Concise Review

From blood to bile: recent advances in hepatobiliary transport

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Abstract

Transport of endogenous and exogenous substances from blood to bile is an essential function of the liver. In the last decade a still growing number of specific transport proteins present at the sinusoidal and canalicular membrane domains of hepatocytes and cholangiocytes have been cloned and functionally characterized. Studies assessing the molecular expression and function of these hepatobiliary transport proteins under different experimental conditions has helped to define the adaptive responses of hepatocytes to certain physiological states and to cholestatic liver injury and to a better understanding of the physiology of bile formation and of the pathophysiology of certain cholestatic diseases. Particularly relevant is the elucidation of the molecular bases of several forms of inherited cholestatic liver disease, which may help to the development of better diagnostic tools or to the design of new therapeutic strategies. In the present review we summarize recent experimental and clinical data involving hepatobiliary transport mechanisms.

Key words: Bile, bile salts, organic anions, transporters, bile secretion.

Bile secretion from the liver has a pivotal physiological role as an excretory route for endo- and xenobiotics and in the digestion and absorption of lipids from the intestinal lumen. Molecular identification and cDNA cloning of liver and intestine membrane transport proteins that determine bile formation have allowed a better understanding of the processes involved in bile formation as well as of the pathophysiology of human cholestatic diseases.1 In this review we summarize the current views on the nature of hepatic transport systems and their regulation under physiological and pathophysiologica conditions.

The physiology of bile formation

Hepatocytes, are polarized secretory epithelial cells with two distinct domains: the basolateral (sinusoidal) domain and bile canaliculus defined by the presence of junctional complexes that establish a sealed apical compartment. Bile is formed from the active secretion of osmotically active compounds by hepatocytes into the canalicular space followed by the passive movement of water through the tight junctions.2 Major osmotically active compounds include organic anions like BS, glutathione, glutathione-conjugated compounds, and glucuronide-conjugated substances and some inorganic anions like bicarbonate and chloride. These solutes are secreted against steep concentration gradients and thus require to be actively secreted. BS are the major solutes in bile and are considered to be the major osmotic driving force in the generation of bile flow.3,4 Once bile is within the canalicular space further modifications occur along the biliary tree due to the presence of active transport systems in the biliary epithelia.5 In the biliary tree bile composition is modified basically by the addition of bicarbonate. Rapid regulation of bile volume and composition can also occur according to changing physiologic needs. BS and other biliary solutes such as undergo enterohepatic cycling due to the presence of active transport mechanism located in the apical pole of enterocytes at terminal ileum.6,7 This allows the retrieval of those substances from the intestinal lumen to the portal circulation and ultimately to the liver for uptake and re-secretion.

Molecular basis of hepatobiliary transport: function of hepatic transport proteins

Several specific transport proteins for biliary constituents have been identified in both membrane domains of the hepatocytes, biliary epithelia and enterocytes. The number of proteins is continuously increasing and the picture is far to be complete. The study of genetically engi-
neered rodents and the search of molecular defects in human cholestatic diseases had provided important clues on the physiological role of several hepatic transporters. An updated version of the transport systems located in the hepatocytes and biliary cells (cholangiocytes) is showed in figure 1. Available information on the properties of these transport systems is summarized below.

**Sinusoidal uptake of biliary solutes:** The sinusoidal membrane of hepatocytes contains a number of carrier proteins that facilitate the entry of BS and other organic anions into the liver. The major cholephilic compounds in sinusoidal blood are BS (BS). Their uptake is mediated by Na⁺-dependent and Na⁺-independent mechanisms, being the sodium dependent pathway responsible for more than 80% of taurocholate uptake. Several polypeptides have so far been cloned from rat and human liver that are able to confer bile salt transport capacity when expressed in mammalian cells. A high affinity bile salt transporter named sodium taurocholate cotransporting polypeptide (Ntcp) and a growing family of multispecific organic anion transporters are the major proteins involved in this step of bile formation.

Ntcp is the major bile salt uptake system in the basolateral membrane of rat hepatocytes. Ntcp is exclusively expressed in liver and strictly localized on the basolateral membrane of hepatocytes. Transfection of the cDNA into mammalian cells confers the capacity to carry out saturable Na⁺-dependent uptake of conjugated and unconjugated BS with similar kinetic parameters to those previously defined in liver basolateral plasma membrane vesicles. Ntcp transports mostly BS being estrone 3-sulfate the only non-bile salt substrate transported at a significant degree.

