native liver to re-vascularize the engrafted liver. In this case, the blood supply of the native liver is solely given by the hepatic artery, which corresponds effectively to a PCS of the liver. It appears that the blood flow of the hepatic artery can compensate the by-passed portal blood after a PCS and maintain a "functioning liver", so that the effects of PCS do not significantly influence the animal survival. Without handicap of the native liver, the engrafted liver is therefore transplanted parallel with a normal functioning liver. The increased hepatic blood flow will not be sufficient to support proper hepatic function if a further handicap is carried out in the native liver. It is reported that in the case of a 68% PH added to an end-to-side PCS, the rat survival does not exceed 2 days [11]. The results of the present study confirm this data and show, as would have been anticipated, that the combination of a PCS, a 68% PH and a CBDL does even shorten this survival time. In both these circumstances, no animal can be kept alive unless an auxiliary liver graft is transplanted.

The long-term survivors in the HALTx groups make it possible to perform a comparative study of the serum biochemistry between the rats bearing different liver handicaps. When the rats were handicapped only with a 68% PH as in group 4, ALAT increased to a peak level 1 week post-transplant but then returned to normal values after 2 weeks reflecting transitory hepatocellular damage. Both ischemic injury of the graft caused by preservation and the surgical manipulations of the liver at the time of 68% PH are responsible for this initial elevation. On the other hand, TBil of this group only rose slightly after the surgery even in the rats that presented fatal complications. This demonstrates that in case of a sole 68% PH to handicap the native liver, either one of the two livers can still compensate for the functional deficiency of the other. An additional CBDL to the native liver will handicap this organ so profoundly that it can no longer compensate when the engrafted liver is affected by an acute infectious complication: TBil sharply increased in these cases followed by the animal deaths shortly later as shown in group 5. The "double handicap" of the native liver is also responsible for a second peak of TBil around 1 month after HALTx. The rats that developed secondary biliary cirrhosis of the graft presented the highest

values at this time point, when the exhaustion of the regeneration potential and thereby the functional failure of the native liver occurred [19]. The repermeabilisation of the extrahepatic biliary duct of the native liver may presumably explain the normalisation of serum TBil in the long-term survivors.

It has been found that long-standing bile duct obstruction of the native liver impairs the function of reticuloendothelial system, which might lead to serious post-operative complications [20, 21]. The rats handicapped with CBDL (group 5) had indeed a higher incidence of infection and more pronounced post-operative weight-loss. Cholestasis induced by CBDL also inhibits the metabolic functions of liver cells including mitochondrial function. As consequence, regeneration capacity of this organ is compromised [22, 23]. These may be the reasons why a CBDL together with a 68% PH can affect the native liver to such a degree that the post-HALTx animal survival and normal serum biochemical values depend totally upon the functionality of the engrafted liver.

HALTx has advantages over OLTx in treating acute liver failure, inborn errors of metabolism and, exceptionally, chronic liver diseases with high operative risk for OLTx. In the case of acute liver failure, the diseased native liver is expected to recover while the engrafted liver is providing functional support. The engrafted liver will then be removed or left in place to atrophy if the native liver resumes its proper function [1-3]. For patients with chronic liver diseases, regeneration of the engrafted liver and atrophy of the native liver is the rule after a HALTx [24]. Although up to now no clinical experience with HALTx to treat patients with inborn errors of metabolism has been reported, the long-term functionality of both the engrafted and the native livers should be looked for [4]. In view of these different situations, it is indeed important to have a better understanding about the mechanisms of inter-liver competition and the right approaches to control or to modify this process. It is for this purpose, the rat HALTx can be an interesting model to study the insight of this phenomenon, even though it does not replicate the exact clinical situation. It is generally accepted that proper regeneration of the liver can only be achieved when the portal vein is re-vascularized with venous blood or through arterialisation of the portal stump [6, 15, 25]. Interestingly, the partially resected native liver can still regain progressively its volume and function despite the lack of portal blood flow, whereas the engrafted liver eventually atrophies. In case of an additional handicap of the native liver, by means of a CBDL, the functional recuperation of the native liver is completely impaired while the engrafted liver takes over the function and keeps its normal macroscopic and microscopic aspects. These results suggest that besides the known hepatotrophic factors [8, 26], the diseased state of the

native liver also influences the outcome of the inter-liver competition. Yu and co-workers have shown that the atrophy of the engrafted liver can be prevented by its re-arterialisation even when the native liver is not handicapped [5]. Re-arterialisation of the graft in the OLTx model reduces biliary complications, modifies the immunological response and improves micro-vascular perfusion of the graft [27-29]. In the rat model of HALTx, these beneficial effects certainly favour the competitive position of the engrafted liver.

The data of the present study show that in the rat model of HALTx the different manipulations on the native liver do influence not only the post-transplant animal survival but also the serum biochemical values of the liver. Furthermore, handicap of the native liver limited to a 68% PH will lead to the atrophy of the engrafted liver, whereas a 68% PH combined with a CBDL is a handicap that impairs the regeneration of the native liver. In the latter case, the post-HALTx survival and normalisation of the serum biochemistry depend solely upon the viability of the engrafted liver. Although this model has no direct similarity with clinical condition, it allows further approach and understanding to the concept of inter-liver competition.

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3.2. Effects of Re-Arterialisation on Early Graft Function and Regeneration in the Rat Model of Heterotopic Auxiliary Liver Transplantation

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SUMMARY

In the rat model of heterotopic auxiliary liver transplantation, graft re-arterialisation may influence the outcome of inter-liver competition. This was investigated in the current study using two transplanted groups with or without graft re-arterialisation. Immediately after re-perfusion, the re-arterialised grafts showed significantly higher bile flow rate and bilirubin excretion than the grafts without re-arterialisation. DNA synthesis rate was also increased more drastically in the re-arterialised group following the transplantation. Without re-arterialisation, the rats developed more pronounced cytolysis and cholestasis. Among the long-term survivors, all healthy re-arterialised grafts regenerated, whereas 5/6 non re-arterialised grafts atrophied. These data demonstrate that the re-arterialisation does increase graft survival by improving early hepatic function, enhancing regenerative response and preventing post-transplant biliary complications in this rat model.

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INTRODUCTION

In the rat model of heterotopic auxiliary liver transplantation (HALTx), the coexistence of the graft and the recipient's native liver can induce functional inhibition and lead to atrophy of the newly engrafted liver [1]. Besides the role of hepatotrophic factors in maintaining hepatocyte integrity [2], other mechanisms may as well have an influence on the inter-liver competition. In a previous study, we showed that a certain degree of handicap on the native liver was needed to weaken the functional position of the native liver and to prevent atrophy of the graft [3]. It was evidenced that if the native liver was kept intact, the engrafted liver did eventually atrophy and was replaced by connective tissue [4]. By adding a hepatic re-arterialisation to the graft, atrophy could be avoided and regeneration of the engrafted liver was achieved [5].

The studies of orthotopic liver transplantation have demonstrated the beneficial effects of the graft re-arterialisation on improving micro-vascular perfusion [6], decreasing the expression of class I and II MHC antigens [7], maintaining an adequate oxygen supply and tissue ATP [8], reducing the post-transplant biliary complications [9, 10] and increasing animal survival rate [11]. Whether equal mechanisms might be implicated in HALTx remained to be analysed, as the interaction between the graft and the native liver added an important element to this specific rat model.

The current study was conducted to investigate the possible impact of the re-arterialisation on immediate graft function, regenerative response of the engrafted liver and post-operative graft survival and long-term outcome of the recipient animal in the rat model of HALTx.

MATERIALS AND METHODS

Animals and Experimental Groups

Male inbred Lewis rats (Harlan Netherlands B. V., Zeist, the Netherlands) weighing 240-300 g were used in this study. The rats had free access to water and standard pellet food. Housing, humidity and day-night circle were strict according to European guidelines for animal care. Before surgery, food but not water was withdrawn from the recipient rats for 12 hours. Donors and recipients were matched for size.

Two experimental groups were studied: HALTx-A group, HALTx with re-arterialisation of the engrafted liver (n = 58); HALTx-nonA group, HALTx without re-arterialisation of the engrafted liver (n = 58).

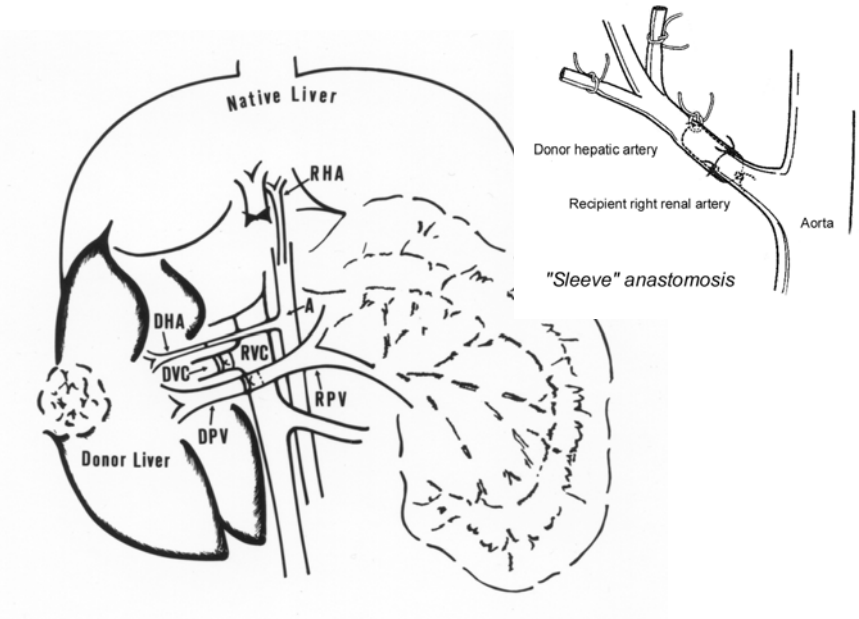
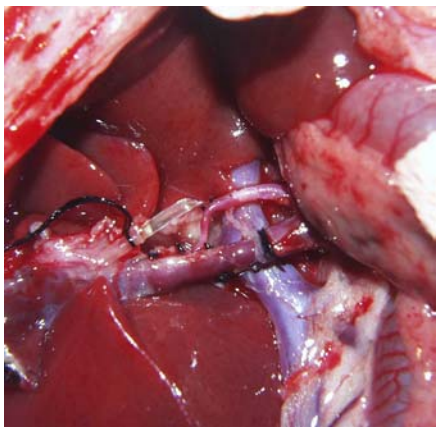


Figure 1. Technique of heterotopic auxiliary liver transplantation with graft re-arterialisation. The portal vein of the donor liver (DPV) was anatomised with the portal vein of the recipient (RPV). Using “sleeve” technique, the hepatic artery of the donor liver (DHA) was re-connected to the recipient’s aorta (A) through the right renal artery. The venous drainage of the donor liver was made between the donor vena cava (DVC) and the recipient vena cava (RVC) via the right renal vein. The recipient native liver received sole hepatic artery supply (RHA).



Surgical Procedure

All operations were carried out with ether anaesthesia, clean but non-sterile instruments and x 12.5 magnification. Body temperature of the rats was maintained at approximately 36°C by using heating pads.

Using 32% of the donor liver, HALTx was performed according to the technique as previously described [12]. The donor liver was perfused with cold (± 4° C) heparinized Ringer's solution: 5 ml via the portal vein and 3 ml via the hepatico-aortic segment. Following a right nephrectomy, the liver graft was implanted in the right paravertebral gutter under the native liver of the recipient. The cuff technique was used for the anastomoses of both the vena cava and the portal vein. The engrafted liver received its portal blood from the portal vein of the recipient. The venous drainage was made through the right renal vein of the recipient to the vena cava. In the HALTx-A group, the hepatic artery of the engrafted liver was re-connected to the recipient's right renal artery with a 10-0 monofilament nylon suture (Tyco Health Care, Belgium) using the "sleeve" technique [13]. The engrafted liver was then re-perfused simultaneously by portal and arterial blood [14]. As the total amount of portal flow went to the engrafted liver, the vascularization of the native liver was limited to sole hepatic artery supply (*Figure 1*). Restoration of the bile drainage was done by insertion of the intubated common bile duct of the donor liver into the recipient's duodenum.

