Molecular and biochemical mechanisms of bile duct injury after liver transplantation
Buis, Carlijn
1 Introduction and outline of this thesis
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Liver transplantation is the ultimate treatment for end-stage liver disease. Survival following liver transplantation has improved substantially over the years due to better pre-transplant care, improved anesthesia and surgical techniques, enhanced intensive care medicine, and more effective immunosuppressant medications. Currently, 1-year patient survival rate is almost 90% and 5-year patient survival rate is 75% (1).

The first attempt to transplant a liver in a human was reported by Starzl in 1963 (2). In the Netherlands, the first liver was transplanted in Groningen in 1979 (3). Nowadays, around 120 livers are transplanted annually in the Netherlands.

In the Netherlands, around 135 patients are currently on the waiting list for liver transplantation. Although transplantation accounts for 77% of the outflow from the waiting list, unfortunately still 12% of the patients die whilst on the waiting list. Worldwide, around 17000 patients are on a waiting list for liver transplantation, while the estimated number of liver transplants performed in 2008 will be less then 14000 (4). The focus on the recruitment of organ donors therefore remains of vital importance in order to continue and improve the success of transplantation.

Posttransplant-related complications can grossly be classified into primary graft dysfunction, vascular complications, graft rejection, recurrent disease, and biliary complications. Reconstruction of biliary drainage is historically considered as the technical 'Achilles heel' of liver transplantation (5). Although the surgical technique of biliary reconstruction has emerged and is now a more or less standardized technique, complications arising from the bile duct and its reconstruction remain a serious source of morbidity. The resulting biliary complications comprise leakage and strictures. Depending on the localization, strictures are classified as anastomotic or non-anastomotic. Non-anastomotic strictures (NAS) are considered to be the most troublesome biliary complication after liver transplantation. NAS are defined as any stricture, dilatation or irregularity of the intra- or extrahepatic bile ducts detected on imaging studies of the biliary tree after liver transplantation. Approximately one in seven patients suffers from NAS after liver transplantation. In patients with NAS graft loss is reported in up to 50% after 2 years (6). Accepted risk factors for NAS are hepatic artery thrombosis, chronic ductopenic rejection, and ABO blood group incompatibility. In 1991 it was first described that NAS may
also occur in the absence of these known risk factors (7). Because of the resemblance of intrahepatic biliary strictures occurring after hepatic artery thrombosis, NAS that appeared despite occlusion of the hepatic artery were also called ischemic type biliary lesions (ITBL). The two names NAS and ITBL are still both used in the literature. A relationship between NAS and the duration of cold ischemia time was discovered soon after. Ever since, research in this area has focussed on identifying pathophysiological mechanisms and implementing therapeutic strategies. Nevertheless, NAS still occur in many patients and in most cases no apparent clinical risk factor can be identified. Therefore, the aim of this thesis was to perform a more fundamental analysis, using genetic, molecular and biochemical methods in an attempt to identify the underlying mechanisms of these biliary complications.

This thesis is divided in three parts, focusing on I) Clinical risk factors for the development and progression of NAS, II) The role of bile salt toxicity in the development of bile duct injury and NAS after liver transplantation, III) The role of heme oxygenase-1 (HO-1) in the protection of liver grafts from ischemia / reperfusion (I/R) injury. The three parts are preceded by a general overview of the causes and consequences of non-anastomotic biliary strictures (chapter 2).

Part I. Non-anastomotic biliary complications after liver transplantation.
The specific aims of this section were to describe the various forms of NAS and the accompanying clinical risk factors as well as to study clinical risk factors for progression of NAS. Chapter 3 describes the non-anastomotic biliary strictures in the Groningen cohort of liver transplant recipients. All imaging studies of the biliary tree were reviewed. Localization and severity of NAS at first presentation were categorized using a newly developed classification. Time interval between transplantation and the initial presentation of NAS were recorded. The purpose of this study was to identify risk factors for the clinical and radiological presentation of NAS, as well as for the timing of NAS after liver transplantation. Chapter 4 concerns the cohort of patients identified with NAS in chapter 3. This chapter focuses mostly on the consequences of NAS. We defined a number of serious complications of NAS, studied their prevalence and risk factors, and evaluated the effects of therapeutic measures.
Part II. Bile physiology after liver transplantation.

