Towards treatment of cholestatic liver disease in children via interference with transcriptional regulation of hepatic transport systems
Mulder, Jaap

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APPENDICES

ENGLISH SUMMARY

NEDERLANDSE SAMENVATTING

LIST OF ABBREVIATIONS

DANKWOORD

CURRICULUM VITAE

BIBLIOGRAPHY
The liver performs many different functions. Besides detoxification of waste products and their subsequent elimination, the liver plays a central role in whole body metabolism and produces a wide range of proteins and other substances involved in e.g., blood coagulation, immunological responses and hormonal actions. Another important function of the liver is the formation of bile. Bile is produced by liver cells (“hepatocytes”) and secreted into a specialized network of minute canaliculi which merge into larger ducts and eventually into the common bile duct. The latter drains into the small intestine. Bile contains a high concentration of bile salts. These detergents (“soap-like”) are required for optimal digestion and absorption of nutrients by the intestine, most importantly for fats and fat-soluble nutrients (e.g. vitamins A, D, E and K). Besides bile salts, bile also contains waste products which can be excreted via the feces.

Impairment of bile formation or bile flow is called “cholestasis”, which has been derived from the Greek words χολη (= “bile”) and στασις (= “stoppage”). Cholestasis can be caused in the liver cells (“hepatocellular cholestasis”) or in the bile drainage system (“obstructive cholestasis”). The consequences of cholestasis is a shortage of bile salts within the intestine and accumulation of bile components in liver and the rest of the body. The former will cause maldigestion and malabsorption of fats and fat-soluble nutrients and possibly nutritional deficiencies. The latter is exemplified by the emergence of jaundice. Bilirubin, a breakdown product of red blood cell hemoglobin, is normally excreted into bile, but can accumulate in the skin and sclera during cholestasis.

As already mentioned, bile salts are detergents. Similarly to their ability to solubilize dietary fats (or lipids) in the intestine, bile salts can also solubilize lipids in the walls of liver cells. This can lead to liver cell injury and death, ultimately leading to impaired liver function (i.e., reduced bile formation, protein synthesis and detoxification). The latter can be defined as cholestatic liver disease.

The above illustrates that bile salts can act as double-edged swords. Bile salts are on one hand indispensable for absorption of essential nutrients, but are on the other hand potentially harmful for the liver and the body. Therefore, local concentrations of bile salts need to be tightly controlled. Several mechanisms exist to provide such a level of control. One mechanism is transcriptional regulation, i.e. regulation of the expression of genes encoding proteins which are involved in bile salt transport and synthesis. Research over the past decade has shown that nuclear receptors (NRs) play an important role in the transcriptional regulation of bile salt homeostasis. NRs are proteins whose activity as transcription factors is regulated by binding of specific compounds (so-called “ligands”). Hence NRs are also called “ligand-activated transcription factors”. Well-known examples of NRs are the classic hormone receptors, e.g. those for glucocorticoids or sex hormones. More recently, it has become clear that the NR family is larger and also includes receptors which regulate intracellular...
processes. The farnesoid X receptor has been identified as an NR whose activity is regulated by binding of bile salts. When the concentration of bile salts within the liver cell increases, FXR will be activated, which leads to the increased expression of genes involved in bile salt export and the reduced expression of those genes involved in bile salt import and synthesis. This response protects the liver cell against an overload of potentially toxic/harmful bile salts. FXR acts as a “thermostat” keeping the intracellular bile salt concentration within a normal range. Besides FXR, several other NRs regulate the expression of genes involved in bile salt homeostasis and bile formation, e.g. the liver X receptor (LXR) and the peroxisome proliferators-activated receptors (PPARs).

As previously stated, cholestasis has various causes, which can be roughly divided into two categories, i.e. hepatocellular causes and obstructive causes. Examples of cholestatic liver disease in children are biliary atresia and parenteral nutrition-associated cholestasis (PNAC). In biliary atresia, bile ducts are obliterated early in infancy by a thus far not entirely clarified process. Recent evidence suggests that it involves an immunological response against the bile ducts provoked by a (viral) insult. PNAC can occur when patients are fed intravenously, but the exact mechanism behind this condition remains to be clarified too. PNAC is known to occur more frequently in children, specifically in premature infants and in infants with a short bowel (due to congenital intestinal anomalies and/or (subsequent) surgery). Several types of treatments for cholestatic liver disease are available, both pharmacological and surgical, depending on the nature of the type of disease. Unfortunately, not all patients benefit (permanently) from these treatments and many of them are eventually bound for liver transplantation. Although transplantation can be life-saving, the number of suitable donors is limited and the procedure remains associated with significant morbidity and mortality. Thus, there remains a need for new treatment strategies. In the studies described in this thesis, the potential application of NR-ligands to intervene in one specific type of cholestatic liver disease, i.e. inflammation-induced cholestasis (IIC), was investigated.