The duration (mean ± SD) of cold ischemia of the liver grafts was 56 ± 7 minutes in the HALTx-A group and 55 ± 8 minutes in the HALTx-nonA group. The duration of warm ischemia of the liver grafts (starting from implantation and ending at blood re-circulation of the engrafted liver) was 25 ± 3 minutes in the HALTx-A group and 25 ± 1 minutes in the HALTx-nonA group.

Analysis of Bile Production

Six HALTx recipients in each experimental group were used to evaluate intra-operative bile secretion. Immediately after the blood re-circulation of the engrafted liver, a tubing of PE-50 was cannulated into the graft common bile duct. The bile production of the initial 15 minutes after re-perfusion was disregarded to obtain a stabilised outflow. Thereafter, the bile was collected at 15-minute intervals up to 60 minutes. All the samples were then frozen for the analysis of bilirubin concentration, which was expressed as bilirubin excretion (μg/min/g liver).

Measurement of DNA Synthesis Rate

DNA synthesis rate was measured as an index of liver regeneration response

on post-operative day 1, 2, 3, 7 and 14. At each time point, 8 HALTx recipients of each experimental group were sacrificed under ether anaesthesia. ^{3}[H] thymidine (specific activity 26 Ci/mmol, Nycomed Amersham plc, England) of 0.2 mCi/g body weight was injected intravenously 2 hours before sacrifice. The patency of the hepatic arterial anastomosis, whenever relevant, was examined. The liver was then flushed with cold physiological saline via the portal vein. The right lobes of the engrafted liver were frozen in liquid nitrogen for determining the DNA synthesis rate by the incorporation rate of ^{3}[H] thymidine into nuclear DNA, while the left lobes and the recipient's native livers were kept in formalin for histological evaluation. The DNA fraction was extracted following the procedures described elsewhere [15]. The radioactivity of ^{3}[H] thymidine was measured in a liquid scintillation counter and the DNA concentration of the same homogenate was determined by the standard method of colorimetric readings at 700 nm, using calf thymus DNA (Boehringer Mannheim GmbH, Germany) as the standard. The DNA synthesis rate was expressed as d.p.m. x 10^{-3}/μg DNA.

Post-operative Follow-ups

Twelve rats in each group were used to evaluate post-operative liver biology, graft and animal survival. Blood samples were taken on post-operative day 1, 2, 3, 7 and weekly thereafter. Serum alanine aminotransferase activity (ALAT), total bilirubin concentration (TBil) and gamma glutamyl transferase activity (GGT) were measured using routine photometric tests on a Hitachi 747 analyser. An autopsy was performed whenever a rat died and all the long-term survivors were sacrificed at the end of the third post-transplant month for histological examination. The liver samples were fixed in 10% formalin. Four-micron thick sections were stained with hematoxylin and eosin (HE) or trichrome and examined under light microscope.

Statistics

The data were expressed as the mean ± standard deviation (SD). The distribution of the data was checked by Kolmogorov test. The Student's *t*-test or the Mann-Whitney rank sum test was applied for normally or not-normally distributed data, respectively. *P* values equal to or less than 0.05 were considered statistically significant.

Table 1. Intra-operative bile flow rate and bilirubin excretion of the engrafted liver after reperfusion in the HALTx-A and HALTx-nonA groups

	HALTx-A (n = 6)	HALTx-nonA (n = 6)	p
Bile flow rate (µl/min/g liver)			
15 min	1.65 ± 0.09	0.97 ± 0.13	< 0.001
30 min	1.78 ± 0.27	0.97 ± 0.10	< 0.001
45 min	1.84 ± 0.30	0.99 ± 0.09	< 0.001
60 min	1.89 ± 0.39	0.97 ± 0.03	< 0.001
Bilirubin excretion (µg/min/g liver)	0.06 ± 0.02	0.02 ± 0.01	0.007

Note: Data are expressed as mean ± SD.

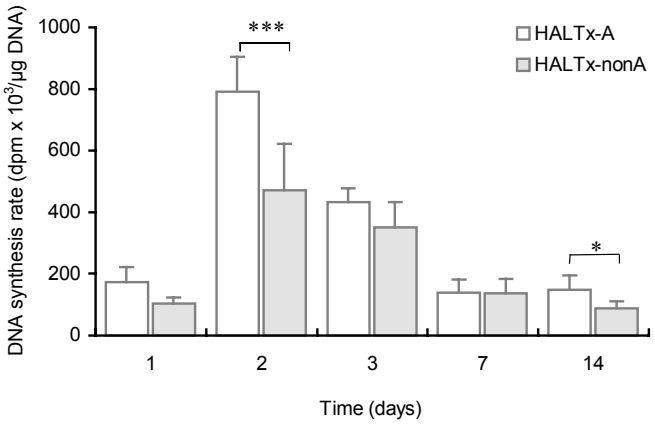
RESULTS

Intra-operative Bile Production

As shown in the *Table 1*, immediately after the blood re-circulation through the engrafted liver, the bile flow rate of the grafts with re-arterialisation was significantly higher than that of the non re-arterialised grafts (1.65 ± 0.09 vs. 0.97 ± 0.13 µl/min/g liver, $p < 0.001$). This remained throughout the observation period of 60 minutes ($p < 0.001$). During this period, the mean bilirubin excretion of the HALTx-A group was more than 3 times higher than that of the HALTx-nonA group (0.06 ± 0.02 vs. 0.02 ± 0.01 µg/min/g liver, $p = 0.007$).

DNA Synthesis Rate

In the two experimental groups, the incorporation rate of ^{3}[H] thymidine into nuclear DNA increased after the transplantation and showed a peak value on the post-operative day 2. The mean value of DNA synthesis rate in the HALTx-A group was significantly higher than that in the HALTx-nonA group (791 ± 113 vs. 471 ± 151 d.p.m. x 10^{-3}/µg DNA, $p < 0.001$). These peak values fell sharply after day 2 and reached residual low levels on day 7. DNA synthesis rates remained steady thereafter with however progressive decline in the non re-arterialised group. On day 14, the values of the 2 groups were 149 ± 45 (HALTx-A) and 87 ± 24 (HALTx-nonA) d.p.m. x 10^{-}



^{3}/µg DNA ($p < 0.05$) **Figure 2**. Post-HALTx course of DNA synthesis rate of the engrafted livers (mean ± SD) in the re-arterialised group (HALTx-A) and the non re-arterialised group (HALTx-nonA). * $p < 0.05$, *** $p < 0.001$.

(*Figure 2*).

Post-operative Follow-ups

In the HALTx-A group, 9 out of 12 rats survived the observation period of three months. One rat died of cholangitis, and another 2 died of abscesses and parenchymal necrosis in the engrafted liver, the latter of which was due to thrombosis of the portal vein and the vena cava. Among the 12 HALTx-nonA rats, 6 died during the follow-up period. Post-mortem examination revealed cholangitis in 3 rats and complete necrosis of the engrafted livers in another 3. This resulted in a 3-month survival rate of 75% in the HALTx-A group compared to 50% in the HALTx-nonA group (*Figure 3*).

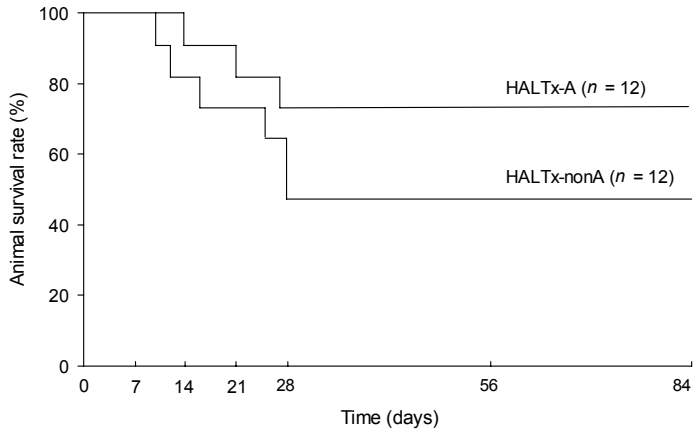


Figure 3. Three-month survival rate after HALTx with graft re-arterialisation (HALTx-A) and without graft re-arterialisation (HALTx-nonA).

After the transplantation, the levels of serum ALAT activity were elevated in the rats of both groups on day 1 and declined on day 2. Thereafter, the mean values of the re-arterialised rats remained in the normal range, while the rats of the HALTx-nonA group showed drastically increased values. The differences between the HALTx-A and HALTx-nonA group were significant on post-operative day 2 (24 ± 10 vs. 54 ± 18 U/l, $p < 0.05$), day 3 (45 ± 18 vs. 115 ± 36 U/l, $p < 0.05$) and day 7 (35 ± 7 vs. 111 ± 56 U/l, $p = 0.01$) (*Figure 4a*). The post-operative serum TBil concentration increased in the rats of both groups and, however, the re-arterialised rats had lower mean TBil values and sooner normalisation compared with that of the rats of the other group. On day 7, the re-arterialised rats had a mean TBil value of 0.11 ± 0.03 mg/dl, which was significantly lower than 0.17 ± 0.04 mg/dl of the non re-arterialised rats ($p = 0.01$) (*Figure 4b*). Serum GGT activity showed even more pronounced differences: mean GGT activity of the rats in the HALTx-nonA group was 5 times higher than that of the re-arterialised rats on the post-operative day 3 and 7. GGT activity of the re-arterialised group was only slightly elevated after transplantation, while this parameter was profoundly altered in the other group (day 3, 0.8 ± 0.9 vs. 5.8 ± 2.9 U/l, $p < 0.001$; day 7, 1.1 ± 1.3 vs. 5.9 ± 2.9 U/l, $p = 0.002$ and day 14, 1.0 ± 1.3 vs. 3.6 ± 3.1 U/l, $p = 0.04$) (*Figure 4c*).

