Chapter 1

GENERAL INTRODUCTION

BACKGROUND

In 1955, Welch performed the first transplantation of the liver in dogs [1]. In 1963, Starzl and his group carried out the first successful hepatic transplant in man [2]. In 1979 a liver transplantation program was started in Groningen, at that time the fourth liver transplant centre in Europe. In 1983 based on the results of the four major centres in the world (Denver, Cambridge, Hannover and Groningen), liver transplantation was considered no longer an experimental technique anymore (NIH consensus development conference in New York 1983). Since 1979 approximately 450 liver transplants have been performed in the Netherlands of which more than 350 were transplanted in the University Hospital of Groningen.

From the beginning of clinical liver transplantation the results have steadily improved. This can be attributed to more careful patient selection, increasing experience in surgical techniques and post-operative care. Also, greater willingness to retransplant after rejection or primary non-function of the graft and better immunosuppression have contributed to the current overall 5 year survival rate of approximately 75%.

The main indication for liver transplantation is irreversible, progressive end-stage liver cirrhosis, either primary or secondary for which there is no acceptable alternative therapy [3]. Patients with end-stage cholestatic liver disease are also excellent candidates for hepatic transplantation. Metabolic disease with the primary metabolic defect in the liver is also a good indication for transplantation since the graft retains the original metabolic capacity of the donor. Acute hepatic failure, the Budd-Chiari syndrome and in selected cases irresectable primary malignancies of the liver are infrequently indications for liver transplantation. The indications for transplantation of the patients of the Groningen liver program are comparable to that of the patients compiled in the European Liver Registry report [4].

One of the main problems in clinical liver transplantation is the absence of a reliable test to assess the viability of the donor liver. This may lead to the unnecessary discard of suitable livers resulting in organ wastage and lengthening of the waiting list for transplantation with all associated negative consequences [5]. Similarly, it can not be prevented that livers that have been severely damaged during donor lifetime or during explantation, cold flush and subsequent cold storage are inadvertently transplanted. In these cases, insufficient recovery of liver function after transplantation leads to more complications, an increased mortality and higher overall costs of a transplantation program.

To assess the cumulated organ damage after explantation with the ultimate goal to predict posttransplant organ function, a viability test of the isolated donor liver is needed. Preferably, such a test should be non-invasive to
maintain the integrity of the donor organ. A number of features of the isolated, cold stored donor liver offer a point of application for non-invasive viability testing.

From literature it is well known that high energy phosphates like adenosine tri phosphate (ATP) play an important role as determinants of cellular viability [6,7]. Therefore, knowledge of the status of the high-energy phosphates could probably serve as a viability indicator of livers to be transplanted. Animal studies indicated that $^{31}$P magnetic resonance spectroscopy ($^{31}$P-MRS) may be used for non-invasive pretransplant viability testing of isolated human donor livers [6,8-11].

Another potential viability test could be the assessment of the tissue hydration state of the isolated liver. In in-vitro animal studies in which the tissue hydration state was assessed with magnetic resonance relaxometry (hereafter abbreviated to relaxometry), the tissue hydration state was found to be related to organ viability [12-13]. Consequently, clinical application of relaxometry for viability testing in human liver transplantation was suggested [12-15].

Furthermore, it is well known from animal studies that during liver preservation, liver tissue switches to anaerobic glycolysis which results in a progressive acidosis [16-17]. Several animal studies have found that tissue acidosis during cold storage affects tissue viability [17-22]. Human donor livers however, have not been the subject of a systematic study of the extent and the consequences of tissue acidosis during cold storage. In theory, non-invasive determination of the extent of tissue acidosis in isolated donor livers could be used for viability testing [23].

Although not related to viability in a strict sense, information about structural abnormalities of parenchyma and vasculature of the cold stored donor liver can be important for transplantation outcome. Donor livers are not routinely checked for structural abnormalities which may prohibit transplantation. Information about parenchyma and vasculature of an isolated liver can be obtained through invasive techniques such as biopsy and injection of contrast fluid. Because of their invasiveness, these techniques endanger the integrity of the liver. A non-invasive imaging like magnetic resonance imaging technique could provide essential anatomical information about the donor liver prior to transplantation.

