Hepatocellular bile salt transport: Lessons from cholestasis

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MOLECULAR MECHANISMS OF BILE SECRETION

Bile secretion starts with the formation of a primary secretion at the bile canalicular level by both bile salt-dependent and -independent mechanisms (‘canalicular bile’, which accounts for 75% of daily bile production), followed by modifications along the bile ductules and ducts (25% of bile secretion) (1). Canalicular bile is formed by osmotic filtration of water and electrolytes in response to osmotic gradients generated by active transport systems located at the basolateral and canalicular membrane of hepatocytes. The major determinant of canalicular bile formation is the excretion of bile salts into the canaliculus (‘bile salt-dependent’ bile flow). In addition, other (nonbile salt) organic anions and cations, and their conjugates osmotically influence bile flow. Canalicular excretion of glutathione and bicarbonate constitute the major components of the ‘bile salt-independent’ fraction of bile flow, although the contri-
Bile formation begins when the liver synthesizes bile acids from cholesterol (Section 3.1). These compounds are then secreted into the liver canaliculi by a sodium (Na\(^+\))-dependent bile salt transport via NTCP (nucleotide binding site-cotransporter protein (NTCP)) (10,11) and a Na\(^+\)-independent organic anion transporting protein (OATP) (12), which also transports a variety of nonbile salt organic anions (e.g., bromosulfophthalein [BSP], estrogen conjugates) and cations (e.g., ajmalium). Na\(^+\)-dependent bile salt transport via NTCP is driven by a Na\(^+\)-potassium (K\(^+\))-ATPase that generates an inwardly directed Na\(^+\)-gradient. Na\(^+\)-K\(^+\)-ATPase activity in turn depends on the membrane potential generated by a K\(^+\)-channel.

The hepatocyte is a polarized epithelial cell with distinct features in its basolateral (sinusoidal) and apical (canalicular) plasma membrane domains (3-5). Two sinusoidal bile salt uptake systems (located at the basolateral membrane) have been cloned in humans (Figure 1) – a sodium (Na\(^+\))-dependent/taurocholate cotransporter (NTCP) (6) and a Na\(^+\)-independent organic anion transporting protein (OATP), which also transports a large variety of other (nonbile salt) organic anions (e.g., bromosulfophthalein [BSP], estrogen conjugates) and cations (e.g., ajmalium). Na\(^+\)-dependent bile salt transport via NTCP is driven by a Na\(^+\)-potassium (K\(^+\))-ATPase that generates an inwardly directed Na\(^+\)-gradient. Na\(^+\)-K\(^+\)-ATPase activity in turn depends on the membrane potential generated by a K\(^+\)-channel.

The canalicular membrane contains both ATP-dependent and ATP-independent transport systems (4,5,10) (Figure 2). At least four ATP-dependent transport systems (termed ‘export pumps’) have been identified (Figure 2, left): a multidrug export pump (MDR1) for hydrophobic cationic compounds (e.g., anticancer drugs, calcium channel blockers, cyclosporin A, various other drugs); a phospholipid export pump (MDR3) that acts as a ‘phospholipid flipase’; a conjugate export pump (MRP2/cMOAT) that mediates the canalicular excretion of amphiphilic conjugates formed by phase II conjugation (e.g., bilirubin diglucuronide); and a bile salt export pump (BSEP) that is the ‘sister of P-glycoprotein’ (SPGP). In addition, the canalicular membrane also contains ATP-independent transport systems (right). Canalicular bicarbonate secretion is mediated via the chloride/bicarbonate anion (Cl\(^-\)/HCO\(_3^-\)) exchanger isoform 2 (AE2). Cl\(^-\)/HCO\(_3^-\) exchange via AE2 is driven by a chloride ion channel
MOLECULAR MECHANISMS
OF CHOLESTASIS

Cholestasis may result either from a functional defect in bile formation at the level of the hepatocyte (hepatocellular cholestasis) or from an impairment in bile secretion and flow at the level of bile ductules or ducts (ductular/ductal cholestasis). By using molecular probes for hepatobiliary transport systems in experimental and clinical forms of cholestasis, decreased or even absent expression of transporter proteins has been shown, which may explain the impairment of transport functions with subsequent reduction in bile flow and the development of cholestasis (22). Other mechanisms (not discussed here) that may also contribute to cholestasis are disruption of the cytoskeleton and vesicle transport that normally determine the hepatocyte’s secretory polarity, impairment of signal transduction pathways that normally coordinates cell functions in the hepatic lobule via gap junctions and stimulate bile canalicular contractions, and defects in tight junctional structures that lead to the dissipation of osmotic gradients via ‘leaky’ paracellular pathways (22-25).