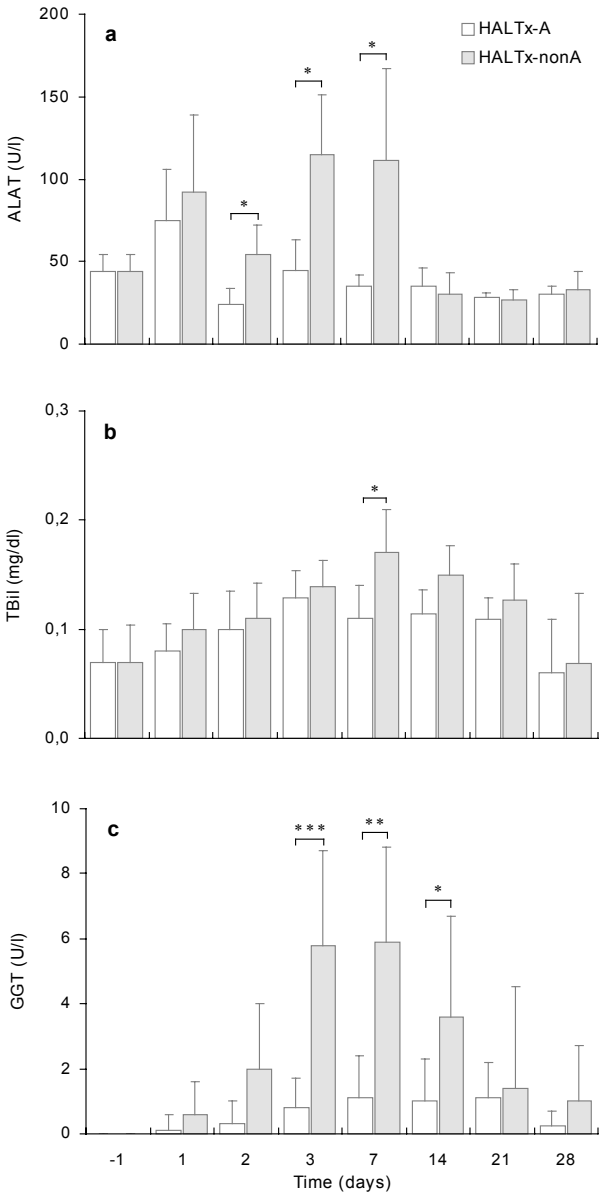
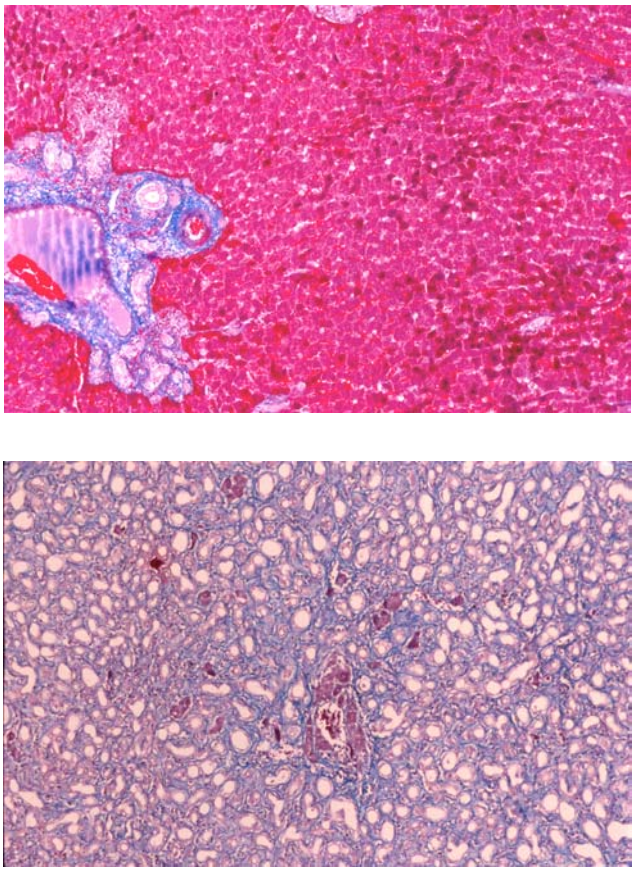


Figure 4. Post-HALTx evolution of serum ALAT (mean ± SD; **a**), TBil (**b**) and GGT (**c**) in the HALTx-A group and the HALTx-nonA group. * $p \leq 0.05$, ** $p < 0.01$ and *** $p < 0.001$.



a.
b.

Figure 5. Histological observations. **(a)** A rat of HALTx-A group sacrificed 3 months after the transplantation: the general lobular architecture of the engrafted liver was well preserved with a patent hepatic artery (Trichrome staining, original magnification x 100). **(b)** A rat of HALTx-nonA group sacrificed at the end of 3rd post-transplant months: the general architecture of the engrafted liver was completely destroyed and replaced by massive ductal metaplasia. Only remnants of hepatocytes were rarely seen around the portal tract (Trichrome staining, original magnification x 100).

At the end of the 3-month follow-up period, amongst 9 long-term survivors of the HALTx-A group, five showed enlarged grafts with well-preserved general lobular architecture (*Figure 5a*), while their native livers were

atrophic. The engrafted livers of the other 4 rats were remained unchanged in size or smaller. Portal vein thrombosis was found in one case and intra-hepatic or intra-peritoneum abscesses were present in the other 3 rats. In contrast, the atrophy of the engrafted liver was found in 5 out of 6 long-term survivors in the HALTx-nonA group, while their native livers appeared normal. Histology revealed that massive ductular metaplasia altered the general architecture of those engrafted livers (*Figure 5b*). Only one graft did not show complete atrophy (engrafted liver: 4.1 g; native liver: 6.7 g) and in this case the engrafted liver had contracted extensive adhesions with the omentum.

DISCUSSION

After a long-lasting debate, the importance of re-arterialisation for the rat model of orthotopic liver transplantation has generally been accepted. The possible impact of graft re-arterialisation on the rat model of HALTx was investigated in the present study with special focus on immediate graft function and post-transplant regenerative response.

The influence of re-arterialisation on early graft function was assessed by the initial intra-operative bile production after re-perfusion of the engrafted liver. This parameter is known to be more indicative for early post-operative hepatic function than standard liver biology [16]. Since bile production is an ATP and an O_2-dependent process [17], it is expected that the hepatic artery should play an important role by maintaining an appropriate intra-hepatic pO_2. The data of this study show that indeed the re-arterialisation of the engrafted liver leads to a significantly higher quantity and quality of bile production of the graft indicating an improved early graft function.

Post-operative biliary complications following non re-arterialised orthotopic liver transplantation were previously attributed to poor surgical technique rather than the lack of the hepatic artery re-construction [18]. Later on, it appeared that it was spontaneous post-transplant graft re-arterialisation that compensated the absence of initial artery supply, which might be accounted for the reported high success rates and an almost normal histology. The development of spontaneous graft re-arterialisation through adhesions of the omentum and the surrounding organs is however a slow process. The earliest sign of collateral arterial blood flow recorded by microspheres as well as by angiography in non-arterialised liver grafts was not seen before the third post-transplant week [19]. Only until the 8^{th} week, significant re-arterialisation could be evidenced by histology, while the regression of bile duct damage and the regression of bile duct proliferation were accomplished

6 months after the transplantation [20]. Using magnetic resonance imaging, we observed that in the rat HALTx, the healthy engrafted livers could already be distinguished from those that would develop post-operative complications (including biliary obstruction) and eventually atrophy as early as in the first post-transplant week (un-published data). This indicates that in the HALTx model the early graft function is crucial for long-term graft survival, since the possible recuperation of the engrafted liver is conditioned not only by the degree of initial hepatic damage but also by proper balance in the inter-liver competition. If a functional advantage is even temporarily given to the native liver, spontaneous graft re-arterialisation, which may occur at a later stage, will not be able to reverse the ongoing graft atrophy. As for the orthotopic transplantation [6, 9], the current results emphasize the importance of re-arterialisation for reducing biliary complications and consequent graft failure in the HALTx model.

The data of the present study also showed that, with or without hepatic artery supply, regeneration was transiently stimulated in the engrafted livers following a HALTx. This increase in DNA synthesis rate was presumably due to a "first pass" of portal hepatotrophic factors flowing through the partial hepatectomized graft. The 24-hour delay of the initial peak compared with that after a standard two-third partial hepatectomy [21] could be due to ischemic damage and operative stress [22]. Remarkably, the re-arterialised rats revealed a more pronounced regenerative response than the non re-arterialised rats despite equivalent portal blood supply. As the donor and recipient rats were syngeneic and the ischemic time was comparable between the two experimental groups in this study, neither acute rejection [23] nor ischemic damage [24] could explain these recorded differences. Thus, it was the re-arterialisation that directly enhanced the first regeneration boost in the engrafted livers. It is known that warm ischemia during the transplantation procedures causes depletion of ATP, which directly induces damage to the hepatocytes [25] and impairs the general hepatic functions such as protein synthesis and bile secretion [26]. The initial regeneration response is also depressed [22]. Re-arterialisation does improve the hepatic oxygen supply needed for an increased ATP synthesis in the liver graft [8], so that the significantly high DNA synthesis rate shown in the re-arterialised rats of this study may directly be attributed to reduced warm ischemic injury of the engrafted liver.

The elevated regenerative response however did not persist as seen in the rats subjected to a two third partial hepatectomy or as recorded in the orthotopic liver transplantation model [27] and fell to low levels immediately after the initial peak. In orthotopic liver transplantation, a loss of the functional hepatocytes caused by cellular necrosis does continue to stimulate

regeneration, whereas in HALTx, the remaining recipient's native liver may compensate in case of a deficient graft function. In addition, this model is remarkable by the fact that after the transplantation the total amount of hepatic mass exceeds the pre-transplant normal liver volume. This could explain the "switch-off" phenomenon of proceeding regenerative process in the engrafted liver. The present results suggest that it takes more than a "first pass" of portal stimulating factors to maintain regeneration. Whether the "switch-off" is due to an increase of specific inhibitory factors or a reduction of stimulating factors circulating through the graft remains to be clarified. The rat model of HALTx may serve as a useful tool to study this phenomenon regularly seen in clinical HALTx and oversized liver transplantation. This oversized liver model may thus provide new paths to gain further insight into the complicated mechanisms of liver regeneration.

Atrophy of the engrafted liver in the HALTx was first recognised as such by Starzl and his co-workers [1]. As this atrophy could be prevented by portal vascularization of the graft [2], the maintenance of portal supply is considered the prime factor for the preservation of graft integrity. In turn, the hormonal hepatotrophic factors are regarded as the key elements in the inter-liver competition of HALTx. This study shows that in this oversized HALTx model, without re-arterialisation, graft survival can not be guaranteed by a sole portal blood supply. The functional position of the engrafted liver in the balance of inter-liver competition is another important factor determining graft regeneration. In addition, the current study demonstrates that re-arterialisation of the engrafted liver can not only significantly enhance the general function of the engrafted liver such as improved bile production and reduce post-operative biliary complications, but also induce a strong post-transplant regenerative response. A better graft and animal survival could then be obtained as compared with non re-arterialised model. We may thus conclude that the re-arterialisation of the engrafted liver indeed plays an important role to prevent graft atrophy and should be considered as necessity in this rat model of HALTx.

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3.3. Effects of Portal Vein Arterialisation on Liver Regeneration after Partial Hepatectomy in the Rat

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SUMMARY

Although portal venous supply is considered essential to preserve hepatic integrity, in this study, effects of portal arterialisation on liver regeneration were evaluated in a rat model of partial hepatectomy. Ninety-six Lewis rats were randomly assigned to four groups of twenty-four each: partial hepatectomy only (group 1), partial hepatectomy with either venous or arterialised portal supply (groups 2 and 3, respectively), and partial hepatectomy without portal supply (group 4). Liver regeneration rate, 5-bromo-2-deoxyuridine (BrdU) labelling index, and liver biological characteristics were assessed on days 1, 2, 3 and 7. Compared with group 1, all the tested rats had a marked body weight loss after surgery, and only the rats in group 4 showed no signs of recovery on day 7. With maintained portal inflow (groups 1, 2, and 3), liver regeneration rate increased steadily to day-7 values of 89.2 ± 11.8%, 81.4 ± 8% and 77.4 ± 9.4%, respectively (p = not significant), and 24-hour peak values of BrdU labelling index were 159 ± 26, 157 ± 42, and 149 ± 48, respectively (p = not significant). Conversely, the rats deprived of portal supply (group 4) showed profound inhibition of these two parameters (14 ± 13, $p < 0.01$; 32.1 ± 7.7%, $p < 0.001$, respectively). These results indicate that proper portal blood supply is essential to initiate and maintain liver regeneration after partial hepatectomy. With an equivalent portal inflow rate of either venous or arterial source, the hepatic regeneration response can be sustained.