In chapter 2, the link between NRs and IIC is reviewed. The mechanisms behind bile formation and NR-action are described. Besides their role as “thermostats” within liver cells, NRs are also crucial for basal expression of various transporter proteins and, therefore, for normal bile formation. Since inflammatory processes, e.g., a bacterial infection such as sepsis or a urinary tract infection, can lead to reduced NR-activity, it comes as no surprise that IIC is (in part) mediated by reduced NR-function. However, the feature of NRs that their activity is regulated by ligands, offers the opportunity to search for and design new ligands that can potently and selectively induce NR-activity. Such ligands could be applied therapeutically. One example would be the use of an FXR-ligand. Such a ligand could be used to boost FXR activation in an earlier stage, i.e., at lower bile salt concentrations. Protective mechanisms might therefore be engaged prior to the emergence of (permanent) liver cell injury.
Several NRs have also been shown to possess anti-inflammatory actions, e.g., PPARγ and LXR. These actions were initially demonstrated in macrophages, which are monocyte-derived immune cells. A sub-group of macrophages resides permanently in the liver. These so-called Kupffer cells (KCs) are known to play an important role in the inflammatory cascade leading to IIC, since they secrete an array of inflammatory mediators (cytokines) in response to various infectious/toxic stimuli. These cytokines subsequently act on liver cells and inhibit bile formation. Based on the assumption that suppression of the inflammatory response in KCs by PPARγ- or LXR-ligands would inhibit the cascade leading to IIC, we performed several animal and cell-culture studies, which are described in chapters 3-5. As an animal model for IIC, we injected mice with lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, which provokes a strong inflammatory response in the liver. As a result of LPS-injection, the gene expression of various proteins involved in bile salt transport and bile formation is suppressed. In chapter 3, our studies with PPARγ-ligand rosiglitazone are described. Pre-treatment with this compound was shown to partially inhibit the cascade leading to IIC, but this effect did not appear to occur at the level of the KCs. Rosiglitazone appeared to act within the liver cells and was shown to preserve the nuclear levels of the NR retinoid X receptor (RXR)-α. RXRα is a special NR, since it is the obligate partner for other NRs to be active. The rapid export of RXRα from the nucleus is one mechanism of reduced transporter gene expression in response to inflammatory stimuli. Preservation of nuclear RXRα levels after LPS by rosiglitazone pretreatment was associated with partially preserved hepatic gene expression. In chapter 4, it was shown that pre-treatment of mice with the LXR-ligand, T0901317, also leads to partial preservation of hepatic gene expression after LPS-injection. This effect also appeared to occur within the hepatocytes, but was not associated with preserved RXRα levels. T0901317 was shown to suppress the effects of another, but not NR, transcription factor, i.e., NF-kB. The drawback of T0901317, however, is the unwanted effect of induction of fatty acid synthesis, which leads to the generation of a massively fatty liver. Considering this side-effect and the apparent lack of an anti-inflammatory effect of T0901317 in KCs, we next investigated whether it would be possible to inhibit the inflammatory response of these cells using T0901317. These studies are described in chapter 5. Kupffer cells were isolated from rat liver and treated with LPS. The subsequent inflammatory response could be partially inhibited by pre-treatment with T0901317. Although this anti-inflammatory effect was less potent and more cytokine-selective than that of the well-known anti-inflammatory drug, dexamethasone, our results indicate that KC-specific LXR-ligands or general LXR-ligands which are pharmacologically targeted at KCs are of potential use as a therapeutic strategy in IIC.

Besides affecting the transport of bile salts, inflammatory processes also affect the transport of other bile constituents, e.g., cholesterol. In chapter 6, we describe that inflammation signalling also leads to reduced cholesterol transport from hepatocytes into bile. The underlying mechanism also appears to involve a transcriptional
suppression, since the gene expression of the half-transporters Abcg5 and Abcg8 is suppressed, but the exact mechanism remains to be elucidated. Again, reduced activity of NRs (i.e., LXR and hepatocyte nuclear factor-4α) may be a central mediating mechanism.

*In conclusion,* this thesis shows that NRs can play a mediating role in the generation of cholestasis, most noticeably of IIC, but that modification of their activity using new ligands may also provide new treatment strategies for cholestatic liver diseases. Although additional investigations remain necessary to further explore these new applications of NR-ligands, they may eventually answer to a dire clinical need for new treatment options. Hopefully, this will lead to an improved outcome of children with cholestatic liver disease.