Expression and regulation of ABC transporter genes during liver regeneration
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Summary and general discussion
Important physiological functions of the liver include the metabolism and disposal of (potentially) toxic endo- and xenobiotics. These compounds are secreted by the liver for removal via bile or urine. This secretion involves specific transport proteins that, in most cases, belong to the ATP-binding cassette (ABC) transporter superfamily. ABC transporters have the capacity to actively transport compounds out of the cell against steep concentration gradients. In this way, liver cells lower the intracellular concentrations of potentially toxic compounds. High expression levels of specific ABC transporters can thus protect the cell from damage.

The liver has the capacity to regenerate after loss of tissue. A commonly used model to study liver regeneration in experimental animals is the surgical removal of ∼70% of the liver (partial hepatectomy, PHx). Under these circumstances, the remaining liver cells will start to replicate but at the same time must maintain their normal metabolic functions as good as possible. In chapter 2 the expression of hepatic transport systems involved in bile formation during liver regeneration after PHx in rats has been studied. Initial studies showed maximal DNA synthesis at 24 hours after PHx. Therefore, transporter expression and bile formation were analyzed in detail at this timepoint. Serum bile salt levels were highly increased after PHx, as a result of the reduced hepatic transport capacity. The expression of hepatic uptake transporters was markedly decreased, limiting the uptake of bile salts into the hepatocyte. The expression of proteins involved in bile salt secretion was, however, maintained at a normal level. As bile flow and bile salt secretion were increased when expressed per gram liver, the remnant liver was not cholestatic. Hepatocytes in the remnant liver showed highly increased levels of Mdr1b mRNA, which may increase the resistance of hepatocytes against products of cellular oxidative stress reactions, such as lipid peroxidation. In addition, a number of cytochrome P450 proteins are known to be down-regulated after PHx, possibly to reduce formation of reactive oxygen species and risk of DNA damage during cell division. Up-regulation of Mdr1b by the cell could be a compensatory pathway to enable transport of substrates, not metabolized via cytochrome P450, directly into the bile.

Liver damage results in activation of hepatic progenitor cells when proliferation of hepatocytes is suppressed. These progenitor cells are located in the canals of Hering, which form the connection between the hepatocytic canaliculi and the bile ductules. Activation of these progenitor cells results in the generation of bipotential oval cells, able to differentiate into hepatocytes or into cholangiocytes. As these oval cells provide a mechanism for the liver to regenerate after severe injury, they must be well protected against toxic damage. High expression levels of specific ABC transporters would be beneficial in this respect. However, ABC transporter gene expression had thus far not been characterized in oval cells. In rats, oval cell proliferation can be achieved by treatment with 2-acetylaminofluorene (2-AAF), which inhibits proliferation of hepatocytes, after which regeneration is induced by PHx. Chapter 3 describes the expression profile of ABC transporters in 2-AAF/PHx-treated rats. In total liver the mRNA levels of Mdr1b were highly increased. In addition, mRNA levels of Mrp1 and Mrp3 were increased. Using immunohistochemistry, we could localize the increased Mdr1b expression to the periportal hepatocytes whereas oval cells expressed Mrp1 and Mrp3.
The oval cells were further characterized by comparing isolated Thy-1 positive cells with isolated cholangiocytes and hepatocytes. It has been shown that Thy-1 expression in the liver is specific for oval cells. This allows the specific isolation of these cells by flow cytometry. Thy-1 positive cells highly expressed Mrp1 and Mrp3 mRNA, while the hepatocyte-specific transporters Mdr2, Bsep, Mrp2, and Mrp6 were minimally expressed. The expression pattern of ABC transporters in Thy-1 positive cells resembled that of cholangiocytes except for Abca1, which was expressed in Thy-1 positive cells but not in cholangiocytes. Thy-1 positive cells showed a surprisingly low expression of Mdr1b mRNA, comparable to resting hepatocytes and cholangiocytes. These results are summarized in Figure 1. The rat liver epithelial cell line RLE φ13, often used as a model for hepatic oval cells, was found to retain the ABC expression profile of oval cells by and large, but did have a high mRNA expression level of Mdr1b. Data are interpreted to indicate that hepatocytes are protected by high levels of Mdr1b, while oval cells are protected by high levels of Mrp1 and Mrp3. Via Mrp1, the latter cells are able to efficiently remove products of oxidative stress reactions such as GSSG or the GSH conjugate of 4-hydroxynonenal. High expression of Mrp3, on the other hand, allows for the export of bile salts and glucuronidated compounds.