The specific aims of this section were to evaluate the contribution of bile composition to the development of bile duct injury. Bile salts have potent detergent properties and may damage cells of the biliary tract by affecting the integrity of the membranes. The detergent properties of bile salts are normally counteracted by phospholipids. By forming mixed micelles of bile salts, phospholipids and cholesterol, phospholipids “neutralize” bile salts thereby protecting against cellular injury. In a previous study our group has shown that bile produced early after transplantation has an abnormal composition characterized by a low phospholipids-to-bile salt ratio (8). Based on these findings we hypothesized that bile salt toxicity early after liver transplantation contributes to the formation of NAS.

NAS are a frequently encountered complication after non-heart-beating (NHB) liver transplantation. Aim of chapter 5 was to study the role of bile salt toxicity in the pathogenesis of bile duct injury after NHB liver transplantation. We hypothesized that NHB liver transplantation is associated with increased bile salt toxicity early after liver transplantation depending on the length of the warm ischemia time in the donor. To test this hypothesis we studied bile composition, graft survival and the degree of bile duct injury in a porcine liver transplant model.

Chapter 6 describes the role of altered bile composition in the development of NAS after human liver transplantation. In a large clinical study in 111 patients bile composition and the development of NAS were studied in a prospective fashion. The aim was to test whether bile composition is involved in the pathogenesis of NAS.

Chapter 7 concerns the genetic variations in hepatobiliary transporters. These transporter proteins are responsible for bile secretion. The bile salt export pump (BSEP, official name ATP binding cassette, subfamily B, member 11. ABCB11) mediates ATP-dependent secretion of bile salts across the canalicular membrane of hepatocytes. Multidrug resistant protein 3 (MDR3, official name ATP binding cassette, subfamily B, member 4. ABCB4) acts as a primary active phospholipid flippase and translocates phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane. Multidrug resistant related protein 2 (MRP-2, official name ATP binding cassette, subfamily C, member 2. ABCC2) acts as a multispecific organic anion transporter that mediates biliary excretion of a broad spectrum of divalent organic anions, including bilirubin and glutathione. Via the subsequent passive diffusion of water into the bile, this process is the most significant contributor to the bile salt–independent bile flow. Aim of this study was to assess whether genetic variations in the above described transporters, present in the donor liver, are associated with the occurrence of NAS in the recipient after transplantation.
Part III. HO-1 and hepatobiliary injury after liver transplantation.

HO-1 has been proposed as a graft survival gene. Upregulation of HO-1 is considered to be one of the most critical cellular protection mechanisms during cellular stress such as ischemia and reperfusion occurring during a transplant procedure. The specific aim of this section was to study the role of HO-1 expression in relation to postoperative hepatobiliary injury and graft function.

Chapter 8 concerns endogenous HO-1 expression levels in human liver transplants. We studied changes in HO-1 expression levels during liver transplantation and correlated this with immediate postoperative hepatobiliary injury and graft function after transplantation.

Chapter 9 describes two genetic polymorphisms in the promoter influencing the inducibility of HO-1: a (GT), polymorphism and a single nucleotide polymorphism (SNP), A(-413)T. We analyzed these two functional HO-1 promoter polymorphisms in donor genomic DNA in relation to hepatobiliary injury and outcome after human liver transplantation. Furthermore, we studied the functional relevance of these polymorphisms by measuring hepatic messenger ribonucleic acid (mRNA) expression.

Finally, in Chapter 10 the results as described in this thesis are summarized and future perspectives are discussed.

References

1. www.unos.org; www.eurotransplant.nl