This review focuses on molecular alterations of hepatocellular transport systems in cholestasis.

Several experimental models of cholestasis have been used to study the mechanisms of human cholestatic liver diseases, such as sepsis-induced cholestasis (endotoxin [lipopolysaccharide (LPS)]-treated rats), oral contraceptive-induced cholestasis or cholestasis of pregnancy (ethinylestradiol [EE]-treated rats) and extrahepatic biliary obstruction (common bile duct ligation [CBDL]) (26,27). Administration of LPS, EE or CBDL to rats results in a marked reduction of mRNA and protein levels of the basolateral NTCP and OATP, as well as MRP2 and BSEP (28-36). Decreased expression of these transporters may explain impaired hepatocellular uptake and canalicular excretion of bile salts and other (nonbile salt) organic anions (eg, bilirubin diglucuronide) in cholestasis. In contrast, the expression of other transport systems such as the MDR1 at the canalicular membrane and MRP-isoforms at the (baso)lateral membrane (MRP1 and 3) increases following CBDL and LPS administration (35-39). Upregulation of these transport systems in cholestasis may be a compensatory mechanism that prevents further accumulation of potentially toxic biliary constituents within cholestatic hepatocytes.

Recent findings suggest that the downregulation of transporter gene expression occurs primarily at the transcriptional level (28,33), and that the reduction in gene transcription may be due to alterations in the quantity or function of regulatory nuclear transcription factors (33). For example, LPS administration in vivo reduces the activity of critical, liver-specific transcription factors (eg, hepatocyte nuclear factor 1) that normally regulate the NTCP promoter, thus providing a molecular mechanism for decreased NTCP gene transcription in a rat model of sepsis-induced cholestasis (33).

These experimental findings in rat models of cholestasis are confirmed by data obtained from clinical forms of cholestasis. For example, mRNA levels of the basolateral bile salt transporter NTCP are decreased in patients with extrahepatic biliary atresia, which subsequently increase if complete biliary drainage by portoenterostomy (Kasai procedure) is performed (40). On the other hand, MDR1 and MDR3 mRNA levels are increased in patients with obstructive cholestasis (41). Expression of the Cl⁻/HCO₃⁻ anion exchanger isoform AE2 is reduced in the livers of patients with primary biliary cirrhosis (42,43). Because Cl⁻/HCO₃⁻ exchange activity contributes to the secretion of both canalicular and ductular bile, decreased hepatic expression of AE2 can lead to impaired bile flow. Upregulation of AE2 mRNA and protein levels has been reported in primary biliary cirrhosis patients treated with ursodeoxycholic acid (UDCA), indicating that the improvement of hepatobiliary excretory function under UDCA treatment may be, in part, mediated by the stimulation of gene expression of defective hepatobiliary transport systems (42,43). UDCA may also increase the number of transport proteins contained in the canalicular membrane by stimulating vesicular exocytosis and targeting transporters to the canalicular membrane (44,45). Future studies need to investigate whether UDCA also stimulates the expression of hepatobiliary transport systems for the canalicular excretion of bile salts and conjugated bilirubin (eg, BSEP, MRP2).

In addition to acquired alterations of hepatobiliary transport systems, hereditary mutations of transporter genes can also result in cholestasis (5,22) (Figure 3). Progressive familial intrahepatic cholestasis (PFIC) is a severe type of cholestatic liver disease found in infants and children that is inherited in an autosomal recessive fashion (46,47). Three types (PFIC 1-3) have been identified. PFIC-1 (also known as Byler disease) is characterized by low gamma-glutamyl transpeptidase (γ-GT) serum levels, elevated serum bile salts, normal serum cholesterol and low biliary bile salt levels. This form of PFIC has been mapped to chromosome 18q21-22 (48). Benign recurrent intrahepatic cholestasis (BRIC), a recurrent cholestatic disorder in adults, also
known as Summerskill syndrome, has also been mapped to chromosome 18q21.22 (49), indicating that there may be a familial cholestasis gene that is responsible for both PFIC-1 and BRIC. Recently, a gene encoding a ‘P-type ATPase’ (FIC1) likely to be involved in the enterohepatic circulation of bile salts and mutated in both PFIC-1 and BRIC has been described (50). The mutations were found in different regions of the FIC1 gene, possibly explaining the different phenotypic appearances of PFIC-1 and BRIC.