Hepatic progenitor cells are also activated in various human liver diseases, for instance during viral infections. In chapter 4, we studied the expression of ABC transporters in human liver specimens from patient diagnosed with primary biliary cirrhosis, chronic hepatitis C, or submassive liver cell necrosis and compared these with normal liver. By using dilution series of specific antibodies, we were able to quantify the degree of up- or down-regulation of protein expression. In normal liver, hepatocytes and cholangiocytes expressed MDR1. MRP3 was expressed in cholangiocytes and in hepatocytes surrounding the central vein. MRP2 and BSEP expression was, as expected, hepatocyte-specific. There
was no detectable expression of MRP1. Under conditions of regeneration after massive hepatocyte loss, remaining hepatocytes expressed high levels of MDR1, MRP1, and MRP3. Expression of MRP2 and BSEP was only decreased in hepatocytes with severe cholate stasis. At the same time, expression of MDR1, MRP1, and MRP3 was highly increased in ductular structures close to the parenchyma. It has been postulated that these ductules form a reservoir in which toxic bile can accumulate. MRP3 may function as a basolateral transporter, extruding bile salts back into the systemic circulation.

A striking difference in ABC transporter gene expression between the rat model described in chapter 3 and the human liver specimens studied in chapter 4 relates to the expression pattern of Mdr1b versus MDR1. Whereas progenitor cells in rats do not show high expression levels of any of the P-glycoproteins, MDR1 is highly expressed in human progenitor cells. This may be related to the severity of liver damage, as the human liver specimens were obtained from patients with end-stage liver disease. In addition, MDR1 levels in humans are in general higher than the Mdr1a/Mdr1b counterparts in laboratory animals.

The most pronounced effect of PHx in rats was on the expression of Mdr1b (chapter 2). In chapter 5, we aimed to gain insight in the regulatory mechanism involved in this induction. As the cytokine tumor necrosis factor-alpha (TNF-α) has an essential role in liver regeneration after PHx, we hypothesized that Mdr1b expression after PHx was, at least in part, induced by TNF-α. We developed an in vitro system, using cultured primary rat hepatocytes or a rat hepatoma cell line, in which Mdr1b mRNA levels could be induced by TNF-α. We selectively used constructs expressing dominant negative Fas-associated death domain protein (FADD), TNF receptor associated factor-2 (TRAF2) or IκB to inhibit pathways downstream of TNF receptor-1. Inhibition of NF-κB activation prevented induction of Mdr1b gene expression by TNF-α, demonstrating the essential role of NF-κB in the induction of Mdr1b by TNF-α. Because p53 is up-regulated by TNF-α in an NF-κB-dependent manner and the Mdr1b promoter contains a p53 binding site, we used liver cells expressing a dominant negative p53 to show that TNF-α up-regulation of Mdr1b is independent of functional p53. Using transient transfection assays we were able to show that Mdr1b up-regulation correlated with activation of the promoter. Mutation of the NF-κB site in the Mdr1b promoter prevented its induction by TNF-α, showing that activation of the rat Mdr1b gene by TNF-α is a direct result of NF-κB binding to the promoter.

From these studies we conclude that there is increased expression of specific ABC transporters in different hepatic cell types during liver regeneration. Proliferating hepatocytes express high levels of Mdr1b. Likewise, hepatocytes in severely damaged livers express Mdr1b/MDR1. Rat hepatic oval cells express high levels of Mrp1 and Mrp3, whereas human progenitor cells express high levels of MDR1, MRP1, and MRP3. We hypothesize that expression of these transporters help these cells to withstand the unfavourable conditions associated with severe liver damage. Understanding the protective function of these transporters, together with elucidation of the regulatory mechanisms involved, may contribute to the development of novel therapies for severe liver diseases.