The usefulness of the four above mentioned features for clinical viability testing can be studied non-invasively by applying nuclear magnetic resonance (NMR) techniques on cold stored isolated donor livers. Some of these NMR techniques have been tested in isolated small animal livers but so far, human livers destined for transplantation have not been studied with NMR.

In the following paragraphs a short introduction is given on current clinical viability testing, liver preservation and metabolism during cold storage. Next, a concise description is given of nuclear magnetic resonance. Finally, the specific aim of the study is presented.
CURRENT CLINICAL METHODS OF VIABILITY TESTING FOR LIVER GRAFT SELECTION

Viable means: capable of surviving. Viability testing in the liver transplant setting means the assessment of the capacity of an organ to regain its original function after transplantation.

The selection criteria for liver donation intend to obtain a graft which will provide adequate liver function after transplantation. Current selection of liver donors is based on clinical parameters such as hemodynamic stability, absence of sepsis, and a negative past medical history coupled with serum parameters of adequate liver synthetic function and no signs of significant parenchymal damage [5,24-25]. However, although these criteria are strictly adhered to, in practice they do not differentiate between grafts with "good" and "poor" function after transplantation [5,26-27].

Additional dynamic liver function tests before explantation have so far only been used in limited clinical trials. At present, three such tests of liver function in the donor are available; the monoethylglycinexylidide (MEGX) test after lidocaine infusion, the indocyanine green clearance, and galactose elimination capacity. The lidocaine-MEGX test gives an estimation of the hepatic Cytochrome P-450 capacity to remove toxic substances. This test may reflect the potential of an organ to withstand the metabolic stress of a transplantation. Controversial reports on the usefulness of this test have made that it is not widely used for viability assessment[28-33].

The two other dynamic tests, indocyanine green clearance and galactose elimination capacity, appear to have limited clinical application because the necessity to take donor blood samples at regular intervals is cumbersome [34].

Another potential indicator of liver viability is the arterial ratio between acetoacetate and B-OH-butyrate (KBR=ketone body ratio). This ratio reflects the mitochondrial NAD+/NADH-ratio in the liver and it thus reflects the energy producing capacity of the mitochondria [35]. Although initial results appear to be promising in assessing graft function after transplantation [36], the usefulness of this test for routine clinical viability testing has yet not been fully elucidated.
Chapter 1

LIVER PRESERVATION

One of the key factors in organ preservation is hypothermia. Hypothermia during organ preservation decreases the degradation rate of essential cellular components which are necessary for organ viability. Hypothermia also reduces the energy requirements of the tissue and with that the demand for oxygen. However, hypothermia also interferes with energy dependent functions and slows down cellular ionic pumps which induces osmotic and ionic shifts within tissues. This results in cell swelling and expansion of the interstitial space and leads to decreased cellular viability [37]. Thus, suppression of cell swelling as well as expansion of the interstitial space using so-called impermeants is essential for good organ preservation. For that reason the currently used UW preservation solution (University of Wisconsin, DuPont Critical Care, Waukegan, IL) contains the impermeants raffinose, hydroxyethyl starch and lactobionate (see Table 1).

<table>
<thead>
<tr>
<th>Impermeants</th>
<th>K-lactobionate 100 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raffinose 30mM</td>
</tr>
<tr>
<td>H⁺ buffer</td>
<td>KH₂HPO₄ 25 mM</td>
</tr>
<tr>
<td>Colloid</td>
<td>Hydroxy ethyl starch 5 g%</td>
</tr>
<tr>
<td>other components</td>
<td>MgSO₄ 5 mM</td>
</tr>
<tr>
<td></td>
<td>Adenosine 5 mM</td>
</tr>
<tr>
<td></td>
<td>Glutathione 3 mM</td>
</tr>
<tr>
<td></td>
<td>Allopurinol 1 mM</td>
</tr>
<tr>
<td></td>
<td>Insulin 100 IU/L</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone 8 mg/L</td>
</tr>
</tbody>
</table>

Table 1 Composition of the UW solution.