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INTRODUCTION

The concept of arterialisation of the portal vein has drawn a constant attention since the 1960s because of its various clinical applications. In an attempt to reduce post-shunt encephalopathy, portal arterialisation was first used clinically in combination with an end-to-side portosystemic shunt to treat patients with liver cirrhosis.[1-3] Because the increased oxygen supply after portal arterialisation can improve hepatic energy metabolism and liver regeneration, a technique of partial arterialisation has also been favoured in extended hepatectomy for patients with advanced hepatobiliary malignancy.[4] Temporary use of portal arterialisation during liver transplantation surgery has shown beneficial effects on shortening warm ischemia time of the donor liver and consequently reducing the incidence of immediate post-operative graft failure.[5, 6] In the case of extensive portal and splanchnic vein thrombosis, or absence of the portal and mesenteric vein because of anatomic variations, portal arterialisation is an option to preserve sufficient hepatic blood supply in orthotopic liver transplantation.[7-11] More recently, encouraging results have been reported with the use of this procedure in heterotopic auxiliary liver transplantation to treat patients with acute liver failure.[12]

The quality of the portal venous inflow has generally been regarded as the key elements for the preservation of hepatic integrity.[13-15] With a relative low concentration of hormonal hepatotrophic factors, an arterialised portal supply would hamper the regeneration capacity of the liver. In the present study, liver growth and DNA synthesis rate were compared among rats with either venous or arterialised portal inflow, or without portal supply after partial hepatectomy. The post-operative evolution of body weight and liver biological characteristics was also analysed. A modified technique of arterial-venous anastomosis was used to obtain an arterialised portal supply with an inflow rate similar to that of portal venous supply following partial hepatectomy.

MATERIALS AND METHODS

Experimental Animals

Male inbred Lewis rats (IFFA CREDO Belgium sa, Brussels, Belgium), weighing 240-300 g, were used in this study. Animals received humane care according to European guidelines (Het Belgisch Staatsblad, Feb. 29th, 1992). All animals were kept in a temperature-controlled environment with a 12-hour light-dark cycle and had free access to food and water. After an

acclimation period of one week, all rats were fasted for 12 hours before surgery.

Ninety-six rats were randomly assigned into four groups of twenty-four rats each. A standard 68% partial hepatectomy was performed in the rats of all four experimental groups to induce liver regeneration. In group 1, only partial hepatectomy was performed. In group 2, with the exception of a period of portal vein occlusion to reproduce the transitory splanchnic congestion similar to group 3, portal venous supply of the liver remained intact. In group 3, portal supply of the liver was arterialised through an arterial-venous anastomosis. In group 4, portal supply of the liver was completely by-passed by a portacaval shunt.

Surgical Procedure

Under ether anaesthesia, all the operations were performed using clean but non-sterile instruments. A heating pad was used to maintain a stable body temperature at approximately 36°C throughout the surgery.

A 68% partial hepatectomy (68% PH) was performed according to the technique described by Higgins and Anderson.[16] Through a midline incision, the median and the left lobes of the liver were excised after placement of a 5-0 suture ligature on the corresponding pedicle. Rats of all groups were also subjected to a right nephrectomy, which was needed for portal arterialisation in group 3. In the rats of group 2, after a 68% PH, the portal vein was cross-clamped for a period of ten minutes (equivalent to the average time needed to perform portal vein arterialisation in the rats of group 3). A portacaval shunt (PCS) was placed in the rats of group 3 and 4 using the technique introduced by Lee and Fisher:[17] after dividing the pyloric vein, the portal vein was ligated and transsected. Using hand-sutured technique, the distal part of the portal vein was anastomosed to the vena cava in an end-to-side manner. Arterialisation of the portal vein (PVA) in the rats of group 3 was performed with the stent technique: after a right nephrectomy, a polyethylene tubing (24 Ga) of 0.6 cm was interposed between the right renal artery and the proximal part of the portal stump, and then secured with a 5-0 suture. This technique had the advantage of allowing arterial-venous anastomosis to be conducted with a stable inflow rate by using a stent with a standard inner diameter. It was measured in our laboratory that, through a stent of 24 Ga (0.5 mm inner diameter), the average flow rate of an arterialised portal supply was between 8 ± 3 ml/min and 9 ± 3 ml/min, which was similar to the recorded portal venous inflow after a 68% PH (7 ± 2 ml/min, minimum; 8 ± 2 ml/min maximum). The peritoneal cavity was closed in two layers.

At the end of operation, 1.5 cc of heparinized physiological saline solution was transfused intravenously and 10 mg Na Cefazolin was injected intramuscularly. The pre-operative diet of food pellets were resumed two hours after operation.

Assessment of Liver Regeneration

Six rats from each group were sacrificed on the post-operative day 1, 2, 3 and 7. One hour before sacrifice, all examined rats were weighted and 5-bromo-2-deoxyuridine (BrdU, 50 mg/Kg body weight) was injected intraperitoneally. Each liver was removed and immediately weighed. The ratio of liver weight to body weight (LW-BW) was calculated. From the resected liver weight at surgery and the remnant liver weight at autopsy, the liver regeneration rate (LRR) was determined according to the formula of Child:[18]

$$\frac{\text{(liver weight at autopsy – estimated residual liver weight at the time of surgery)}}{\text{resected liver weight}} \times 100\%$$

The resected liver specimens were fixed in 4% formalin. Five-micron thick sections were stained with haematoxylin and eosin for light microscopic examination. To allow calculation of BrdU labelling index, an indicator for DNA synthesis in hepatocytes, immunohistochemical staining of incorporated BrdU was obtained using Ventana system (Ventana Medical Systems, Inc. Tucson, Arizona, USA). After deparaffinization, the sections were sequentially treated with inhibitor for four minutes and protease 1 for six minutes. The sections were then incubated with monoclonal IgG1 anti-BrdU antibodies (Medical & biological laboratories co., Naka-ku Nagoya, Japan) for thirty-two minutes, after which they were incubated consequently with amplifier A, amplifier B, biotinylated Ig, avidin-hrpo, AEC and AEC H2O2 for eight minutes each. The sections were counterstained using haematoxylin for six minutes and bluing reagent for two minutes. All steps were carried out at the temperature of 37°C. Hepatocytes containing nuclei with brown staining were considered labelled. The number of positive stained cells was counted in randomly selected fields of ten each in periportal, midzonal and centrolobular areas using high-power fields (original magnification x 320). The number of BrdU-labelled nuclei per 1000 hepatocytes was designated as the BrdU labelling index.

Liver Biochemical Tests

At sacrifice, blood samples were drawn from the inferior vena cava. Serum alanine aminotransferase activity (ALAT) and albumin concentration (Alb) were analysed for subsequent assessment of hepatic injury and liver function, respectively.

Statistics

Data were expressed as mean ± standard deviation (SD). Data distribution was checked by the Kolmogorov test. The Student's *t*-test or the Mann-Whitney rank sum test was applied for normally or not-normally distributed data, respectively. *P* value of 0.05 or less was considered statistically significant.

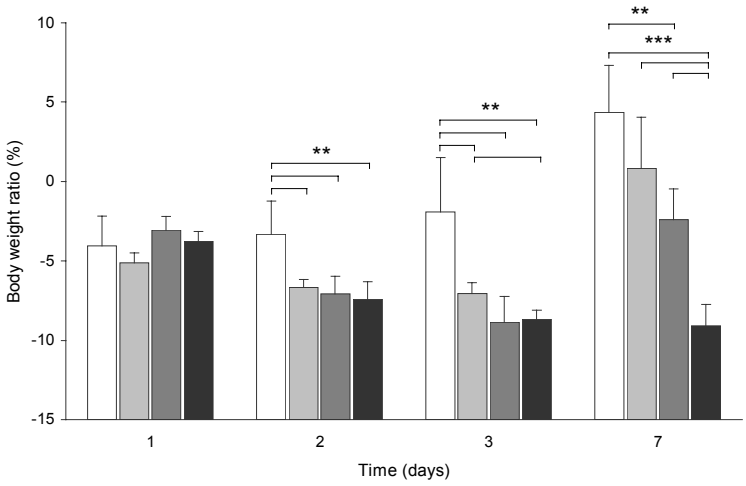

Figure 1. Body weight ratio (*BWR*) 1, 2, 3 and 7 days after 68% partial hepatectomy. Compared with the control rats (group 1, □), on day 2 and 3, the rats either with portal inflow (venous, group 2, ■; arterialised, group 3, ■) or without portal inflow (group 4, ■) had marked body weight loss. On day 7, only the rats deprived of portal supply (group 4, ■) showed no signs of recovery. (*n* = 6 each group). Values were expressed as mean ± SD. Asterisks indicated statistically significant differences between the experimental groups. ** $p < 0.01$; *** $p < 0.001$

RESULTS

Body Weight

Post-operative changes in body weight were expressed as the ratio to the initial value immediately after surgery (day 0) (*BWR*). As shown in *Figure 1*, the mean BWR of the rats subjected to partial hepatectomy (group 1) increased steadily from -4.06 ± 1.89% on day 1 to 4.35 ± 2.97% on day 7. The different surgical manipulations in the rats of group 2 and 3 showed similar effects on BWR evolution: BWR values of these two groups declined during the first three days after surgery. They were significantly lower than that of group 1 on the second ($p < 0.01$, group 2 and 3) and the third post-operative day ($p < 0.01$; $p = 0.001$, respectively). On day 7, though the values of group 2 and 3 were still lower than that of group 1 ($p < 0.01$, group 3), they nearly recovered from the initial loss, and the difference between these two groups was not significant. Without portal blood supply, however, the mean BWR value of the rats in group 4 remained at a low level even seven days after surgery (-9.09 ± 1.35%), which was significantly lower than the three other groups (4.35 ± 2.97%, 0.83 ± 3.37%, -2.42 ± 1.93%, respectively; $p < 0.001$).

Ratio of Liver Weight to Body Weight (*LW-BW*)

After the operation, the mean LW-BW value of the rats in group 1 rose from 1.57 ± 0.1% on day 1 to 2.93 ± 0.18% on day 7, an increase of 213% from its initial value of 0.94 ± 0.08% (day 0). A sharp increase of LW-BW was also observed in the rats of both group 2 and 3: from the day-1 value of 1.57 ± 0.13% (group 2) and 1.50 ± 0.13% (group 3), they reached 2.73 ± 0.19% and 2.69 ± 0.20% on day 7, which corresponded to a 180% and 177% increase from the initial values (0.98 ± 0.06%, 0.97 ± 0.04%, respectively). Compared with those of group 1, the values of group 2 and 3 were significantly lower on day 2 ($p < 0.001$, group 2; $p < 0.01$, group 3) and day 3 ($p = 0.001$ group 2), whereas the differences were no longer significant on day 7. No marked differences could be recorded between the values of group 2 and 3. In the absence of portal supply, the rats of group 4 showed the lowest increase rate of LW-BW among the four groups. Throughout the one-week observation period, the LW-BW values of group 4 were markedly lower than those of the other three groups ($p < 0.001$). On day 7, the value of 1.80 ± 0.2% was only an increase of 94% from its initial value of 0.93 ± 0.03% (*Figure 2*).

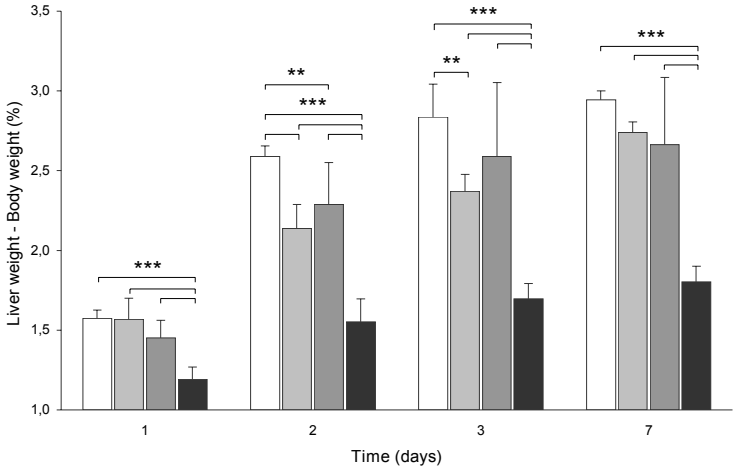

Figure 2. Ratio of liver weight to body weight (*LW-BW*) 1, 2, 3 and 7 days after 68% partial hepatectomy. Compared with the control rats (group 1, □), the increase of LW-BW was slower in the rats with either venous portal inflow (group 2, ■; day 2 and 3) or arterialised portal inflow (group 3, ■; day 2). Throughout the one-week observation period, LW-BW in the rats deprived of portal supply (group 4, ■) was significantly lower than those in the rats of other groups. (n = 6 each group). Values were expressed as mean ± SD. Asterisk indicated statistically significant differences between the experimental groups. ** $p < 0.01$; *** $p < 0.001$.