Several lines of evidence suggest that Ntcp is the predominant and probably the exclusive Na⁺-dependent transporter BS on the basolateral membrane of the hepatocyte. Ntcp’s cDNAs have been identified and cloned from several species other than rat including mouse, rabbit, hamster and human (NTCP). In the mouse two isoforms resulting from alternative splicing have been identified and named Ntcp1 and Ntcp2. Ntcp2 lacks the last 45 amino acid residues compared with the normal or “wild type” Ntcp and its physiological function is unknown. Recent studies on the sorting mechanisms of Ntcp suggest that truncated forms of the transporter, like Ntcp2, may loose the fidelity of basolateral membrane sorting and led to intracellular accumulation. Human NTCP is a 349-aminoacid protein with similar substrate specificity than that observed for rat Ntcp but higher affinity for BS. All Ntcp’s use the transmembrane inwardly directed sodium gradient, maintained by the N⁺/K⁺ ATPase located at the sinusoidal membrane of the liver cell.

Na⁺-independent transport systems located at the sinusoidal membrane of hepatocytes have a wider substrate affinity than Ntcp and are able to transport a great variety of organic anions other than BS. Thus, in addition to fulfill the physiological need of taking up unconjugated BS and non-bile salt endogenous organic anions from sinusoidal blood, these transport systems play an important role in the uptake of xenobiotics by the liver. Sinusoidal Na⁺-independent transport of BS and organic anions is mainly mediated by the so-called organic anion transporting polypeptides (Oatp’s). Oatp’s are a family of polyspecific transporters, with overlapping substrate affinity that is able to mediate the sodium-independent uptake of BS,

**Figure 1.** Schematic representation of hepatobiliary transport proteins involved in bile formation. The transport proteins are represented as circles with the arrows showing direction of transport. Ntcp = sodium taurocholate cotransporting polypeptide, oatp1, 2 and 4 = organic anion transporting polypeptides 1, 2 and 4, oct1 = organic cation transporter 1, Bsep = Bile salt export pump, mdr1 and 2 = multidrug resistance P-glycoprotein 1 and 2, Mrp2 and 3 = canalicular multidrug resistance associated protein 2 and 3, FIC1: Familial Intrahepatic Cholestasis type 1 protein, ASBT = Apical Sodium-dependent Bile acid Transporter, CFTR = Cystic Fibrosis Transmembrane conductance Regulator, AE2 = Anion Exchanger, BS⁺ = bile salts, OA = Organic Anions, PC = Phosphatidylcholine.
particularly unconjugated species. In addition, Oatp’s mediates the uptake of a large number of other compounds differing in charge and structure. These compounds include bromosulphalein, thyroid hormones, cardiac glycosides, neutral steroids and numerous drugs. Several Oatp’s have been identified in both rat and humans (OATP’s). Carriers with predominant expression in the rat liver include Oatp1, 2 and 4 which are responsible for the majority of sodium independent sinusoidal bile salt transport in the rat liver. Oatp4, is the most recently cloned family member. Data on human liver OATP’s is also growing. Several proteins with similar substrate characteristics have been cloned although some of them are not truly orthologues of the rat gene products. For that reason a different nomenclature has been adopted designing the identified human OATP’s with capital letters from A to E. Three members of the family are predominantly or exclusively expressed in the liver and have similar functions than rat Oatp’s (OATP-A, -B, and -C).

Information on bilirubin uptake from blood into hepatocytes is limited. Sinusoidal membrane transporters belonging to the Oatp’s family transport bilirubin monoglucuronide. However, a transporter of unconjugated bilirubin in the sinusoidal membrane has not as yet been identified. It has been suggested, from in vitro experiments using artificial membranes, that unconjugated bilirubin cross the hepatocyte sinusoidal membrane by a diffusion process.

The uptake of cationic compounds at the sinusoidal membrane of rat hepatocytes is thought to be mediated by the polyspecific organic cation transporter Oct1. Oct1 belongs to a superfamily of transporters including multidrug-resistance proteins, facilitative diffusion systems, and proton antiporters. Oct’s mediate electroneutral transport of small organic cations with different molecular structures, independently of sodium and proton gradients.