Phosphate is the main buffer in UW and counteracts the acidosis that develops during cold ischemia. Also, adenosine is added as a precursor of adenosine-triphosphate (ATP). Adenosine and phosphate are believed to act synergistically to stimulate ATP synthesis [38]. Allopurinol serves as xanthine-oxidase inhibitor and may limit the formation of oxygen-derived free radicals. Glutathione which is also a component of UW serves as a powerful anti-oxidant. Furthermore, UW contains a balanced composition of electrolytes in which potassium is the main component for osmotic support. Immediately prior to use Penicillin G, Insulin, and dexamethasone are added. Penicillin prevents bacterial overgrowth and organ infections. The addition of insulin is somewhat theoretical and is believed
Introduction

to enhance glucose and/or potassium uptake by the preserved organs. Dexamethazone is thought to stabilize the hepatic and/or sinusoidal cell membranes and render them less vulnerable to an acidic environment. Also, lysosomal activation due to membrane destabilization may be limited.

The introduction of UW has had significant impact on liver transplantation [39-41]. It has helped to develop liver transplantation from an emergency procedure into a semi-elective procedure. UW has facilitated liver transplantation on an international scale, allowing long-distance procurement, organ sharing by split-liver procedures, and improved recipient preparation. These advantages explain why at present UW is the most commonly used preservation solution in liver transplantation.

A liver preservation for clinical transplantation is started with in-situ cold flush through aorta and portal vein with 2 litres of UW solution during the explantation operation. At the same time, the abdominal cavity is filled with cold saline to provide adequate surface cooling of the liver. Approximately 4 L of UW is flushed through the organ, 1.5 L through the portal vein, 2.5 L through the aorta/hepatic artery. The in-situ cold flush takes approximately 25 minutes. Then, after removal of the liver on the backtable an additional amount of UW is flushed through hepatic artery and portal vein until the outflow from the vena cava and the common bile duct is clear. The biliary system is also additionally flushed with UW. Livers are then packed in sterile plastic bags and stored in UW solution on melting ice in styrofoam containers. Safe cold ischemia time for a UW preserved liver is between 15 and 20 hours but the definite ultimate cold storage time is not known. A case of a successful liver transplantation after 34 hours of cold ischemia has been reported by the Pittsburgh group [42]. In general, extended cold ischemia time is associated with primary dysfunction (PDF) but if no other risk factors for PDF are present, extended preservation with all its advantages, such as elective surgery, is justified. When a number of risk factors for PDF must be accepted in a specific patient, preservation time is the only variable that can be controlled and should therefore be kept as short as possible [40].

LIVER METABOLISM DURING COLD STORAGE

At the onset of cold ischemia when warm oxygenated blood flow is interrupted and replaced by cold preservation solution with minimal oxygen content, aerobic metabolism of the liver rapidly slows down. It has been calculated that when temperature is reduced from 37°C (body temperature) to 0°C (preservation temperature) metabolism is suppressed approximately 12 fold [37,43]. Initially a very low level of aerobic metabolism may still be present but the remaining oxygen in the tissues and in UW is consumed within minutes [44]. Due to the lack of oxygen to serve as a H⁺ acceptor, aerobic breakdown of pyruvate ceases and aerobic ATP synthesis stops. The remaining ATP is dephosphorylated, first
into adenosine-diphosphate (ADP), and with continuing demand for energy into adenosine-mono-phosphate (AMP). At that point, the lack of oxygen forces the cells to switch to the anaerobic breakdown of glucose (glycolysis) via the activation of phosphofructokinase. Phosphofructokinase is an enzyme which is rate-limiting in glycolysis. ATP is an inhibitor whereas AMP is an activator of phosphofructokinase. After oxygen is depleted, the AMP/ATP ratio increases which results in activation of phosphofructokinase and the effect is an increase of the overall rate of glycolysis (the Pasteur Effect) [45]. At the end of the glycolytic enzyme cascade, pyruvate is converted into lactate by the action of lactate dehydrogenase. Hydrolysis of ATP to ADP and subsequently to AMP (proton generating steps) and generation of lactate result in a progressive tissue acidosis [6,17,46]. The continuing acidification surpasses the buffering capacity of the (preserved) liver cells. The resulting low pH causes feedback inhibition of phosphofructokinase with in turn a decrease of glycolytic ATP generation [17,47-48]. The ultimate cessation of glycolytic ATP generation is one of the reasons why organs have a certain limit in the tolerance of cold ischemia.