Liver Regeneration Rate (*LRR*)

As shown in *Figure 3*, hepatectomised livers regenerated at a similar rate in the rats of group 1, 2 and 3 during the first post-operative week. From the day-1 values of 26.3 ± 4.8% (group 1), 24.8 ± 8.9% (group 2) and 23.7 ± 5.8% (group 3), LRR values increased to 89.2 ± 11.8%, 81.4 ± 8% and 77.4 ± 9.4% on day 7, respectively. No significant differences were found among the values of these three groups. Complete portal by-pass in the rats of group 4 hampered liver regeneration profoundly: compared with those of group 1, 2 and 3, the respective LRR values of group 4 were significantly lower on day 1 ($p \leq 0.01$), day 2 ($p < 0.001$) and day 3 ($p \leq 0.001$). On day 7, the value of 32.1 ± 7.7% in the rats of group 4 was 64%, 61% and 59% less than those of group 1, 2 and 3, respectively ($p < 0.001$).

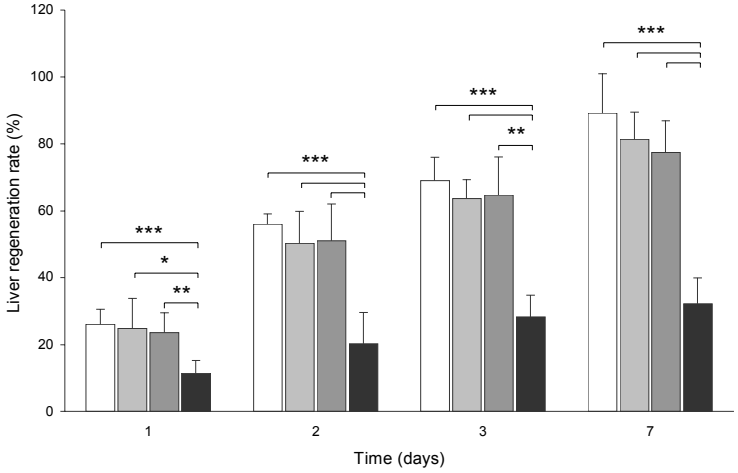

Figure 3. Liver regeneration rate (*LRR*) 1, 2, 3 and 7 days after 68% partial hepatectomy. Similar to the control rats (group 1, □), the rats with persistent portal inflow (venous, group 2, ■; arterialised, group 3, ■) had significantly higher liver growth than the rats deprived of portal supply (group 4, ■) during the first post-operative week. (n = 6 each group). Values were expressed as mean ± SD. Asterisk indicated statistically significant differences between the experimental groups. * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$.

BrdU Labelling Index

Immunohistochemical staining for BrdU in livers of the four experimental groups is shown in *Figure 4*. The post-operative evolution of BrdU labelling index was similar in the rats of group 1, 2 and 3: the number of positively stained cells peaked twenty-four hours after PH (159 ± 26, 157 ± 42 and 149 ± 48, respectively) and declined thereafter. Differences among these three groups were not significant. In contrast, the BrdU index was drastically suppressed in the rats without portal blood supply. The recorded 24-hour value of 14 ± 13 in the rats of group 4 was less than one tenth of those in the rats of the other three groups ($p < 0.001$, compared with group 1 and 2; $p < 0.01$, with group 3). On day 2, it was still significantly lower than those of other groups ($p < 0.001$, compared with group 1 and 2; $p < 0.05$, with group 3) (*Figure 5*).

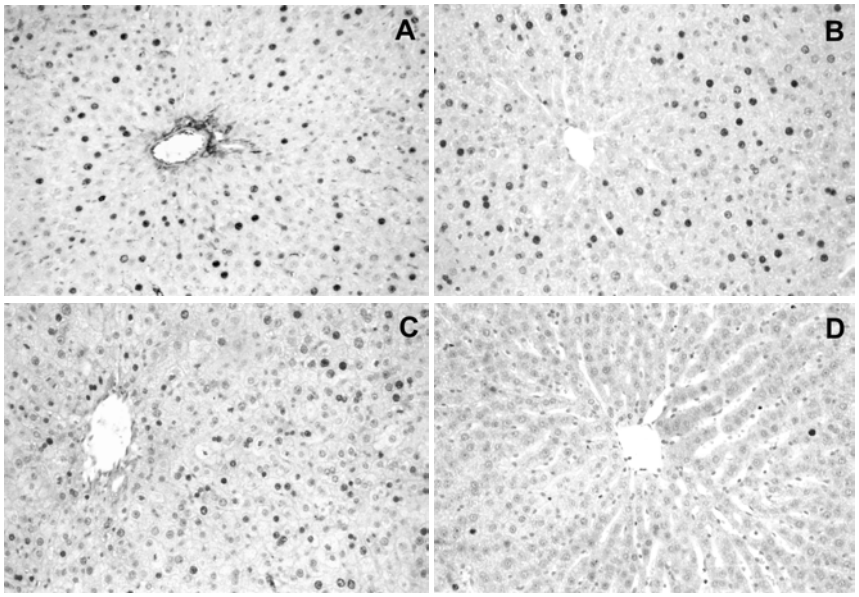

Figure 4. Immunohistochemical staining for 5-bromo-2-deoxyuridine (BrdU) 24 hours after 68% partial hepatectomy. Similar to the control rats (group 1, **A**), the rats with persistent portal inflow (venous, group 2, **B**; arterialised, group 3, **C**) had a significantly higher percentage of BrdU-positive staining hepatocytes (nuclei with deposition of brownish pigment) than the rats deprived of portal supply (group 4, **D**). (original magnification x 400).

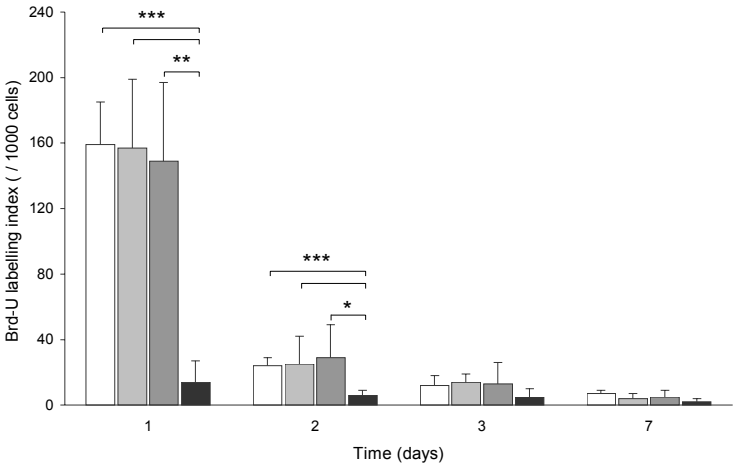

Figure 5. 5-bromo-2-deoxyuridine (BrdU) labelling index on 1, 2, 3 and 7 days after 68% partial hepatectomy. On day 1 and 2, similar to the control rats (group 1, □), the rats with persistent portal inflow (venous, group 2, ■; arterialised, group 3, ■) had a significantly higher percentage of BrdU-positive staining hepatocytes than the rats deprived of portal supply (group 4, ■). (n = 6 each group). Values were expressed as mean ± SD. Asterisk indicated statistically significant differences between the experimental groups. * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Liver Biochemical Tests

The mean values of serum albumin concentration decreased in the rats of all four groups after surgery (*Table 1*). Serum albumin concentration (Alb) of the rats in groups 1, 2 and 3 recovered one week later and no marked differences were found among the values of these three groups. Without portal supply, the rats of group 4 showed a prolonged decrease in serum Alb levels, and the value of 2.7 ± 0.3 on day 7 was significantly lower than the level of 3.2 ± 0.3 of group 1 ($p = 0.01$). The mean levels of serum alanine aminotransferase activity (ALAT) peaked one day after surgery in all rats of the four groups and declined thereafter. Compared with the value of group 1, significantly higher ALAT levels were found only in group 3 on day 2 ($p < 0.05$) and in group 4 on day 1 ($p < 0.01$).

Table 1. Post-operative evolution of serum albumin concentration (Alb) and alanine aminotransferase activity (ALAT)

	Day -1	Day 1	Day 2	Day 3	Day 7
Alb (g/dl)	3.3 ± 0.2				
group 1 (n = 6)		2.9 ± 0.2	3.0 ± 0.2	3.0 ± 0.5	3.2 ± 0.3
group 2 (n = 6)		2.7 ± 0.2	2.8 ± 0.3	3.0 ± 0.3	3.0 ± 0.3
group 3 (n = 6)		2.7 ± 0.1	2.8 ± 0.2	2.7 ± 0.2	2.9 ± 0.3
group 4 (n = 6)		2.9 ± 0.2	2.8 ± 0.2	2.7 ± 0.3	2.7 ± 0.3*
ALAT (U/l)	41 ± 9				
group 1 (n = 6)		292 ± 62	109 ± 37	68 ± 17	56 ± 7
group 2 (n = 6)		360 ± 188	130 ± 87	65 ± 11	49 ± 9
group 3 (n = 6)		357 ± 59	156 ± 47*	62 ± 19	54 ± 11
group 4 (n = 6)		454 ± 70**	131 ± 24	54 ± 7	50 ± 26

Note: Values were expressed as mean ± SD. Asterisk indicated statistically significant differences versus the values of group 1. * $p \leq 0.05$; ** $p < 0.01$.

DISCUSSION

The importance of portal blood supply to regeneration of the liver has been noted since the 1920s, based upon the observation that total liver mass and hepatocyte number are larger with intact portal inflow and atrophy occurs in liver deprived of portal venous blood.[19] It is further evidenced as such by the discovery that a liver lobe transplanted in an auxiliary position will regenerate, if the engrafted liver instead of the native liver receives the portal blood.[20, 21] The data from the current study clearly show that adequate portal inflow is essential for liver regeneration after a partial hepatectomy. Corresponding well with previous reports,[22, 23] complete diversion of portal blood realised by a portacaval shunt not only results in prolonged post-operative body weight loss, but also delays or abolishes the expected sharp increase in DNA synthesis induced by a standard 68% partial hepatectomy and impairs the restoration of the liver mass. Absence of portal flow induces hepatocyte degeneration characterized by cell volume decrease and cell death,[24] and deprives of the portal hepatotrophic factors. [25, 26] These may be responsible for the hampered regeneration capacity. On the other hand, a significant decrease in total portal inflow is known to lead to a rapid increase

in the blood flow in the hepatic artery, through which general liver function can be sustained.[11, 27] However, the data from this study reveal that, deprived of portal supply, the peak value of DNA synthesis rate is reduced to one tenth of the expected normal value, and liver regeneration rate one week after partial hepatectomy is only one third of that recorded in the rats with normal portal supply. This finding may indicate that the degree of the compensation from the hepatic artery is insufficient to initiate a proper hepatic regeneration response and maintain the regenerative process after partial hepatectomy, which would confirm the theory that this compensatory mechanism of the so-called hepatic artery buffer response is only partial.[28, 29]

To date, much speculation exists, in particular, on whether the quality of the portal blood (contents) or the quantity of the portal blood (inflow rate) is of major importance for liver regeneration. It is suggested that the diversion of hormonal hepatotrophic factors, such as endogenous insulin, from the portal tract to the systemic circulation, is responsible for the atrophy seen after portal shunt.[14, 30] Conversely, other investigators have shown that when the portal tract is perfused by systemic venous blood rather than the blood flowing from the intestines and pancreas, liver size can be maintained[18, 31] and regenerative hyperplasia is within the normal range.[32] The evidence in this study that hepatic regeneration response can be initiated, even with an arterialised portal supply after a partial hepatectomy, further supports the later argument. Moreover, when the inflow rates are equivalent, the hepatectomised livers with an arterialised portal supply can regain their weight as fast as those with a portal venous supply. By separately measuring hepatic venous and arterial inflow (per gram of tissue mass) after a left portal vein stenosis, Rocheleau et al. recently demonstrated that either portal venous or hepatic arterial flow could maintain viable liver mass in proportion to the amount of blood flow delivered.[33] The data from the present study may therefore indicate that the initiation and maintenance of liver regeneration induced by a partial hepatectomy do not necessarily require highly concentrated hepatotrophic factors, or that elements other than those classical splanchnic factors may be the initial trigger of the regenerative process.

