Assessment and preservation of liver function in hepatic ischemia and reperfusion

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Chapter 1

General introduction and outline of the thesis
Introduction

Ischemia can be defined as 'a condition in which an organ is deprived of sufficient blood supply'. Ischemia is accompanied by lack of oxygen and substrates, essential sources for cellular energy metabolism and a failure to remove end products of metabolism. Since energy is needed for maintenance of cell homeostasis and integrity, the lack of adenosine 5'-triphosphate (ATP) initiates a cascade of damaging effects that leads to cellular dysfunction, cellular and interstitial oedema and ultimately, to cell death. Plasma membrane changes lead to an influx of sodium, drawing with them a volume of water to maintain osmotic equilibrium, resulting in cell swelling. The energy consuming resynthesis of the cytoskeleton will be hampered by the shortage of ATP, resulting in damage to the cytoskeleton. Rupture of lysosomes and release of hydrolases lead to intracellular injury as well as activation of calcium-dependent proteases and phospholipases. Eventually, membrane integrity cannot be maintained and the cell dies.

Although under normal conditions one would try to avoid a state of ischemia of an organ, in hepatic surgery, ischemia is often deliberately introduced to reduce the risk of excessive bleeding. During major liver resections, blood flow to the liver may be temporarily occluded to prevent major blood loss. This maneuver, first described by Pringle in 1908 as a measure to control blood loss in liver trauma, reduces intra-operative blood loss and significantly improves post-operative outcome. A second factor contributing to intraoperative blood loss during resection is back-bleeding from the hepatic veins. This problem can be tackled by occluding the supra- and infrahepatic caval vein in conjunction with vascular inflow occlusion. This technique of total hepatic vascular exclusion (THVE) has been reported to effectively reduce blood loss during extensive resectional procedures of the liver. At the same time, these maneuvers induce ischemia of the liver by vascular inflow occlusion of the portal vein and hepatic artery. Subsequent reperfusion is initiated when the clamps are released and the circulation to the liver is restored, giving rise to ischemia/reperfusion (I/R) injury.

Reperfusion can be defined as 're-establishment of blood flow'. Reperfusion has two beneficial consequences for ischemic tissue; the oxygen supply needed for energy metabolism is restored and toxic metabolites are removed. Although reperfusion is a prerequisite to reverse the progression towards ischemic cell death, paradoxically, it can also enhance liver injury by initiating a mosaic of biochemical processes, in which a number of cells, mediators and enzymatic systems take part. Three important pathways can be recognized: production of reactive oxygen species (ROS), microcirculatory dysfunction and an inflammatory response.
Reactive oxygen species formation:

ROS formation has been the focus of a large body of studies and is considered to be an important contributor to liver I/R injury. Production of ROS takes place at the intracellular and extracellular level. At the intracellular level, the respiratory chain in mitochondria and cytoplasmic xanthine oxidase were identified as the main sources of ROS. During ischemia, xanthine oxidase (XO), which is produced by the conversion of xanthine dehydrogenase (XD), accumulates in the cell. Under non-ischemic conditions, xanthine oxidase converts hypoxanthine to xanthine, releasing superoxide in the process. Therefore, during ischemia, tissue levels of hypoxanthine also increase. With the introduction of oxygen on reperfusion, the reaction proceeds, releasing large quantities of superoxide. However, several studies showed little evidence for enhanced intracellular ROS formation during reperfusion, except after relatively long ischemic periods. Debate about the importance of intracellular ROS production continues since cultured sinusoidal endothelial cells (SEC) and hepatocytes are capable of generating significant quantities of ROS.

Several studies provided evidence that in post-ischemic livers, ROS formation occurs predominantly in the extracellular space. The most potent toxins are hydroxyl radicals (OH•). Under normal conditions, Fe2+ is bound to ferritine. However, superoxide promotes the release of free ferrous iron, which catalyses the Haber-Weiss reaction resulting in hydroxyl radical production. These aggressive oxygen free radicals lead to lipid peroxidation, which results in structural and functional cell damage. However, the contribution of lipid peroxidation to total cell destruction in liver I/R is still controversial.

Other oxygen radicals such as nitric oxide (NO•), superoxide anion (O2•-) and hydrogen peroxide (H2O2) have important physiological functions. The role of NO• as an important determinant in vascular tone has been postulated by several authors and can play an important role in the attenuation of liver I/R injury, although Jaeschke et al. could not show any role for NO in liver I/R injury. Excessive prolonged production of NO contributes to tissue damage in septicaemia, ischemia/reperfusion injury, and other inflammatory conditions.

