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Concise Review

Cholestasis: human disease and experimental animal models

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Abstract

Cholestasis may result from a failure in bile secretion in hepatocytes or ductular cells, or from a blockade to the free bile flow. Human cholestasis may be induced by many drugs, being antibiotics the more common. Other types of cholestasis seen in humans are a group of familial cholestatic disorders, obstructive cholestasis, primary biliary cirrhosis, extrahepatic biliary atresia, primary sclerosing cholangitis, cholestasis of pregnancy, oral contraceptive-induced cholestasis, and sepsis-induced cholestasis. Experimental animal models allow the understanding of pathophysiological mechanisms involved and their clinical correlates. The most common experimental models of intrahepatic cholestasis are estrogen-induced, endotoxin-induced and drug-induced cholestasis. A well known model of extrahepatic biliary obstruction is common bile duct ligation. Drug-induced cholestasis were described using different drugs. On this regard, alpha naphthylisothiocyanate treatment has been extensively used, permitting to describe not only cholestatic alterations but also compensatory mechanisms. Congenital defficiency of transport proteins also were studied in natural rat models of cholestasis. The experimental animal models allow to define down-regulated alterations of hepatocyte transport proteins, and up-regulated ones acting as compensatory mechanisms.

In conclusion, animal model and transport protein studies are necessary for the progressive understanding of congenital and acquired human cholestasis, and regulatory mechanisms that operate on liver cells.

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Bile secretion normally depends on the function of a number of membrane transport systems in hepatocytes and cholangiocytes, and on the structural and functional integrity of the bile-secretory apparatus. Cholestasis (from the Greek *chole*, bile, and *stasis*, standing still) is a bile flow stagnation which may result from a failure in the secretory transport in the hepatocytes or in the ductular cells, or from a blocking in the free bile flow excretory pathway outside the liver.[1,2] The two former are considered intrahepatic, and the latter, extrahepatic cholestasis.

Cholestasis is defined, clinically and biochemically, with varying degrees of jaundice (at the expense of conjugated bilirubin), pruritus and elevated serum levels of alkaline phosphatase, GGT (γ-glutamyl transpeptidase), 5'-nucleotidase, bile acids, and cholesterol. As hydrophobic bile acids are strong detergents they may cause membrane injury and impairment of membrane function. In turn, retained bile acids down-regulate new bile acid synthesis, which results in a reduction of the bile salt pool and of the enterohepatic recirculation. In addition, retention of cholesterol originates increased cholesterol content of membranes that reduces their fluidity and impairs the function of integral membrane proteins.

Cholestasis may be caused by acute or chronic interruption in the mechanism of bile flow generation, and specific transport proteins for biliary constituents have now been identified in membrane hepatocytes and bile duct epithelia.[3] Advances in the molecular cloning of membrane transport proteins that determine bile formation have facilitated studies of the molecular mechanisms of cholestatic liver disease.[4] Moreover, bile salt transport proteins undergo adaptive responses during cholestasis, that serve to protect the liver from bile salt retention and facilitate bile salt excretion through extrahepatic routes.[5]

It seems useful to analyze the causes that lead to cholestasis in humans, and then the experimental animal models that allow a better understanding of human pathophysiology.

Human disease

Intrahepatic cholestasis results from impairment of bile formation by liver cells, whereas obstructive or extra-

hepatic cholestasis results from blockage of bile ducts that carry the bile from the liver to the intestine. Although each process may induce cholestasis, many times a combination of factors is present.

Main causes of adult intrahepatic cholestasis are medications and pregnancy. Currently, antibiotics are the most frequent type of drugs causing intrahepatic cholestasis. Agents known for many years to cause cholestasis include estrogens, cyclosporin A, rifamycin SV, rifampicin, glibenclamide, chlorpromazine, erythromycin, and the oxypenicillins. The spectrum of drug-induced cholestasis ranges from reversible cholestasis to chronic forms as the vanishing bile duct syndrome. Drugs like bosentan-an endothelin antagonist and potentially useful cardiovascular agent-bind to or disable the bile salt export protein and generates a dose-dependent increase in serum bile acids and alkaline phosphatase causing cholestasis; however, little cell injury occurs.[6] Flucloxacilin, an isoxazolyl-penicilin excreted in the bile can injure the human bile duct and also produce cholestasis.[7]