Based on the discoveries of the last decades, the phenomenon of liver regeneration has been defined as a precise and highly orchestrated response induced by specific external stimuli, which involves sequential changes in morphologic structure, gene expression and growth factor production. Although many growth factors and cytokines, such as hepatocyte growth factor, epidermal growth factor, transforming growth factor-α, interleukin-6, tumour necrosis factor-α insulin, and norepinephrine, appear to play an important role in this process, none of these substances seem to be a primary

mitogen for hepatocytes.[34] It has been realised that before replication, hepatocytes have to be sensitised in order to be competent and fully responsive to the trophic action of growth factors.[35, 36] It is hypothesized that this so-called "priming" phase, meaning the transition of hepatocyte from a G_0 resting state to a G_1 replicative state, needs gene activation, and that more than one pathway may be involved, which may require growth factors and cytokines such as tumour necrosis factor and interleukin 6.[37] As the priming begins simultaneously in all hepatocytes almost immediately after partial hepatectomy,[38] one can assume that it may be triggered either by hormonal factors already present in the blood or liver, or by hemodynamic factors. In parallel to an early and persistent increase in portal pressure,[39, 40] researchers have found that changes in hepatocyte membrane potential can be recorded as early as within five minutes following partial hepatectomy.[41] This has been linked to a series of alterations that include increases in sodium-potassium adenosine triphosphatase pump activity, sodium fluxes into hepatocytes, and sodium-dependent amino acid transport.[38, 42-44] A recent report showed that the blood-flow-activated sodium and potassium current in cardiac microvascular endothelial cells is flow-rate dependent.[45] In addition, an increase in portal pressure is also related to such ultra-structural changes in the liver as sinusoid dilatation and disappearance of the sieve-plate arrangement of small endothelial pores, thus leaving the surface of the parenchymal liver cell directly exposed to any circulating hepatotrophic substances present in the portal blood.[40] We speculate that a significant increase in relative blood flow and consequently an even slight increase in portal pressure induced by partial hepatectomy may itself be one of the first factors triggering the entire process of liver regeneration. Further clarification of the exact role of changing portal inflow rate and pressure in the priming phase may sort out the earliest signals associated with the initiation of the hepatic regeneration response.

The data from the present study demonstrate that proper blood perfusion in the portal tract is essential for initiating and maintaining liver regeneration after a partial hepatectomy in the rat. In case of an equivalent inflow rate, the immediate hepatic regeneration response can be initiated and the one-week liver regeneration rate can be sustained by an arterialised portal supply at the same level as by a portal venous inflow.

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CHAPTER 4

GENERAL DISCUSSION

Evaluation of graft viability following heterotopic auxiliary liver transplantation

As assessed in the present work, various surgical manipulations on the recipient's native liver in the rat model of heterotopic auxiliary liver transplantation (HALTx) do affect post-transplant animal survival, graft survival and liver biology differently.

Although a normal rat can tolerate a 68% partial hepatectomy (68% PH) without major complications, this manipulation becomes a significant functional handicap to the liver whose portal supply is completely deprived by a portacaval shunt (PCS).[1, 2] The partial hepatectomy greatly increases the metabolic load at the hepatocellular level,[3] while the portal influx of nutritive elements needed to response to the high energetic metabolism is totally by-passed by a PCS.[4, 5] This results in an energetic deficit,[6] and, consequently, hepatic metabolic activities and active biosynthesis of cellular components are drastically hampered.[2] However, as shown in the current work, with the support of an auxiliary liver, the portal deprived native liver can recover from this surgical handicap and even take over the engrafted liver to regenerate. This indicates that functional reduction of the portal deprived liver induced by a 68% PH is still reversible.

The combined handicap of a 68% PH and a common bile duct ligation (CBDL) on a portal deprived liver is so severe not only that hepatic regeneration capacity is completely impaired leading to native liver atrophy in all long-term HALTx survivors, but also that the rats can not survive this handicap without the presence of a functioning auxiliary liver. In this case, the post-transplant animal survival relies solely upon the viability of the engrafted liver, and the values of serum liver biochemistry therefore reflect directly the functionality of the engrafted organ.[7] It is recognized that, in cholestasis, the intracellular retention of biliary constituents and the diminution of bile acids excretion inevitably leads to the imbalance between hydrophobic and hydrophilic bile salts in the biliary system.[8-10] The known hepatotoxic potential of the hydrophobic bile salts, which play a central role in the pathogenesis of bile-salt-induced liver injury, is then unmasked.[8-12] Along with high biliary pressure,[13, 14] hydrophobic bile salts cause hepatocyte death mainly through cytolysis.[8, 10] This is characterised by morphologic alterations including membrane disruption and cytoplasmic enlargement accompanied with increased hepatocellular enzyme release.[15-17] Apoptosis, a form of programmed cell death, has recently been found to be the predominant mechanism of hepatocyte death under moderate bile acid toxicity.[17-19] Hydrophobic bile salts also interfere with the mitochondrial function thereby decreasing ATP production,[20-22] and inhibit directly hepatocyte proliferation [23-25] Furthermore, the activation of Kupffer cells

following extra- or intra-hepatic bile duct obstruction is linked to increased generation of reactive oxygen species and free-radical formation, which may indirectly injure the hepatocytes.[26-32] The subsequent activation of hepatic stellate cells by Kupffer cells induces the net deposition of collagen and other components of the extra-cellular matrix perpetuating liver necrosis, fibrosis and cirrhosis.[29, 33-37]

Although the damaging effects of this combined handicap of 68% PH and CBDL on the native liver is apparently fatal and irreversible, the data of post-transplant monitoring by magnetic resonance imaging (MRI) from the current work show that, in the presence of a viable auxiliary liver, the expected drastic functional decline of the native liver (reflected by T_1 relaxation times) becomes obvious only four weeks after transplantation. This new evidence demonstrates that beyond its life-supporting role, a well-functioning auxiliary liver also reduces the mechanical and the biochemical aggression of the handicap imposed on the native liver. This slows down the process of the physiological deterioration of the handicapped organ. Liver biology, especially during the initial phase after a HALTx, is then a global representation of the viability of both the engrafted and the native livers. Extra caution should thus be taken when this parameter is used to assess graft viability in the rat model of HALTx. Obviously, this specific approach cannot be applied in the experimental studies where the survival of the native liver is expected, as the imposed inflicted handicap will completely abolish its regenerative potential.

The possible advantages of using MRI to monitor post-HALTx hepatic viability are for the first time evaluated and clearly demonstrated in the current work. It allows a separate identification of post-transplant complications such as abscess and necrosis in the two individual livers. The analysis of the relation between signal intensity and hepatic viability brings further interesting information. Corresponding well with a previous clinical report,[38] it is found that the higher the signal intensity on a T_1 weighted image, the better the hepatic viability. The viability of each individual liver can thus be assessed by its signal intensity at any given time. In the current work, the post-transplant evolution of T_1 relaxation times is compared between the healthy engrafted livers and the livers presenting infectious and ischemic complications. It reveals that the eventual outcome of the engrafted and the native liver, either regeneration or atrophy, can already be predicted at an early post-operative stage by examining the pattern of their T_1 evolution. A non-invasive measurement as such can facilitate systematic post-transplant follow-up of the evolution of the viability of both livers. MRI is hence a very promising tool for the studies on inter-liver functional competition. In the situations as pre-surgical evaluation of transplant candidates in fulminant or sub-fulminant hepatic failure, and post-transplant

or post-hepatectomy assessment of liver viability, the clinical potential of MRI could be proved beneficial and indeed warrants further prospective human studies.

Study of inter-liver functional competition following heterotopic auxiliary liver transplantation

After a clinical auxiliary liver transplantation, portal blood supply is shared between the engrafted liver and the recipient's native liver. It has generally been accepted that most of the common post-transplant events, such as reperfusion injury, allograft rejection, allograft viral infection and native liver regeneration, cause changes in intrahepatic portal venous resistance modifying the amount of portal blood delivered to the liver. The liver with less portal resistance receives more portal blood and consequently hepatotrophic factors, and therefore holds a more favourable position in the inter-liver competition. In this way, an alteration of the relative portal blood distribution will eventually lead to regeneration imbalance between the two livers.[39-42]

Notably, in the rat model of HALTx used in the current work, the recipient's portal supply is completely shunted towards the engrafted liver, while the native liver viability is only sustained by hepatic artery supply. Despite the fact that there is no sharing of portal blood between the two livers, a change in the degree of surgical handicap on the native liver can still alter the outcome of the engrafted organ from regeneration to atrophy; vice versa, an improvement of the physiological status of the engrafted liver, as obtained by re-establishing its hepatic arterial supply, does result in more pronounced native liver atrophy. Unlike in orthotopic liver transplantation (OLTx) where the progress of hepatic regeneration relays solely upon the number of viable hepatocytes in the newly implanted liver graft, after an auxiliary liver transplantation, the regeneration starts immediately in both coexisting livers to fulfil the body demand for hepatic metabolic function. If the liver is already in a more favourable physiological condition and in addition receives optimal portal supply, its regeneration will be advantaged preferably by "the first wave" of stimulating growth factors influx delivered via the portal tract.[41, 43] In parallel to the progressing regeneration of the one liver, decreased concentration of those stimulating factors in the blood circulation will further bring the other liver into a "dormant" state with eventual atrophy. On the other hand, a proper regenerative response cannot occur in a liver when its general metabolic function is profoundly hampered by either surgical trauma or reperfusion injury, despite proper portal blood supply. In this case, regeneration of the other liver, even deprived of portal supply, can still be stimulated by the "second wave" of growth factors re-

circulating through the hepatic artery.[44]

In contrary to what is seen in human liver transplantation, re-construction of the graft hepatic artery in the rat appears not to be needed for graft and animal survival.[45, 46] Microscopic studies of the non re-arterialised grafts will however reveal a high incidence of bile duct proliferation and biliary complications.[46-48] Regression of bile duct proliferation and bile duct damage is often observed in long-term survival rats, which eventually leads to almost normal liver histology.[49] The mechanism of this compensation to the lack of initial artery supply is mainly attributed to spontaneous graft re-arterialisation,[46, 48-50] which can be seen after the third post-transplant week.[49, 50] In HALTx, the possible recuperation of the engrafted liver is conditioned not only by the degree of initial hepatic damage but also by the balance of the inter-liver competition. Good early graft function is hence crucial for long-term graft survival in this model. Though the absence of arterial supply in the engrafted liver gives, in principle, only temporarily functional advantage to the native liver, spontaneous progressive graft re-arterialisation, which may occur at a later stage, will not be able to reverse the ongoing graft atrophy. In addition to the beneficial effects of graft re-arterialisation reported in previous studies,[51-56] the data of the current work further reveal that re-arterialisation improves early hepatic function, induces a more marked early regenerative response and reduces post-transplant biliary complications. The engrafted liver is thus functionally advantaged in the inter-liver competition with better graft survival compared with the non re-arterialised model.