Finally, the mechanisms underlying the uptake of other biliary solutes are less clear. However, a major advance has been recently made in the elucidation of the uptake mechanism for high-density lipoproteins (HDL). Since biliary cholesterol originates predominantly from cholesterol present in this lipoprotein this process is relevant for bile formation. Experiments on cloning and characterization of a hepatocyte HDL receptor, the scavenger receptor class A type 1 or SRBI, have suggested that this protein may have a critical role in controlling both serum and biliary cholesterol levels.

**Intracellular transport.** The mechanisms by which biliary solutes are transported across cells remain poorly understood. Available information pertains mainly to the intracellular movement of BS. These solutes undergo rapid monomeric movement to the canalicular pole of the hepatocyte probably bound to intracellular binding proteins. Several proteins have been identified as intracellular bile salt binders. In rat liver 3-hydroxysteroid dehydrogenase represents the major cytosolic bile salt binding protein, whereas in humans the predominant protein is a dehydrodiol dehydrogenase. The current body of evidence does not support a role of vesicular transport in the intracellular movement of BS. Sterol carrier protein 2 and phospholipid transfer protein may be involved in the intracellular transport of cholesterol and phospholipids, respectively but the exact details of their action are not well defined.

**Canalicular transport of biliary solutes:** Transport of biliary solutes across the canalicular membrane of the hepatocyte provides the primary driving force for generation of bile flow and is critical for the excretory function of the liver (i.e. body disposal of endo- and xenobiotics, including drugs). The elucidation of the important physiological role of the so-called ABC (for ATP Binding Cassette) transporters, which function as ATP-dependent export pumps and have a common ATP-binding motif in their protein sequences have led to the identification of at least four ABC transporters in the canalicular membrane which act as unidirectional, ATP-dependent export pumps for BS, amphiphilic anionic conjugates, lipophilic cations and phospholipids. Information on these transport systems is briefly summarized below.

**Bile salt transport.** Secretion of BS takes place against a high osmotic and chemical gradient (~5 µM inside the cell versus 1000 µM in the canalicular space). Gerloff, et al. provided convincing evidence that a novel canalicular ABC transporter, named sister of the P-glycoprotein (sPgp,40), effectively mediates ATP-dependent BS transport when overexpressed in the insect S9 cell line. Consequently to this work sPgp was renamed as Bsep (Bile Salt Export Pump, 20). The rat Bsep is a 160kDa protein closely related to the multidrug resistant (mdr 1 and 2) genes and exclusively located at the canalicular domain of hepatocytes. cDNAs from rat, mouse and humans have been recently cloned. The identification of mutations in the BSEP gene as playing a role in human cholestatic liver disease (see section on “Clinical implications”) provided further support to the concept that BSEP may be the predominant canalicular BS transporter. However, additional bile salt transporters may exist at the canalicular pole of liver cells as suggested by the phenotype seen in mice with targeted inactivation of Bsep gene. Bsep null mice have a dramatic impairment in biliary secretion of taurocholate when challenged intravenously with this bile salt but do not have a major decrease in basal bile secretion. Thus, these data suggest that Bsep is the main export pump for hydrophobic BS and that alternative transport mechanisms exist at the canalicular membrane of hepatocytes.

**Transport of non-bile acid-organic anions:** the canalicular transport of non-bile acid organic anions (including conjugated bilirubin) as well as sulfated and glucuronidated BS, is carried out by a 190kDa protein member of the multidrug resistant protein family (MRP), MRP2. MRPs are hepatocellular ABC export pumps that transport amphiphilic substrates to the extracellular space. MRP2 (previously named canalicular Multispecific Organic Anion
Transporter, cMoat) is the canalicular isoform which was first functionally identified in naturally occurring mutant strains of rats that lack the capacity to excrete several organic anions and conjugated bilirubin. Physiologically relevant substrates of MRP2 include glutathione-S-conjugates such as leukotrienes, monoglucuronosyl-bilirubin, bis-glucuronosyl-bilirubin, 17-β-glucuronosyl-oes-tradiol and glutathione disulfide. Evidence supporting an MRP2-mediated low-affinity transport of reduced glutathione, a major driving force for the so-called bile salt-independent bile flow, have been published.