Microcirculatory dysfunction:

Attempts to reperfuse ischemic tissue might not always be successful due to progressive microcirculatory obstruction, leading to the process called 'no-reflow phenomenon'. Microcirculatory dysfunction is the primary event in the development of liver I/R injury. In the early stages of reperfusion, failure of transmembrane transport mechanisms leads to
intracellular oedema and cellular swelling particularly of the sinusoidal endothelial cells, thereby narrowing the sinusoids. Furthermore, the concentration of endothelin (ET) in plasma and hepatic parenchyma increases during reperfusion, eliciting contraction of stellate cells and hence a further decrease in liver blood flow. The accumulation and consequent stasis of neutrophils in the sinusoids further hamper the hepatic microcirculation by increasing flow resistance. This cessation of flow, leading to intravascular hemoconcentration, constitutes a continuation of the ischemic period.

**Inflammatory response:**

Kupffer cells play a central role as the initial cytotoxic cell type and as a source of many pro-inflammatory mediators. Kupffer cells become activated during liver I/R. Kupffer cells have been postulated as the main sources of ROS in the early reperfusion phase, leading to recruitment of neutrophils into the liver. Furthermore, activation of Kupffer cells leads to the release of inflammatory mediators, such as interleukins (IL-1 and IL-6) and tumor necrosis factor-α (TNFα). TNFα and IL-1 are potent pro-inflammatory cytokines responsible for up-regulation of the expression of adhesion molecules (selectins and B-integrins), giving rise to enhanced leukocyte-sinusoidal endothelial cell interactions. They also play a role in neutrophil chemotaxis and activation, possibly through activation of complement. After ischemia, TNFα and IL-1 appear in plasma only after 5 min of reperfusion. The important role of these mediators in liver I/R injury was confirmed by blockade of the IL-1 receptor, which attenuated oxygen-derived free radical production and microcirculatory disturbances and reduces TNFα production, tissue injury and mortality after hepatic ischemia-reperfusion.

The inflammatory cascade after I/R injury induces the release of platelet activating factor (PAF). PAF is thought to activate neutrophils by promoting the production of TNFα and cytokine induced neutrophil chemoattractant (CINC). Since rats pretreated with a PAF receptor antagonist showed a decreased elevation in TNFα and CINC levels during reperfusion, pre-treatment of rats with a specific PAF antagonist improved hepatic erythrocyte flux and function and decreased hepatocyte and SEC damage. Only Chavez-Cartaya and co-workers failed to show reduced reperfusion injury after normothermic in vivo ischemia in rats with another PAF antagonist.

The complement system, which consists of a number of plasma proteins, is part of the humoral defence system. These proteins circulate in their inactive form and are activated by proteolytic cleavage. Complement activation leads to the generation of opsonins and
anaphylotoxins and the formation of the membrane attack complex. In many organs, ischemia is a crucial initiator of complement-mediated tissue damage \(^{56-62}\). Complement activation has been proposed to play a role in the development of liver I/R injury \(^{63,64}\). Depletion of complement before ischemia \(^{64}\) or blocking of complement activation with either soluble complement receptor 1 \(^{65,66}\) or C1 inhibitor \(^{67}\) attenuated liver I/R injury. The precise mechanism of action has not been elucidated until now, but a role for the stimulation of Kupffer cells and neutrophils has been proposed \(^{64}\). Activation of the classical pathway of complement was found in ischemic myocardium, which was associated with the deposition of C-reactive protein (CRP), suggesting a role for this acute phase protein in the activation of complement in ischemic tissue \(^{68,69}\).

There is no single factor responsible for liver injury after temporary ischemia and reperfusion. One deals with a mosaic of biochemical processes, in which a number of cells, mediators and enzymatic systems take part. Single intervention therapies based on physiological (superoxide dismutase, nitric oxide, catalase), pharmacological (allopurinol, antioxidants) and physical (ischemic preconditioning, hypothermia) approaches have been explored to reduce the consequences of liver I/R injury. However, combined efforts using antioxidants, anti-inflammatory mediators, cytokine receptor antagonists, soluble complement receptors may prove more useful than single target strategies to ameliorate the effects of liver I/R. However, care should be taken to design safe interventions, without completely blocking the natural healthy defence mechanisms.