Warm ischemia is considerably more harmful than is cold ischemia [49]. A liver can sustain relatively long periods of cold ischemia as compared to warm ischemia. One reason for the existence of a different time limit for cold and warm ischemia is the extent to which ATP is degraded. ATP, ADP, and AMP do not pass the mitochondrial membrane. As long as the degradation of ATP is largely halted at AMP, as is the case in cold ischemia [49]. AMP is available for mitochondrial resynthesis of ATP [50]. This explains why after a moderate period of hypothermic storage, organs can regain their original metabolic function after reperfusion [37]. In contrast, during warm ischemia AMP is further degraded into non-phosphorylated compounds like adenosine, inosine, hypoxanthine, xanthine, and urate to which the mitochondrial membrane is freely permeable. These compounds can leak out of the mitochondria [51] and may not be available for resynthesis of ATP once the blood flow has been re-established. Furthermore, since the ischemic liver contains a large amount of xanthine-oxidase, high concentrations of xanthine produced during ATP degradation could enhance superoxide (free radical) production and thus cause additional detrimental effects on liver viability [43,49].

From the results of the numerous studies on liver viability after reperfusion there is general concensus that the regeneration capacity for ATP after recirculation determines the chances for functional recovery and with that, the chances for a successful transplantation [6-7,9,23,50,52-53].
NUCLEAR MAGNETIC RESONANCE

Nuclear magnetic resonance (NMR) is the ability of a nucleus with an unpaired number of protons or neutrons (¹H, ¹³C and ³¹P) to resonate upon the application of a short radiofrequency (RF) pulse in the presence of a strong magnetic field. After cessation of the RF pulse, the nucleus emits radiofrequent energy at its own specific frequency, the so called resonance frequency. This phenomenon was discovered independently by Bloch and Purcell in 1946 [54-55]. For subsequent development of spectroscopic methods for physical chemistry and biochemistry Bloch and Purcell received the Nobel Prize for physics in 1952. The birth of NMR for imaging purposes was in 1973 with the publication of Lauterbur that application of a magnetic field gradient would cause water protons at different sites to resonate at slightly different frequencies [56]. Today, NMR is widely used, in the basic sciences predominantly in its original role as magnetic resonance spectroscopy (MRS) and in diagnostic imaging mainly as magnetic resonance imaging (MRI). The following section is focussed on MRS of ³¹Phosphorus nuclei and MRI of the ¹H nuclei (proton MRI).

³¹Phosphorus Magnetic Resonance Spectroscopy (³¹P-MRS).

After a RF pulse each ³¹P nucleus will absorb and release the energy at specific frequencies depending on how the nucleus is shielded from its immediate environment. The release of RF energy after cessation of the broad bandwidth excitation pulse (free induction decay, FID) is picked up by an antenna (the spectroscopy coil). The FID, which is a time signal, is then Fourier transformed into a frequency signal in which the individual frequencies of the respective ³¹P nuclei can be recognized (Fig 1). These frequencies are characteristic for the type of chemical bond in which the nucleus is located. A graphic display of the frequency intensities along a parts per million (ppm) axis results in a spectrum. This spectrum gives information about the identity of a resonance peak through the position of the peak on the ppm axis relative to a reference signal, the so called chemical shift.

