Introduction and thesis outline
Introduction

The liver is the only human organ that is able to regenerate following injury or loss of mass. The first description of liver regeneration dates back to the ancient Greek myth of Prometheus, in which his regenerating liver allowed an eagle to tear at his liver for eternity as a punishment. Although Prometheus was immortal, the old Greeks knew he still needed his liver to live. Today, liver regeneration is perhaps the most studied biomedical phenomenon. This unique capacity is exploited daily by liver surgeons by performing partial liver resections, which is the preferred treatment for patients with primary and secondary hepatic tumors. These resections can offer a chance of long term survival, however usually only 10-30% of patients ultimately qualify for liver surgery due to both technical and safety limitations.

In the last decade, surgical techniques and perioperative care have improved. Consequently, the indications for resection have greatly expanded resulting in more complex resections being performed in an aging population with more comorbidity. The net result is that extended liver resection is still associated with substantial morbidity and mortality and especially intra-operative blood loss is associated with adverse outcomes. Therefore the afferent hepatic blood supply is often clamped during parenchymal transection to limit blood loss, which is known as vascular inflow occlusion (VIO) or the Pringle maneuver. Although VIO can minimize blood loss, the cessation of hepatic blood supply and subsequent reperfusion induce hepatic ischemia and reperfusion (I/R) injury, which is characterized by a sterile inflammatory response that ultimately leads to hepatocellular necrosis, deteriorated liver function and impaired liver regeneration. The I/R response has been extensively studied, mostly in mouse models and many inflammatory pathways have been identified. However, no effective therapy to reduce hepatic I/R and thereby preserve or enhance liver regeneration has reached clinical application. This might be due to the inaccurate reflection of the characteristics of clinical I/R injury derived from hepatic I/R models in mice. Chapter 1 addresses the differential results in commonly used mouse models by using various durations of hepatic ischemia. The ischemia-time dependent variations in liver function, liver damage and inflammation are investigated. A validated experimental model is essential for translational hepatic I/R research. In chapter 2, we present an example of the discrepancies between experimental models of hepatic I/R and the clinical response in patients. The inflammatory IL-1/IL-23/IL-17A-axis is investigated in both mild and severe mouse models of hepatic I/R and are related to the results from a controlled cohort of patients subjected to liver resection with and without VIO.

Only hypothermia has found broad application to reduce hepatic I/R in patients, in the setting of liver preservation for transplantation and during liver resection using in situ hypothermic perfusion. In Chapter 3, the mechanisms by which hypothermia reduces hepatic I/R are reviewed. In Chapter 4 the protective effect of hypothermia is investigated in
a randomized trial, by using a newly devised in situ hypothermic perfusion technique with retrograde drainage and comparing this to standard treatment during liver resection. The traditional read-out parameters for liver parenchymal injury are plasma transaminase levels, however, the relevance of postoperative transaminase levels have recently been questioned in literature. Therefore Chapter 5 addresses the relevance of postoperative transaminase levels for postoperative morbidity and mortality following liver resection.

Sufficient liver tissue must remain after liver resection in order to avoid induction of post-hepatectomy liver failure (PHLF), for which no effective treatment is available and consequently is associated with a high mortality rate. To minimize these risks, the future liver remnant (FLR) is preoperatively quantified which can be performed by measurement of liver volume, or alternatively though quantitative assessment of liver function using techniques such as hepatobiliary scintigraphy. In patients with an insufficient FLR to safely perform partial hepatectomy, portal vein embolization (PVE) can be performed to enhance the FLR. The current standard is embolization with permanent embolic materials, however when patients are found to be unresectable intra-operatively the state of permanent embolization may give rise to complications. Reversible PVE might therefore be favorable in selected patients and a method to establish reversible portal vein embolization is provided in Chapter 6 using a rabbit model of PVE. Clinical translation of experimental results from rabbits is essential and therefore the translational value of the rabbit model of VPE is elaborated in Chapter 7.

Perihilar cholangiocarcinoma (PHC) is a biliary tumor that typically obstructs major bile ducts causing obstructive cholestasis which is known to reduce the regenerative capacity of the liver. Therefore, PHLF is more frequent in these patients and preoperative assessment of the FLR and selection of patients for PVE is essential. In Chapter 8 a cut-off value for FLR volume is presented to select PHC patients for VPE in conjunction with a risk score to predict PHLF. Liver volume does however not necessarily reflect liver function, therefore the predictive value of $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy for PHLF in PHC patients is evaluated in Chapter 9. Besides liver failure, overall morbidity and mortality rates following hepatic resection for PHC are high. Adequate preoperative selection of patients is therefore essential and a general surgical risk-score to predict postoperative mortality is validated in a cohort of PHC resections in Chapter 10.