MRP2 is the best studied representative of the so-far identified members of the MRP transporter family located in hepatocytes. MRP1 is expressed at a very low level in normal liver cells while MRP3 and MRP6 are located, in contrast with MRP2, mainly at the basolateral/lateral membrane of hepatocytes. In addition to MRP orthologs in mammals (human, rat, rabbit, and mouse), MRP family members have been identified in invertebrates such as the nematode Caenorhabditis elegans. The MRP family of proteins may have a role in resistance against nucleoside analogues used in cancer chemotherapy. In the liver, MRP’s seem to play a widespread role in detoxification and in the regulation of paracellular and/or transcellular solute movement from blood into bile. In addition, due to the transport capacity of glutathione conjugates and reduced glutathione they might play a role in the hepatocyte’s defense against oxidative stress.

**Transport of biliary lipids.** Biliary lipid secretion serves as an excretory pathway for body cholesterol disposal and play a major role in the intestinal absorption of dietary lipids through the formation of micelles from biliary phospholipids (mainly phosphatidylcholine). Moreover, a cytoprotective role of biliary lipids against bile acid-induced injury to hepatocytes and biliary cells has been suggested. Secretion of biliary cholesterol and phosphatidylcholine is a very complex process that involves lipid supply to the canalicular pole of hepatocytes from either preformed or neosynthetic hepatic sources, and probably the detergent action of bile and have significant liver pathology characterized by oxidative stress.

**Transport systems in cholangiocytes.** The organic and inorganic components of bile may be significantly modified by an array of absorptive mechanisms on the apical membrane of cholangiocytes. Biliary epithelial cells account for only 3-5% of the overall population of liver cells. However, they may contribute as much as 40% of the daily production of bile depending on the species by adding fluid and electrolytes to canalicular bile. Recently, a wealth of information is accumulating on the biology of cholangiocytes from normal adult rodent and human livers. Particularly relevant is the identification of several specific transport proteins in different membrane domains of biliary epithelia that seem to be relevant for ductal bile formation.
The cystic fibrosis transmembrane conductance regulator (CFTR) mediates secretion of chloride into the biliary tree. This process is tightly coupled to bicarbonate secretion. As chloride exits the cell, the cholangiocyte depolarizes and facilitates bicarbonate entry through the action of an electrogenic sodium-bicarbonate cotransporter. The rise in bicarbonate concentration stimulates the activity of an apical chloride/bicarbonate exchanger, in which luminal chloride is exchanged for intracellular bicarbonate resulting in the secretion of bicarbonate into bile. In addition to CFTR-coupled bicarbonate secretion the apical sodium dependent bile salt transporter (ASBT), recently shown to be present in cholangiocytes, may contribute to ductular secretion through the so-called “colehepatic shunt”. This pathway involves reabsorption of unconjugated BS that first became protonated and once inside the biliary cell act as proton donors promoting the formation and secretion of bicarbonate from carbonic acid. An anion exchanger (AE isoform 2, AE2) can also contribute to bicarbonate secretion.

Finally, it has been demonstrated that cholangiocytes also express isoforms of the membrane water channels, aquaporins, at the apical and/or basolateral domains. This supports the concept that transcellular water transport in biliary epithelia takes place through pore-forming intrinsic membrane proteins.

**Regulation of hepatobiliary transporters: insights for the pathophysiology of cholestasis**

Adaptive responses of hepatocytes to certain physiological states and to choolestatic liver injury includes changes in the molecular expression and function of the hepatobiliary transport proteins. Assessment of the expression of transport proteins in experimental models of cholestasis such as bile duct ligation (obstructive cholestasis) and ethinylestradiol or endotoxin administration (hepatocellular cholestasis) are the most commonly used models. Thus, down-regulation of the expression of Ntcp and Oatp1 has been reported in all three forms of cholestasis. Decreased transcription rates of the Ntcp gene have been observed in obstructive cholestasis and decreased binding activity of a critical nuclear transcription factor required for basal Ntcp gene expression occurs upon the injection of endotoxin. The expression and function of canalicular transporters such as MRP2 and Bsep is also down regulated in both extrahepatic and hepatocellular cholestasis. However, decreased expression of MRP2 is more intense than Bsep. The relative preservation of Bsep expression may contribute to diminish the extent of liver injury produced by bile salt retention. In contrast, studies assessing the expression of other canalicular transport proteins such as mdrlb-P-glycoprotein have shown that this protein is up regulated in obstructive cholestasis. In addition, an up-regulation of a basolateral ABC transporter, MRP3 is seen in bile duct ligation. Interestingly, MRP3 is also up regulated in a non-cholestatic experimental model such the Eisai hyperbilirubinemic rats, which may also be regarded as a compensatory mechanism occurring when function of MRP2 is impaired.