In in-vitro studies where small-bore high-field (=high homogeneity) magnets are used, the intensity of a peak is linearly proportional to the concentration of the respective ³¹P metabolite. In clinical studies which use large-bore low-field (=low homogeneity) magnets, the area under the curve of a peak is used for estimation of the amount of ³¹P nuclei. The use of peak areas compensates for slight differences in magnetic field homogeneity due to differences in dimension and position of different subjects in the magnet. In living cells, only the mobile ³¹P bearing molecules can be detected. The ³¹P nuclei
which are present in bone or DNA are bound in a relatively solid

Fig 1. Free Induction Decay, before (left) and after (right) Fourier transform.

state. These $^{31}\text{P}$ nuclei have very short FID's and with present clinical spectroscopy equipment they appear only as a faint broad hump in the baseline of the spectra. Since $^{31}\text{P}$ metabolites are present in tissue at a low concentration (Pi concentration is approximately 25 mmol/L) and because of their relatively low gyromagnetic sensitivity (1/15 of the sensitivity of protons in a comparable magnetic field), a relatively large number of signal averages has to be accumulated to obtain adequate signal-to-noise ratios.

Spectroscopic research on isolated human organs has become accessible to clinical researchers with the implementation of spectroscopy hard- and software in large bore, whole body imaging systems. At a field strength of 1.5 Tesla a number of $^{31}\text{P}$ bearing compounds are of special interest with respect to organ transplantation which are discussed next.

The phosphomonoesters (PME) peak is a multicomponent peak which is regarded to reflect glycolytic intermediates like glucose-6-phosphate, fructose-1-
Introduction

6-diphosphate and AMP. Furthermore, the PME peak represents phosphocholine (PC) and phosphoethanolamine (PE), two monophosphorylated organic compounds which may reflect the rate of phospholipid (membrane) biosynthesis [57-58].

The inorganic phosphate (Pi) may in a UW preserved donor liver spectrum be observed as two peaks, one from Pi from the liver itself (hepatic Pi) and one from the inorganic phosphate which is a component of UW preservation solution (Pi-UW).

The phosphodiester peak (PDE) corresponds with glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE) which are catabolic products of PE and PC respectively [57-60]. In contrast to PME, the role of PDE in cellular metabolism is not clear but it may be dependent on nutritional status of the donor [9].

NAD⁺/NAD(H) and NADP⁺/NADP(H) are involved in oxidation-reduction reactions.

Fig 2. Spectral resonances of the alpha, beta and gamma phosphorus atoms.
reactions in which they act as co-enzymes for a variety of dehydrogenases. They also serve as proton and electron donator/acceptor at the end of the electron transport chain.

Phosphorylated adenosine is present in three appearances: adenosine mono, di and tri phosphate (AMP, ADP and ATP respectively). AMP is present in the spectrum hidden in the PME peak. ADP shows up at two resonance frequencies corresponding with the alpha and gamma positions of the phosphate in the molecule (Fig 2). ATP is present at three frequencies in the spectrum corresponding with the alpha, beta, and gamma positions of the phosphate in the molecule. In clinical spectroscopy practice, when estimating the amount of ATP, only the beta-atp is considered since this signal is coming solely from ATP.

Tissue pH can be calculated from the chemical shift of the hepatic inorganic phosphate (Pi) peak relative to a reference peak which is pH-independent, for example the NAD(H) peak [61]. Hepatic inorganic phosphate and inorganic phosphate in the preservation fluid can be discriminated from each other by their different chemical shifts.

Using $^{31}$P-MRS, the presence of the above mentioned compounds can be assessed in the living cell without risks for the sterility and storage temperature. As such, $^{31}$P MRS is particularly suited for research on cold stored donor organs while the integrity of the organ is maintained [62-68].

**PROTON MAGNETIC RESONANCE IMAGING.**

The introduction of magnetic field gradients in combination with a phase encoding gradient allows to determine the spatial location of the origin of an NMR signal, in other words, image formation based on the spatial distribution of NMR susceptible nuclei is possible. Since the original observation of image formation through NMR by Lauterbur in 1973 [56], an enormous development of MRI techniques has taken place and is still continuing. At present, only proton MRI has found clinical application thanks to the overwhelming abundance of proton nuclei in the human body.