In the last decade, bile acids (BAs) have been identified as crucial mediators of liver regeneration through activation of the nuclear BA farnesoid X-receptor (FXR) in both liver and intestine (Figure 1). The importance of the enterohepatic circulation of BAs following resection for liver regeneration is demonstrated in Chapter 11, in which the association of
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postoperative external bile drainage with PHLF is investigated. Following the implications of FXR in many diseases in the field of gastroenterology, potent FXR agonists have been developed, one of which is obeticholic acid (OCA). OCA is modified from a natural occurring BA and thereby gained a ~100-fold increased potency for the FXR receptor. The effect of obeticholic acid on liver regeneration is tested in Chapter 12 by using our rabbit model of PVE. Since especially patients who experience obstructive cholestasis are vulnerable to PHLF, obeticholic acid was tested in a rat model of extrahepatic cholestasis and partial hepatectomy, which is the subject of Chapter 13. Part of the effects of OCA might be attributable to overexpression of fibroblast growth factor 15 (FGF15 or human homologue FGF19). FGF15/19 might have potent anti-cholestatic effects, however, the exact effects and mechanisms are largely unknown. One of the major difficulties in this line of research is the expression of FGF19 in humans under cholestatic conditions, which is not the case in rats. In addition, there are large interspecies differences in the bile acid pool composition. An experimental model of obstructive cholestasis similar to the human characteristics obviously is of major interest. The hamster model characterized in Chapter 14 might be a valuable model to study cholestatic liver disease and develop new treatment modalities.

Figure 1: The enterohepatic circulation with regard to liver regeneration. FXR activation in hepatocytes represses bile acid synthesis by repression of CYP7A1 and CYP8B1. Bile acid export is upregulated through BSEP and OSTβ. In addition, direct stimulation of liver regeneration is effected through Foxm1b. Intestinal FXR activation results in the production of FGF19, which acts on FGFR4 on hepatocytes and exerts similar effects on bile acid homeostasis as hepatic FXR. In addition foxo1 is repressed and though JNK STAT3 and NF-κB are activated which both potentiate liver regeneration.

Another new approach to stimulate liver regeneration is associating liver partition and portal vein ligation for staged hepatectomy (ALPPS, Figure 2). This new type of two-stage hepatectomy induces more extensive and rapid liver growth compared to other procedures.
such as PVE. The unaudited introduction of this new surgical technique was, like many surgical innovations, implemented in clinical practice without any evidence of its safety and efficacy. This had led to several controversies regarding the reported high morbidity and mortality rates, and questionable oncological outcomes. From a physiological point of view, the functional value of the observed rapid increase in liver volume is also debated. These issues are highlighted in Chapter 15. Using again the rabbit model, we examine the effects of parenchymal transection during PVE on the increase in liver volume and function compared to PVE alone in Chapter 16. These results in rabbits are confirmed in patients in Chapter 17, by examining the increase in liver volume and function after ALPPS.

ALPPS has increased technical resectability which could potentially be of value in the treatment of patients with colorectal liver metastases, but its additional value ultimately depends on the oncological outcomes achieved. In Chapter 18, overall survival after ALPPS for very advanced CRLM is compared to a matched cohort of patients who received palliative systemic therapy. ALPPS has also been performed in a limited number of patients with PHC, in whom resection is regarded a high-risk procedure. In Chapter 19, the outcomes of ALPPS for PHC are described and related to the outcomes of standard liver resection for PHC.

Figure 2: Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS). ALPPS is typically used for patients with bilateral colorectal liver metastases (Baseline). During the first stage, the remnant liver is cleared of tumor, the portal vein to the diseased segments is ligated and the liver parenchyma is transected between the future remnant and diseased liver. In the inter-stage interval the remnant liver rapidly hypertrophies and therefore the resection can usually be completed after 7-14 days, in the second stage.

This thesis examines the various conditions that lead to enhancement of liver regeneration in an effort to increase resectability, improve outcomes and ultimately improve survival of patients who require liver resection for cure of their disease. Both experimental and clinical approaches are used with the aim of reducing intra-operative liver damage, improving patient selection, improving liver regeneration, and in the end, making liver resection a safer operation.
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REFERENCES