Besides stressing the importance of the accessibility of the portal supply with its stimulating growth factors and nutritive elements, these results emphasize that the coexisting two livers after a HALTx are also competing for the regenerative capability based on their physiological status. The balance between the supply of regeneration stimulating factors and the hepatic capability to response to those factors will determine the final fate of the two livers. Up to date, little is known about whether any specific signals are transmitted between the two livers to regulate the post-HALTx process. If so, further exploration of the nature of those signals will bring more detailed insights about the mechanisms of the inter-liver competition phenomenon and may also have important clinical implications, in case of acute liver failure where temporary functional support of the auxiliary graft is required,[57] or in case of metabolic diseases where coexistence of two well-functioning livers is needed.[58]

Portal blood supply has been proved to play a vital role in liver regeneration. As presented in this work, complete lack of portal supply will lead to a significantly impaired regenerative response and restoration of liver

mass. When the liver is deprived of its portal vascular blood, hormonal growth factors may only reach the liver through the arterial circulation.[44] Through vasodilation, hepatic arterial inflow increases instantly after a PCS to compensate the reduction of total liver blood supply. However, the data of this work show that the degree of the compensation from this so-called "hepatic artery buffer response" is insufficient to restore a proper hepatic regeneration response and delays the regenerative process after a standard partial hepatectomy. This might be due to the lower concentration of the hepatotrophic factors in the arterial blood. Interestingly, it is observed in the current work, that with a re-established same amount of blood inflow, the hepatic regeneration rate in the hepatectomized rats is equal, irrespective of the portal supply of either venous or arterial nature. These data suggest that the "required concentration" of hepatotrophic factors may not be essential, or rather that the presence of blood in the portal tract itself is a prerequisite of initiating and maintaining a normal liver regeneration. This may explain why, in ALTx, a liver graft with an arterialised portal supply is sufficient to sustain the life of the patient with acute liver failure, while, with an un-interrupted portal venous supply, the recovery and regeneration of the diseased native liver can go on unaffected.[59]

Liver regeneration can be induced by a great variety of stimuli ranging from dietary alteration, to toxic damage and to loss of liver mass as after partial hepatectomy.[60] Determining what factors are responsible for initiating the regenerative process is one of the core issues in understanding the molecular bases of liver regeneration.[61] Amongst the so-called "hepatotrophic factors" discovered during the last three decades, some growth factors and cytokines were hypothesized to be the "initiators".[62-64] Although the role of most of those growth regulators has been identified as crucial for a specific step of hepatocyte proliferation either as a stimulator or as an inhibitor, none of them seems to be a primary mitogen.[64, 65] Moreover, it has recently been shown that hepatocytes need to be "sensitised" before replicating, which means that they have to transit from a G_0 resting state to a G_1 replicative state, in order to be competent and fully responsive to the trophic action of the growth factors.[66 67] It has been found that this so-called "priming phase" begins simultaneously in all resting hepatocytes almost immediately after partial hepatectomy.[68] As the production of growth factors and cytokines is not an immediate event, early hepatic regenerative response following partial hepatectomy is more likely to be activated either by hormonal factors already present in the blood or in the liver, or by changing hemodynamic factors. The most remarkable event instantly occurring after partial hepatectomy is the substantial increase of the portal blood flow to the residual liver mass with the consequent rise of portal pressure.[69, 70] This significant hemodynamic change could be one of the first triggers that

initiate the regeneration cascade in the hepatectomised liver. Studies have shown that, in parallel to an early and persistent increase in portal pressure, changes in hepatocyte membrane potential can be recorded as early as within the first five minutes after liver resection.[71] This is linked to a series of events essential for hepatocytes replication.[68, 72-74] In addition, following partial hepatectomy, consequent alterations in hepatic ultra-structure have been documented involving sinusoid dilatation and disappearance of the sieve-plate arrangement of small endothelial pores. This leaves the surface of the parenchymal liver cell directly exposed to the circulating hepatotrophic substances in the portal blood.[70] Moreover, an increased portal pressure reflecting wall shear stress of sinusoids causes nitric oxide release.[75] This leads to an increased mRNA expression of c-*fos*, an immediate-early gene,[76, 77] the protein product of which is known to participate in the stimulation of cell growth.[78] It has however been shown that, in a portal branch ligation model, c-*fos* as well as other factors such as c-*myc*, c-*jun*, NF-κB, STAT3 and IL-6 are similarly induced in both proliferating and atrophying liver lobes, and even in the livers of sham operated animals. Only the actual liver mass present during the period corresponding to mid- to late G_1 phase seems, in this case, to determine the magnitude of the proliferative response.[79-82] This could suggest that the observed early regenerative response after liver resection is not specifically related to either increased portal flow or loss of liver mass; or that still other not yet identified factors are the true regenerative triggers. Further clarification on how the hemodynamic changes and / or the degree of liver mass reduction modulates the extent of hepatic regeneration after partial hepatectomy may elucidate another piece of the complex puzzles in the liver regeneration process, which has still kept some of its intriguing secrets.

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SUMMARY

Although auxiliary liver transplantation does offer advantages over orthotopic liver transplantation, in particular in treating patients with acute hepatic failure and children with hepatic-based inborn errors of metabolism, its results are often compromised by the interactions between the coexisting engrafted liver and native liver. To date, it is generally accepted that the process and final outcome of the so-called inter-liver competition is governed by the supply of portal blood and, most crucial of all, the distribution of hormonal "hepatotrophic factors" between the two livers. However, other factors such as the physiological status of each individual liver might also play an important role in this mechanism. It was, therefore, the purpose of the current work, to investigate whether a change in the functional condition of each individual liver would influence the fate of the two livers using a rat model of heterotopic auxiliary liver transplantation (HALTx), and further to re-examine the controversy on whether changing the content of the portal blood would alter the hepatic regeneration capacity after a standard partial hepatectomy.

To define indicative biological parameters for post-HALTx evaluation of graft viability, a 68% partial hepatectomy (68% PH) and a common bile duct ligation (CBDL) were performed to hamper the native liver's functionality. Commonly used serum biochemical liver parameters were analysed. It was found that the surgical handicap on the native liver did efficiently induce lethal hepatic failure, as, in this case, animal survival relied solely upon the viability of the engrafted liver. The liver biology therefore reflected directly the graft functionality. The post-operative values of serum bilirubin concentration, gamma glutamyl transferase activity, alkaline phosphatase, alanine aminotransferase activity and aspartate aminotransferase activity were significantly lower in the transplanted rats than in the control rats. These data reveal that a viable engrafted liver can indeed reduce the deleterious effect of cholestasis caused by the surgical handicap on the native liver (Chapter 2.1.).

In a further study, for the first time, magnetic resonance imaging (MRI) was tested for its potential to monitor post-transplant hepatic viability in this rat model of HALTx. The results showed, firstly, that post-transplant hepatic complications as abscess and necrosis could be depicted separately in the coexisting two livers on MR image. Secondly, it was found that the better the hepatic viability, the higher the signal intensity on T_1 weighted image. The viability of each individual liver could thus be assessed by its signal intensity at any given time. Thirdly, in the presence of a viable engrafted liver, the expected drastic functional decline of the native liver reflected by

its T_1 relaxation times became obvious only 4 weeks after transplantation. This showed clearly the role of the well-functioning auxiliary liver in reducing the mechanical and biochemical aggression of the inflicted handicap on the native liver. Fourthly, as soon as after the first post-transplant week, the healthy engrafted livers could already be distinguished from those that would develop complications by a significant decrease in T_1 relaxation times. This non-invasive measurement does facilitate systematic follow-up of the evolution of the two livers after HALTx. MRI is thus a very promising tool for the studies on inter-liver functional competition. Prospective human studies based on these new discoveries may further demonstrate the beneficial potential of MRI in clinical situations such as pre-surgical evaluation of transplant candidates in fulminant or sub-fulminant hepatic failure and post-transplant or post-hepatectomy assessment of liver viability (Chapter 2.2.).

In order to study specifically the effects of various hepatic physiological conditions on the inter-liver competition, the possible influence of portal blood distribution to the native liver was abolished by completely shunting it to the engrafted liver. This allowed focusing on how the different handicaps of the native liver could affect animal survival and the regeneration capacity of both the engrafted and the residual native livers. It was observed that a viable auxiliary liver was able to prevent high animal mortality caused by a 68% PH on a portal deprived native liver. The native liver could recover from this functional reduction and even regenerate, while the engrafted liver atrophied despite its proper portal blood supply. An additional handicap on the native liver, through a combined 68% PH and CBDL, resulted this time in atrophy of the native liver and regeneration of the engrafted organ (Chapter 3.1.). In another experimental setting, the effects of graft re-arterialisation on liver regeneration were evaluated. It was shown that, the re-arterialised grafts had significantly higher bile flow rate and bilirubin excretion immediately after re-perfusion compared with that of the grafts without re-arterialisation. Re-arterialisation also increased DNA synthesis rate of the engrafted liver, and the rat developed less cytolysis and less cholestasis. This led to a significantly improved graft growth, which was paralleled to a more pronounced native liver atrophy. Re-arterialisation could indeed enhance the functional advantage of the engrafted liver (Chapter 3.2.).

In the last part of this work, it was found that complete absence of portal blood supply to a partial resected liver caused a marked inhibition of early regenerative response and liver growth rate. The degree of the compensation from the “hepatic artery buffer response” was insufficient to restore normal hepatic regeneration after a standard partial hepatectomy. Notably, if the amount of portal blood inflow was re-established by arterialising the portal

stump, the DNA synthesis rate as well as liver growth could be sustained at a level equivalent to that observed in the rats of control group (Chapter 3.3.).

The data of the current work emphasize that, besides the portal blood supply, the change of the physiological condition of each individual liver does affect the balance of the inter-liver competition after a HALTx. Rather than the high concentration of the hepatotrophic factors, the presence of sufficient blood flow in the portal tract, of either venous or arterial origin, appears a prerequisite to maintain the normal chronology and the intensity of the regeneration process.

SAMENVATTING

Een auxilliaire levertransplantatie biedt duidelijke voordelen aan bij de behandeling van patiënten lijdende aan acuut leverfalen, alsook bij kinderen met congenitale metabole ziekten. Ondanks de conceptuele aantrekkelijkheid van deze type transplantatie, zijn de resultaten hiervan niet steeds éénduidig en dit ondermeer omwille van het ontstaan van een functionele competitie tussen de eigen of natieve lever en de getransplanteerde lever. Vandaag wordt het algemeen aanvaard dat de portale bevloeiing van de lever met zijn hepatotropische factoren de functionele status van de 2 levers in grote mate beïnvloedt. Buiten deze extrinsieke factoren moet men nochtans ook rekening houden met levergebonden intrinsieke factoren wanneer gesproken wordt over de functionele competitie tussen de 2 levers. De fysiologische status van de 2 levers speelt inderdaad een belangrijke rol in het min of meer receptief staan van het orgaan ten opzichte van externe stimulatiefactoren. Het was dan ook het doel van ons onderzoek om op een rattenmodel van heterotope auxilliaire levertransplantatie (HALTx) verschillende functionele beperkingen van donor- en recipiëntlevers te testen en na te gaan in welke mate deze factoren de competitie tussen de recipiëntlever en de auxilliare lever zouden kunnen beïnvloeden. Hiernaast hebben wij ons eveneens de vraag gesteld of extrinsieke factoren met dan hier meer expliciet hemodynamische factoren implicaties zouden kunnen hebben op de lever regeneratie capaciteit na een standaard partiële hepatectomie.