Liver regeneration is also often associated with cholestasis. The underlying molecular mechanisms seem to be related to down-regulation of hepatic transporters. Thus, several studies have assessed the expression of some transport proteins after partial hepatectomy in the rat. Basolateral transporters including Ntcp and Oatp1 and Oatp2 are markedly down regulated during early stages of regeneration. In contrast, protein and mRNA expression of two ABC transporters Bsep and MRP2 remained unchanged. These modifications are transient and returned to control values 7-14 days after partial hepatectomy. The differential regulation of basolateral and canalicular organic anion transporters after partial hepatectomy provides a potential molecular mechanism for regenerating liver cells to protect replicating liver cells by reducing the uptake of BS and maintain biliary secretion of biliary constituents.

Injury that occurs after ischemia/reperfusion (I/R) of the liver is also associated to cholestasis and is a clinical problem in liver transplantation, hepatic surgery with inflow block for trauma and cancer, and various types of shock. Bile production is frequently diminished when livers are reperfused following cold ischemia in patients undergoing orthotopic liver transplantation and, in fact, bile flow appears to be one of the most reliable parameters of hepatic ischemic damage. ATP depletion, alterations in intracellular calcium regulation and the activation of phospholipases and proteases participate as mechanisms of ischemic injury. The reperfusion of the ischemic organ may lead to the aggravation of ischemic injury through the action of reactive oxygen species and other proinflammatory mediators produced by Kupffer cells and neutrophils. In addition to hepatic injury by ischemia and reperfusion, other factors such as graft rejection, immunosuppressive therapy, biliary obstruction and sepsis can contribute to posttransplantation cholestasis. Whether altered expression or function of hepatobiliary transport proteins is affected under I/R of the liver is currently under investigation in our laboratory.

In summary, the molecular expression and function of several hepatobiliary transport proteins is altered under different pathophysiological conditions. Collectively, information on changes of hepatobiliary transporters in experimental models suggests that a general pattern of response take place under conditions where bile secretion is impaired. This response involves down-regulation of the uptake of BS and other potentially toxic organic anions with a relative preservation of bile salt excretion and up-regulation of excretory proteins such as P-glycoprotein and MRP3. Thus, decreased expression of Ntcp and other sinusoidal uptake proteins could represent a protective mechanism to prevent further uptake of BS. On the other hand, preservation of Bsep and...
increased expression of P-glycoproteins could represent a secondary response in an attempt to eliminate potentially toxic substances into bile. Up-regulation of MRP3 may be regarded as a compensatory mechanism occurring when the canalicular secretion of anionic conjugates by MRP2 is impaired. The underlying molecular mechanisms of the above-mentioned changes are unclear. However, recent evidence on the role of nuclear receptors as bile acid “sensors” inside the cell have important implications in understanding the regulation of both bile acid synthesis and transport. Nuclear receptors are able to act in concert to turn on and off bile acid synthesis. They also seem to be key regulators of bile salt transport through controlling the expression of membrane transport proteins regulating the uptake and export of BA in the hepatocyte. This leads to an overall implication that hepatocyte can protect itself from excess of BS by reducing both bile acid synthesis and import.

**Clinical implications**

Molecular studies using experimental models of is rapidly bridging basic science with clinical medicine. The concept that defects in gene expression of membrane transporters may result in cholestasis led to the search of genetic defects that cause or predispose to cholestatic disease. Thus, the identification of specific mutations in the so-called progressive familial intrahepatic cholestasis (PFIC) has established the molecular basis of a clinically important group of cholestatic disorders of infancy. On the basis of clinical findings, clinical-laboratory observations, morphologic studies and genetic analysis, three types of PFIC’s are now recognized. PFIC subgroups types 1 and 2 are characterized by cholestasis and a low to normal serum gamma-glutamyltransferase activity, whereas PFIC type 3 have an elevated serum activity of the latter enzyme.