Signal intensity in MRI is, apart from proton density and motion, largely determined by the relaxation process in which the nuclei return to their equilibrium state after cessation of the RF pulse. This relaxation process consists of two components, each with their own relaxation time constants. $T_1$, or spin-lattice relaxation indicates how fast energy is transferred from the resonant nuclei to the molecular environment by random thermal motions of molecules. $T_2$ or spin-spin relaxation indicates the loss of phase coherence among resonant nuclei. For biological tissues it is generally assumed that $T_1$ is determined predominantly by intermolecular interactions between macromolecules and a
Introduction

single bound hydration layer, and that tissue $T_2$ is governed mainly by exchange diffusion of water between the macromolecular-bound and free water phases [69]. Thus, MR images are based on the distribution and the physico-chemical environment of the $^1$H nuclei in the body. Since these characteristics differ in the various tissues of the body, images with excellent contrast between the different tissues can be obtained [70]. Image contrast can be further enhanced by using RF pulse sequences which exploit the differences in relaxation times of the different tissues. Manipulating instrumental parameters such as repetition and echo times allows to obtain images in which $T_1$ differences are highlighted or images in which $T_2$ differences are highlighted. A variety of combinations of echo and repetition times is possible with more or less $T_1$ or $T_2$ weighting. Thus, it is possible to use pulse sequences tailored to a specific tissue. This unique capability of MRI is particularly evident in its ability to differentiate between soft tissues like brain, muscle, fat and connective tissue. Furthermore, in contrast to other tomographic techniques (e.g. Computed Tomography), MRI offers the opportunity to obtain images in any desired plane of interest.

Relaxometry is a technique in which $T_1$ and $T_2$ relaxation times are calculated. The combination of relaxometry with MRI by using a sophisticated RF pulse sequence results in a spatial distribution of relaxation times. In such an image, the tissue relaxation times are displayed as grey scale values.

SCOPE AND AIM OF THIS THESIS

At present, there is no reliable test to assess the viability of a donor liver with predictive capacity for transplantation outcome and for that reason there is still a considerable risk of discarding suitable donor livers on ill-founded grounds. For the same reason there is also the risk of transplantation of "bad" donor livers. A recent report by Mirza and co-workers elegantly demonstrated that early graft function and 1 year graft survival of "marginal quality" donor livers was similar to that of "good livers" [5]. In their opinion, usable donor livers are being unnecessarily refused on medical grounds. On the other hand however, approximately 20% of the transplanted grafts show primary dysfunction (PDF), either presenting as initial poor function (IPF) in 16% of cases, or as primary non-function (PNF) in 6% [31,40]. IPF represents borderline liver function immediately after transplantation and livers with IPF will either recover after a prolonged period of dysfunction or lead to death or retransplantation at a later date. PNF is defined as a state of immediate non-reversible lack of functional activity of a liver graft leading to death of the recipient. Therefore, PNF requires immediate re-transplantation which is associated with a 50% mortality risk. For obvious reasons, a test which can identify PNF livers would have significant clinical impact.

The time point at which a viability test is performed is a fundamental
element in the discussion of clinical viability testing. All currently used donor
criteria apply to the liver when the organ is still in the donor. Even if the
lidocaine-MEGX test or comparable tests would turn out to be reliable indicators
for liver viability, that would only apply to the viability of that liver in the donor.
The impact of the cold preservation on liver metabolism and the unpredictable
events during induction of anaesthesia and the surgical explantation procedure
itself are completely bypassed when using the current donor viability indicators
only. Therefore, a prerequisite for a reliable viability test of a donor liver is that it
includes the cumulative organ damage that has been inflicted during
explantation and preservation in addition to damage sustained during donor
lifetime. That would mean that liver viability after explantation and cold
preservation i.e. during cold storage, should be tested. Such attempts were
made by assessment of trypan blue exclusion and taurocholic acid uptake
capacity in isolated hepatocytes obtained from donor livers [71-72]. However,
hepatocyte isolation from biopsies failed in more than 50 % of the cases [72].
Moreover, the tests could not discriminate hepatocytes of discarded livers from
hepatocytes of accepted livers [71].