The effects of rifamycin SV and rifampicin on human liver organic anion transporting proteins (OATPs) have been studied. OATP8 and OATP-C represent the major uptake systems for sulfobromophthalein and bilirubin, and OATP8 is the predominantly involved in the hepatocyte uptake of rifampicin in humans. Because OATP8 and OATP-C can transport a wide spectrum of amphipatic organic compounds including drugs and peptides, their inhibition may have wide consequences regarding the toxicity of xenobiotics or the possible prevention of toxic liver injury.[8]

The pharmacokinetics of many drugs is modulated by human canalicular multidrug resistance associated protein 2 (MRP2), and its expression and activity may be altered by certain drugs and disease states. It transports conjugates, cancer chemotherapeutics, uricosurics, antibiotics, leukotrienes, glutathione, toxins, and heavy metals.[9]

Human basolateral multidrug resistance associated protein 3 (MRP3)-which transports bile salts and conjugated compounds from hepatic cells into the blood-is markedly up-regulated during cholestasis. Livers of patients treated with omeprazole showed higher MRP3 protein expression compared with the remainder of the population.[10] MRP3 up-regulation may be important for liver function preservation, since genetic defects in MRP3, in combination with hormones, may promote cholestasis during pregnancy or during treatment with estrogen-containing medications.[11]

The identification of defective transporters in some familial cholestatic disorders has led to improved understanding of the molecular mechanisms of human cholestasis. On this regard, progresive familial intrahepatic cholestasis (PFIC) is a group of severe genetic cholestatic liver diseases of early infancy exhibiting a primary retention of bile salts[12] due to a defect in canalicular bile acid transport. It was seen that PFIC patients with normal GGT levels (type 1) have a defect that can be due to mutations in familial intrahepatic cholestasis type 1 gene (FIC1). In some cases, it was associated with extrahepatic features.[13] PFIC type 2, shows low levels of serum GGT and did not express BSEP in the canalicular domain. However, in PFIC type 3, GGT serum levels are high and the pathophysiology is different. Mutations in human multidrug resistance P-glycoprotein 3 (MDR3)[14]-which translocates phosphatidylcholine-were identified in such patients as responsible, and the analysis of bile showed very low concentrations of phospholipids.

Estrogens can produce intrahepatic cholestasis of pregnancy (ICP) and oral-contraceptive-induced cholestasis.[15] Cholestasis also occurs with androgenic anabolic steroids and in men who received estrogens for therapeutic purposes.[2] In ICP, organic anion transport is reduced during the last trimester of pregnancy and serum levels of bile salts and conjugated bilirubin are usually elevated. This cholestasis is particularly common in Chile and in Scandinavia, and may reflect a genetical predisposition.[2] Moreover, ICP is linked to PFIC type 3 and to other genetic defect, the benign recurrent intrahepatic cholestasis (BRIC), which in turn appears to be related to PFIC type 1.[16]

Basolateral and canalicular bile acid and organic anion transport are markedly impaired in endotoxemia, producing the so-called sepsis-induced cholestasis.[1] Impairment of canalicular secretion of conjugated bilirubin explains jaundice of sepsis and the decreased secretion of bile acids explains a decrease of bile flow during sepsis.[17] Lipopolysaccharides (LPS) in the outer membrane of gram-negative bacteria and LPS-induced cytokines may also impair hepatobiliary excretion during total parenteral nutrition associated intrahepatic cholestasis as well as during alcoholic and viral hepatitis.[2,18,19] It was also reported that cholestasis may be a side effect of cytokine therapy with TNF-α and Il-2 in man.[2]

In some hereditary molecular changes as Dubin-Johnson syndrome, specific mutations in the MRP2 gene result in failure to insert the protein in the apical membrane of the hepatocyte.[20] However, this is a benign disease and biochemical or histologic signs of cholestasis are not seen.

Other causes of human cholestasis are obstructive cholestasis, extrahepatic biliary atresia (EBA), primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), and its variant autoimmune cholangitis.