PFIC-2 is similar phenotypically to PFIC-1, but is different genetically because the gene locus is on chromosome 2q24 (51). PFIC-2 is caused by mutations in the BSEP/SPGP gene (16), resulting in the absence of the canalicular BSEP in the liver of these patients (52).

In contrast to PFIC-1 and PFIC-2, the third subtype, PFIC-3, is characterized by high γ-GT serum levels, as well as bile duct proliferation and inflammatory infiltrates in portal areas. This type of cholestasis is caused by mutations of the MDR3 gene that results in the absence of canalicular MDR3 and a marked reduction of biliary phospholipid levels (53,54). Because phospholipids in bile normally protect bile ductular epithelial cells from bile salt toxicity by the formation of mixed micelles, the marked reduction or even absence of biliary phospholipids may explain bile duct injury in these patients (Figure 4). UDCA may benefit some patients with PFIC-3 by exerting its protective effect from the biliary lumen, presumably by counteracting the toxic effects of other (more hydrophobic) bile salts in bile (55). PFIC-3 provides an important link between a hepatocellular (canalicular) transport defect and the development of cholangiopathies. Many human neonatal and adult cholangiopathies and cholestatic syndromes are being re-evaluated for possible MDR3 defects (56). Of note, patients with primary biliary cirrhosis have normal MDR3 mRNA levels (57), suggesting that decreased MDR3 gene expression is not involved in the pathogenesis of this vanishing bile duct syndrome. Heterozygotes for hereditary transporter mutations may have an increased susceptibility to exogenous cholestatic injuries (eg, drugs, hormones). Of interest, some mothers of PFIC-3 patients had recurrent episodes of cholestasis during pregnancy (54).

Dubin-Johnson syndrome is caused by mutations of the human MRP2 gene that results in the absence of MRP2 in the liver (58,59). Although patients with Dubin-Johnson syndrome are usually hyperbilirubinemic rather than cholestatic, this is yet another important example of how a mutation of a hepatocellular transporter gene can impair bile excretory function. This syndrome is characterized by abnormal biliary excretion of various endogenous and exogenous substances (eg, bilirubin diglucuronide, bromosulphophthalain conjugates, oral cholecystographic agents) that are normally excreted by MRP2 (60).

Cholangiocytes are also the primary cellular target in various cholestatic diseases. Mutations of the CFTR gene result in the impairment of ductal Cl− and water secretion. This defect is associated with mucus obstruction of intrahepatic bile ducts, and can lead to focal areas of biliary fibrosis and cirrhosis. The molecular mechanisms of cystic fibrosis and the pathophysiology of immune-mediated, infectious and drug-induced cholangiopathies have recently been reviewed elsewhere (18,19,61,62).

**SUMMARY AND CONCLUSIONS**

Hereditary mutations in transporter genes or exposure to substances that cause cholestatic injury, such as drugs, hormones or proinflammatory cytokines, result in the decreased or even absent expression of the basolateral and canalicular transport systems (Figure 5). These molecular changes may explain the impaired hepatocellular uptake, and excretion of bile salts and other organic anions in cholestasis. The increasing information on the molecular regulation of hepatobiliary transport systems should bring new insights into the pathophysiology and treatment of human cholestatic liver diseases. Since submission of this manuscript, additional members of the OATP gene family

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**Figure 4** Congenital MDR3 defect in a subtype of progressive familial intrahepatic cholestasis (PFIC-3). Left Under normal conditions phospholipids (PL) in bile protect bile ductular epithelial cells from bile salt (BS) toxicity by the formation of mixed micelles. Right Mutations of the MDR3 gene result in decreased biliary phospholipid levels (broken line). Bile salts are still excreted and cause bile duct injury (cholangitis) further downstream.

**Figure 5** Molecular mechanisms of cholestasis. Decreased expression of hepatobiliary transport systems may result from either hereditary mutations in transporter genes or exposure to cholestatic injury such as drugs, hormones, proinflammatory cytokines and biliary obstruction. Decreased or even absent expression of hepatobiliary transport proteins may explain impaired transport function causing cholestasis.
have been cloned (for a continuous update see http://www.med.rug.nl/mdl/tab3.htm).

REFERENCES

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Hepatobiliary transport

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