PFIC type 1 is associated to mutations in a single gene named FIC1 were found. FIC1 gene product is the first human member of a recently defined subfamily of P type ATPases that are involved in ATP-dependent aminophospholipid transport. FIC1 is expressed in many epithelial cells including the liver, the biliary tree and the intestine. Its function is not yet known and could participate in the transport of aminophospholipids from the outer to the inner leaflet of liver or biliary cell membranes. This could be relevant to the regulation of BS transport or the maintenance of the lipid composition of the canalicular membrane but this assumption remains speculative. It has been also reported that some familial forms of recurrent intrahepatic cholestasis are also linked to specific mutations in the FIC1 gene.

PFIC type 2 is related to mutations in the canalicular bile salt transporter (BSEP) gene. Most of mutations result in undetectable BSEP protein on the canalicular membrane which is in line with the very low biliary BS levels seen in these patients. Thus defective canalicular transport result in ongoing liver injury through accumulation of BS inside the hepatocytes.

Patients with PFIC type 3 have different clinical and histological characteristics than those seen in other groups of PFIC’s. A markedly elevated serum gamma-glutamyltransferase and extensive bile duct proliferation and portal fibrosis are hallmarks that resemble the phenotype seen in the mdr2 knockout mice. In fact, genomic DNA analysis of MDR3 gene (which encodes for the canalicular phospholipid transporter) in two PFIC3 patients showed gene mutations resulting in stop codons leading to complete absence of the gene product in the liver. Since phospholipids have a cytoprotective role against bile acid-induced injury to hepatocytes and biliary cells MDR3 deficiency leads toxic damage of these cells because reduced formation of mixed micelles and high concentrations of monomeric BS into the bile.

Information on the occurrence of mutations of specific transporters in PFIC patients has prompted the search of such molecular defects in other forms of cholestatic diseases like adult and neonatal cholangiopathies. Interestingly, MDR3 gene expression is normal in patients with primary biliary cirrhosis suggesting that defective expression of this gene are not involved in the pathogenesis of this disease. It has been also reported that mutations of MDR3 can be associated to recurrent familial intrahepatic cholestasis of pregnancy.

Dubin-Johnson syndrome is another example of how mutations in hepatic transporter affect hepatic excretory function. This syndrome is a rare autosomal recessive disorder characterized by chronic conjugated hyperbilirubinemia and impaired hepatobiliary transport of non-bile salt organic anions which is associated to mutations in the MR2 gene. As previously mentioned (see section on “canalicular transport”), MRP2 mediates ATP-dependent transport of a broad range of endogenous and xenobiotic compounds, including conjugated bilirubin, across the canalicular membrane of the hepatocyte.

In addition to hereditary alterations of hepatobiliary transport systems, data from experimental models of cholestasis suggest that decreased expression of membrane transporters may explain the impaired hepatic uptake and excretion of BS and organic anions seen in several cholestatic conditions. Clinical scenarios such as sepsis- and drug-induced cholestasis, intrahepatic cholestasis of pregnancy and obstructive cholestasis are examples where hepatic transporters can be defective.

Defects in cholangiocyte transport protein may also play a role in some human diseases. Thus, it has been shown that the expression of AE2 is reduced in patients with PBC but not with other forms of cholestasis or liver cirrhosis, suggesting that AE2 alteration is not a secondary effect due to inflammation or cholestasis. In addition, mutations and/or functional defects of CFTR might have a role in acquired cholangiopathies as it occurs in cystic fibrosis.

An interesting and still under-explored area of research is the potential existence of genetic polymorphisms in xe-
nobiotic transporters such as P-gp (MDR1) and MRP2. These polymorphisms may have great impact as cholestasis susceptibility factors as well as factors modulating body drug disposal. Genetic epidemiological evaluation of candidate genes that may predispose to cholestasis is needed.

Concluding remarks

The identification and functional characterization of a growing number of specific transport proteins present at the sinusoidal and canalicular membrane domains of hepatocytes and cholangiocytes represent a great advance in the understanding of the pathophysiology of certain cholestatic diseases. Continuous progress on the field is expected in the next several years which may help to the development of better diagnostic tools or the design of new therapeutic strategies for human liver cholestatic diseases.

References