Om met routine biologische parameters de post-HALTx viabiliteit en functie van het getransplanteerd orgaan te kunnen volgen, werd de natieve lever met een 68% partiële hepatectomie en galwegen ligatuur belast. Deze heelkundige manipulatie van de natieve lever veroorzaakt een dodelijk leverfalen in de afwezigheid van een getransplanteerde auxillaire leverent. Indien de overleving van de ratten rechtstreeks afhankelijk is van de auxilliaire lever, zal de biologie grotendeels de functie van deze ent weerspiegelen. De serumconcentraties van bilirubine, gamma GT, alkalische fosfatase, gamma glutamyl transferase, alanine aminotransferase activiteit en aspartaat aminotransferase activiteit van de getransplanteerde groep zijn inderdaad duidelijk lager dan deze van de controlegroep. Deze gegevens tonen aan dat in dit model de functionele levertransplant het toxisch effect van de cholestase op de natieve lever kan tegenwerken (deel 2.1).

Om de evolutie en de viabiliteit van de 2 levers na auxillaire levertransplantatie snel en herhaaldelijk te kunnen volgen werd voor de eerste keer nucleair resonantie-onderzoek gebruikt (MRI). Het onderzoek maakte het mogelijk om in eerste instantie de postoperatieve levercomplicaties zoals abcessen en parenchymateuze aantastingen van elke

individuele lever vroegtijdig te kunnen diagnosticeren. Ten tweede kon men via de signaalintensiteit op de T_1 gewogen beelden de viabiliteit van elke lever beoordelen, waarbij hogere intensiteitsbeelden werden bekomen bij betere leverfunctie. Eindelijk maakte deze studie het mogelijk om de evolutie van de T_1 relaxatietijden van de 2 levers te vergelijken in een normale toestand en in geval van levercomplicaties. De finale evolutie van de 2 levers kon op deze manier reeds vanaf de eerste postoperatieve week voorspeld worden. Deze gevoelige en niet-invasieve meting vergemakkelijkt duidelijk de follow-up van de 2 levers na HALTx. MRI is dus een veelbelovend werkinstrument in de studie van de functionele competitie tussen 2 levers. Prospectieve studies bij de mens zouden op dit vlak zeker interessante resultaten kunnen leveren bij de selectie van patiënten lijdend aan fulminant of subfulminant leverfalen en voor de bepaling van de leverfunctionaliteit na transplantatie of na partiële leverresectie (deel 2.2.).

In het gebruikte model van HALTx was de portale bloedtoevoer van de recipiënt lever volledige geshunt naar de getransplanteerde lever, terwijl de viabiliteit van de natieve lever enkel verzekerd werd door bevloeiing via de arteria hepatica. Ondanks het feit dat het portaal bloed exclusief naar de transplantlever geshunt wordt, blijkt de evolutie van deze ent naar regeneratie of atrofie rechtstreeks gebonden aan de graad van de handicap aangebracht aan de eigen lever. En een 68% PH, en een galwegen ligatuur van de eigen lever is nodig om atrophie van de ent te voorkomen (deel 3.1.). Anderzijds, konden wij tonen dat een rearterialisatie van de getransplanteerde lever toegevoegd een de portale revascularisatie een duidelijk meer uitgesproken stimulatie van de galexcretie na reperfusie met zich meebrengt; rearterialisatie gaf ook een hogere DNA synthese, minder uitgesproken cytolyse en cholestase. Met rearterialisatie kan men dan ook een significant betere entoverleving bekomen. De functionele optimalisatie van de ent heeft een rechtstreekse repercussie op de functionele competitie tussen de 2 levers (deel 3.2.).

In het laatste deel van het werk (deel 3.3) konden we aantonen dat in een klassiek model van partiële leverresectie een volledige portale derivatie de regeneratieve capaciteit van de lever significant verminderd en aldus het herstel van de levermassa na leverresectie verhinderd. De spontane arteriële compensatie na de portale shunt was onvoldoende om een normale regeneratieve respons na partiële hepatectomie te verzekeren. Restitutio van het totale leverdebiet via areterialisatie van de portale stomp kon anderzijds, merkwaardig genoeg, wel de normale respons herstellen. Deze resultaten suggereren dat hoge concentraties van hepatotrofische factoren niet essentieel zijn bij de initiële fase van leverregeneratie.

In conclusie, het zijn dus niet enkel de portale stimulerende groeifactoren en nutritieve elementen, maar het is ook en vooral de algemene functionele status van elke lever welke een rechtstreekse invloed heeft op de regeneratieve capaciteit van elk individueel organ. Een voldoende debiet, dat het weze met arterieel of veneus bloed, via het portaal systeem naar de lever moet verzekerd worden om een normale regeneratiechronologie na partiële leverresectie te bekomen.

RESUME

Si la transplantation auxiliaire du foie présente des avantages théoriques évidents chez les patients souffrants d'insuffisance hépatique aigue ainsi que chez les enfants atteints de maladie métabolique congénitale, les resultats après ce type de transplantation ne sont pas toujours aussi clairement positifs. L'interaction qui a lieu entre le foie donneur et le foie receveur joue ici, parmi d'autres, un rôle important. Il est généralement admis que l'apport de sang portal contenant les facteurs hormonaux hépatothrophiques influence directement le devenir des foies mis en compétition fonctionelle. D'autres facteurs tel que l'état physiologique propre de chaque foie individuel joue également un rôle dans le devenir des organes mis en compétition. Ce fut donc le but du présent travail d'étudier l'influence de l'état physiologique et de l'état fonctionel des foies en présence sur la balance compétitive interhépatique, et ce, à l'aide du modèle de transplantation auxiliaire hétérotopique chez le rat (HALTx). En annexe nous avons tenté de réexamimer la controverse au sujet de la question concernant le rôle prépondérant, soit qualitatif, soit quantitatif de l'apport portal du sang sur la régénération hépatique après hépatectomie partielle standard.

Afin de suivre dans le modèle de transplantation auxiliair la fonction et la viabilité du greffon à l'aide de paramètres biologiques courants, le foie hôte fut soumis à un hépatectomie de 68% et une ligature de la voie biliaire principale. Ces manipulations chirurgicales du foie hôte sont responsables d'une insuffisance hépatique mortelle en l'absence d'un greffon auxiliaire. Si la survie des rats est dans ce cas directement dépendant du foie greffé, la biologie de l'animal sera un reflet direct de sa fonction. Comparés aux groupes contrôles, les groupes transplantées ont des concentrations sériques inférieures en bilirubine, gamma GT, fosfatase alkaline, d'activité de gamma glutamyl transferase et alanine aminotransferaset. Ces résultats montrent que dans ce modèle, un greffon fonctionel peut prévenir l'effet toxique de la cholestase du foie hôte.

Pour l'étude suivante la Résonance Magnétique Nucléaire (RMN) fut utilisé pour la première fois afin de suivre la viabilité postopératoire hépatique dans le modèle de HALTx. Ce travail a tout d'abord pu mettre en evidence pour chaque foie individuel, l'apparition de complications postopératoires tels qu'abcès hépatique et nécrose parenchymateuse. Cet examen a montré en deuxième lieu que, plus la fonctionalité hépatique est conservée plus le degré d'intensité du signal des images pesées en T_1 est prononcé. Lévolution de la viabilité des 2 foies pouvait ainsi être documentée et comparée à tout moment du suivi postopératoire. En comparant l'évolution du temps de

relaxation en T_1 des 2 foies, l'RMN peut en outre prédire dès la première semaine postopératoire le devenir des foies qui resteront sains et ceux qui présenteront des complications. L'RMN apparait comme un outil non-invasif fort utile pour le suivi de l'évolution des 2 foies après transplantation auxiliaire et est prometteur dans l'étude de la compétition fonctionelle interhépatique. Des études humaines prospectives en RMN pourraient s'avérer dès lors fort intéressantes non seulement pour prédire le devenir d'un foie atteint d'hépatite fulminante ou subfulminante mais aussi de prognostiquer la viabilité d'un greffon hépatique ou d'un foie restant après résection hépatique étendue (partie 2.2.).

Dans le modèle de HALTx utilisé dans ce travail, l'apport sanguin portal fut totalement shunté vers le greffon alors que le foie natif n'était irrigué que par l'artère hépatique. Malgré le fait que la totalité du sang portal fut dérivé en direction du foie transplanté, l'évolution de celui-ci vers la régénération ou l'atrophie dépendait directement du degré d'handicap du foie hôte (partie 3.1.). Par ailleurs, la réartérialisation du greffon ajouté a sa vascularisation portale influence directement et de façon significative la quantité de production biliaire posttransplantation et celui de cytolyse et de cholestase ainsi que le degré de synthèse d'ADN du foie greffé. La réartérialisation du greffon a permis d'obtenir une survie significativement meilleure des animaux ainsi traités. La meilleure fonctionalité du foie greffé après artérialisation a logiquement un effet direct sur la compétition interhépatique (partie 3.2.).

La dernière partie du travail (partie 3.3.) montre que l'absence totale de l'apport de sang portal affecte significativement la régénération du foie et la restauration de la masse hépatique après hépatectomie partielle. La compensation vasodilatatrice de l'artère hépatique suite à la réduction du débit hépatique total après shunt porto-cave est insuffisante pour maintenir une régénération normale après hépatectomie partielle. Toutefois, la régénération est normalisée si le moignon portal est artérialisé et le débit hépatique total restauré à des valeurs équivalentes à celles des groupes contrôles. Ces résultats suggèrent que de fortes concentrations de facteurs hépatotrofiques ne sont pas indépensable dans la phase initiale de régénération hépatique.

En conclusion, non seulement l'apport portal et son contenu en hormones de croissances stimulantes ainsi que d'éléments nutritifs, mais aussi et surtout l'état physiologique du foie greffé influence la capacité régénérative des 2 foies dans le modèle de HALTx. Une quantité suffisante de sang artériel ou portal doit être véhiculé par la veine porte aux hepatocytes pour assurer une chronologie et intensité normale de la régénération après hépatectomie partielle.

ABSTRACT

This work was conducted in rat models to investigate whether the factors other than known portal hormonal elements could also influence the inter-liver competition in heterotopic auxiliary liver transplantation (HALTx), and whether the quality or the quantity of portal supply was more important for hepatic regeneration.

The results showed that the handicap of a 68% partial hepatectomy (68% PH) plus a common bile duct ligation (CBDL) could drastically hamper native liver's functionality and post-HALTx animal survival relied solely upon graft's viability. Thus, the significantly lower values of serum biochemistry in the transplanted rats indicated directly that the graft was well functioning.

On a T_1 weighted magnetic resonance image, as observed, hepatic complications could be depicted individually in the coexisting two livers and hepatic viability could be assessed by its signal intensity. The healthy grafts could be distinguished from those that later developed complications by a significant decrease in T_1 relaxation times at an early post-transplant stage.

Despite with proper portal supply, the engrafted liver would atrophy if the native liver was handicapped with a 68% PH alone, whereas graft regeneration was assured by a more severe handicap (adding a CBDL). Furthermore, when the graft's physiological status was improved through re-establishing its arterial supply, it had not only a better early function (immediate bile flow rate and bilirubin excretion) and less biliary complications (cytolysis and cholestasis), but also more pronounced liver regeneration (DNA synthesis rate and graft growth) compared with that of control group.

Complete absence of portal supply to a partial resected liver caused a marked inhibition of early regenerative response and liver growth. When an equivalent amount of portal blood inflow was re-established by arterialising the portal stump, DNA synthesis rate and liver growth could be sustained at a level comparable to that of control rats.

These data emphasize that, besides portal supply, a change of the physiological condition of each individual liver does affect the balance of the inter-liver competition after HALTx. Rather than a high concentration of hormonal factors, the presence of sufficient blood flow in the portal tract, of either venous or arterial origin, appears a prerequisite to maintain a normal chronology and intensity of hepatic regeneration.