Apart from metabolic/functional integrity, viability may in a broader sense
also include the morphology of liver parenchyma and the anatomy of the hepatic
vasculature. Parenchymal abnormalities in a donor liver may potentially prohibit
transplantation. There is no common opinion as to whether a liver biopsy from
the donor should be routinely taken from every potential liver donor. However,
since severe steatosis has been associated with an increased incidence of
primary non-function after transplantation, many centres routinely examine the
donor liver histologically before making the final decision whether to use the
graft [31-32,73]. Nevertheless, steatosis can be focal and may as such not be
visible on the surface of the liver. Biopsy from such an area can lead to discard
of the liver on the basis of a sampling error. Similarly, acceptance of a liver with
normal histology based on biopsy but carrying a lesion suspect for malignancy
could be disastrous for the recipient, especially with the potent
immunosuppressive drugs used after transplantation. Furthermore, traumatic
injury to the liver associated with the donors' cause of death, not detectable on
visual inspection can also cause problems after transplantation. As far as the
vascular architecture is concerned, information about the hepatic-artery system
(with the portal-vein branches) can be obtained from workbench arteriography.
The anatomy of the hepatic veins can hardly be visualized but can be very useful
in split-liver and reduced size liver transplantations. In the vast majority of
potential liver donors no special anatomical/morphological evaluation of the liver
is employed and the liver is not routinely checked for structural abnormalities
which may prohibit transplantation.

Altogether, at present no reliable test for the metabolic/functional as well
as the anatomical/morphological viability of human donor liver is available. An
integral post-harvest assessment of liver viability may be provided by applying
NMR on the isolated donor liver.

The first objective of this thesis was to investigate whether $^{31}$P-MRS of the isolated human donor liver was able to provide useful information about the liver metabolic integrity with the ultimate objective to serve as a viability test with predictive capacity for transplantation outcome. The second objective was to investigate whether proton MRI of the isolated human donor liver can detect abnormalities of parenchyma and vasculature which could have consequences for the transplant procedure.

In chapter 2 an overview is given of the logistic and technical problems that had to be solved before the clinical experiments could proceed. Chapter 3 deals with the question whether MRI can detect abnormalities of parenchyma and vasculature of the pretransplant human donor liver. Specifically, it was investigated whether a display of the liver vasculature could be helpful in the preparation of split liver grafts for transplantation. A derived application of "ex-vivo MRI" in extracorporeal liver surgery is also described. Chapter 4 elaborates on the clinical consequences of the unexpected finding (in chapter 3) of large quantities of air in the liver vasculature. Chapter 5 deals with the presence and the posttransplant effects of hypothermia induced tissue edema in human donor livers as studied with relaxometric imaging. A separate note on the relaxation times of the bathing fluid is also presented. Chapter 6 describes the clinical importance of tissue pH for clinical liver transplantation. Specifically, the relation between tissue pH of the donor liver and indicators for preservation\-reperfusion injury is assessed. In addition, selected donor parameters and preservation related events are studied in relation to the tissue pH. Chapter 7 relates the changes in proton relaxation times as a result of alterations in tissue hydration state (observed in chapter 5) with the consequences for the phosphorus spectrum. In chapter 8 the potential role of $^{31}$P-NMR spectroscopy as a viability indicator is presented. Chapter 9 presents a discussion of the integrated findings of the previous chapters. This provides points for consideration and some suggestions for further research. Finally, the summary is presented with the final conclusions.
Chapter 1

References

4. European Liver Registry report 1994
Introduction

International 1993,6,226.

49 Harvey PRC, et al. Adenine nucleotide tissue concentrations and liver allograft viability after cold preservation and warm ischemia. Transplantation 1988,45(6),1016.
50 Hamamoto I, et al. Can adenine nucleotides predict primary nonfunction of the human
Chapter 1

liver homograft? Transpl Int 1994;7,89.


58 Iles RA, et al. 31P magnetic resonance spectroscopy of the human paediatric liver. NMR in Biomed 1990,3(2),90.


64 Weiner MW. The promise of magnetic resonance spectroscopy for medical diagnosis. Invest Radiol 1988,23,253.


71 Sandker GW, et al. Characteristics of human hepatocytes isolated from livers accepted or discarded for orthotopic transplantation. submitted.