**Normothermic versus hypothermic ischemia:**

A distinction has been postulated between the onset of injury after normothermic or hypothermic liver ischemia. In *in vitro* experiments, prolonged normothermic ischemia in rat livers resulted in irreversible damage to the SEC in a much earlier stage than in parenchymal cells. These differences were even more marked during cold ischemia \(^{70,71}\). SEC will undergo various changes, such as rounding and detachment from the sub-endothelial plate \(^{72-74}\). Although these changes occur rapidly after the onset of ischemia, the SEC remain viable for long periods of time \(^{75,76}\). Upon reperfusion however, the SEC rapidly die \(^{72,77,78}\). Recently, evidence has been provided that SEC do not die due to necrosis \(^{73,75}\), but due to apoptosis after cold ischemia \(^{76,79}\). After warm ischemia however, the importance of apoptosis is not yet clear. Results presented by Kohli et al. suggested that apoptosis of endothelial cells followed by hepatocytes is an important mechanism of cell death after ischemia/reperfusion injury in the liver \(^{80}\). In contrast, more recently, Gujral et al. showed that apoptosis does not play a significant role in liver I/R injury and oncotic necrosis.
appears to be the principal mechanism of cell death for both SEC and hepatocytes. The precise role of apoptosis in warm liver I/R injury needs further investigation.

Outline of the thesis

The worldwide effort to elucidate the mechanisms involved in I/R injury and the enormous amount of studies published each year have led to the situation in which multiple animal models and anesthetic methods result in large variation in outcome, rendering many of these studies incomparable. And although one study in itself may be very relevant to the issue of liver ischemia and reperfusion injury (I/R), the fact that it is incomparable to other studies makes it less valuable.

In chapter 2, an overview of the world literature is presented involving liver I/R injury using in vivo rat models and a standardised rat model is proposed. Liver I/R research has been conducted in many animal species, all with their species-specific differences in anatomy and susceptibility to liver I/R injury. Chapter 3 describes a number of experimental models in several animal species and discusses their specific differences. In humans, the need for maintenance of body temperature and physiological pH by ventilation during surgery seems obvious. However, in animal experiments, these issues have not received much attention and the importance of maintaining physiological body temperature and pH seems to be underestimated. In Chapter 4 we, therefore, investigated the effect of minor fluctuations in body temperature on liver I/R injury whereas in Chapter 5, the effects of ventilation and hence blood pH on liver I/R injury were studied.

Inappropriate or excessive activation of the complement system can lead to harmful, potentially life-threatening consequences due to severe inflammatory tissue destruction. These consequences are clinically manifested in various disorders, including septic shock, multiple organ failure and hyperacute graft rejection. Genetic complement deficiencies or complement depletion have been proven to be beneficial in reducing tissue injury in a number of animal models of severe complement-dependent inflammation. This led to the concept that therapeutic inhibition of complement is likely to arrest the process of certain diseases. In Chapter 6, an attempt to inhibit liver I/R injury has been made by application of an endogenous, soluble complement inhibitor (C1-inhibitor). Furthermore, the contribution of C-reactive protein (CRP) to CRP-mediated complement activation in liver I/R has been investigated.

Application of prolonged periods of hepatic pedicle clamping in combination with clamping of the supra-hepatic and infra-hepatic caval vein (total hepatic vascular exclusion=THVE) can be necessary to perform more complicated liver resections. Inevitably, concomitant liver I/R injury is introduced and different approaches for reduction in I/R injury
have been attempted, like intermittent clamping of the hepatic pedicle instead of continuous clamping. Since the influence of temperature on I/R injury has been well established, also in relation with liver preservation and liver transplantation, an attempt was made to reduce the consequences of liver I/R injury by infusion of cold Ringer-glucose thereby decreasing core liver temperature by 10°C during THVE in Chapter 7. Chapter 8 focuses on the microcirculatory consequences and production of oxygen free radicals while the liver was being cold perfused in the latter series of experiments.

A major cause of mortality after liver resection is failure of the remnant liver. Therefore, it is important to estimate total and segmental liver function in the work-up of partial liver resection in order to predict postoperative function of liver remnant. At present, indocyanine green (ICG) clearance is considered one of the most reliable function tests. However, it only estimates global liver function whereas a similar function test, hepatobiliary scintigraphy, is capable of providing segmental information of functional liver mass. In Chapter 9, pre-operative assessment of liver function using both the ICG clearance test and hepatobiliary scintigraphy were compared in a consecutive series of patients considered for partial liver resection.
References