Patients with PBC, an autoimmune cholestatic liver disease, have normal MDR3mRNA in liver biopsy samples, suggesting that decreased MDR3-gene expression may not be involved.[2]

However, reduced levels of anion exchanger 2 (AE2) immunoreactivity at the bile canaliculus and bile ducts and AE2 mRNA levels in liver tissue have been reported.[21]

Obstructive cholestasis is usually the result of physical obstruction of the biliary system at the level of the extrahepatic bile ducts, often by a stone or tumor. However, stric-

ture of bile ducts or compression due to a chronic pancreatitis also may be responsible. MDR1mRNA as well as MDR3mRNA levels are increased in biopsy material of patients with obstructive cholestasis and are well correlated with serum bilirubin and alkaline phosphatase levels.[2,4] In addition, obstruction or paucity of small bile ducts can result in functional obstruction of the entire biliary system. This may be the mechanism involved in the cholestasis observed in Alagille syndrome, an autosomal dominant disorder characterized by jaundice in early infancy. Molecular mechanisms involved in cholangiocyte function are not well known. However, changes in chronic cholestasis with bile duct proliferation might facilitate reabsorption of canalicular bile salts secreted by hepatocytes, by means of ileal sodium-dependent bile salt transporter located in the apical membrane of cholangiocytes.[4] Therefore, bile salts may reenter the blood and be excreted through extrahepatic routes and thus, the bile salt pool may be regulated. This mechanism is known as the colehepatic shunt.[2]

In patients with EBA, mRNA levels of Na^+-taurocholate cotransporter polypeptide (NTCP) are decreased, and are inversely related to the level of total serum bilirubin. This suggests that NTCP is down-regulated in cholestatic liver disease.[4]

PSC is a chronic cholestatic disease in which intrahepatic or extrahepatic bile ducts, or both, become inflamed and narrowed by scar tissue. OATP expression has been shown to be up-regulated in PSC which might help to minimize hepatic concentrations of potentially toxic compounds allowing their transport out of the hepatocytes.[22]

Changes of expression of transport proteins demonstrated in human cholestasis, are summarized in *table I*.

Experimental animal models allowed the understanding of pathophysiological mechanisms involved in human cholestasis. Since experimental cholestasis originates reduction or suppresion of bile flow, retention of bile constituents leads to membrane damage that further impairs bile secretion, thus establishing a vicious cycle of cell injury. Therefore, a simplistic interpretation of the mechanisms involved is not possible.

Experimental animal models

Different animal especies have been used but rats were the most common. Several treatments were applied, such as endotoxin treatment, estrogen treatment, common bile duct ligation and drug administration. Some studies were made *in vivo* in the whole animal, but *in vitro* studies allowed to give an insight in the mechanisms involved.

Animal treatment

This includes any form of injury to the liver that diminishes the osmotic driving force, resulting in the reduction in the rate of fluid secretion. This leads to the development of cholestasis, which may be detected from measurement *in vivo* of the bile flow and the biliary excretion of bile constituents or test compounds. Determination of endogenous bile constituents retained in blood and morphological studies showing changes in the liver tissue allow diagnosis of the cholestasis.

In vitro studies are necessary to understand the mechanisms involved and their application in human pathophysiology. Experimental animal models comprise the endotoxin-treated rat (a model of sepsis-induced cholestasis)[2,23-29] and the ethinyl estradiol-treated rat (a model of oral contraceptive-induced cholestasis and cholestasis of pregnancy).[30,31] These are the most common models that simulate hepatocellular cholestasis. Some drugs also produce hepatocellular cholestasis and are models for drug-induced cholestasis.[1] Other experimental models derived from the mechanical alteration to the bile flow. They include total common bile duct ligation,[32,33] incomplete biliary obstruction,[34-36] and selective biliary obstruction.[37,38] These are models of extrahepatic biliary obstruction.

In vitro studies

The isolated perfused rat liver, a preparation employed either with re-circulation of the perfusion medium or in a single-pass arrangement, is widely used. It allows to

Table I. Molecular changes of hepatocellular transport systems in human cholestasis

Intrahepatic cholestasis			Extrahepatic cholestasis		
PSC	OATP mRNA	↑	PBC	AE2 mRNA	↓*
PFIC-1	mutation FIC1 gene			AE2 protein	↓
PFIC-2	mutation BSEP gene			MDR3 mRNA	↔
				MRP2 mRNA	↓
PFIC-3	mutation MDR3 gene			MRP2 protein	↓
			BO	MDR1 mRNA	↑
				MDR3 mRNA	↑
			EBA	NTCP mRNA	↓
BRIC	mutation FIC1 gene		BRIC	mutation FIC1 gene	

PSC, primary sclerosing cholangitis; PFIC, progressive familial intrahepatic cholestasis (types 1, 2, 3); BRIC, benign recurrent intrahepatic cholestasis; PBC, primary biliary cirrhosis; BO, biliary obstruction; EBA, extrahepatic biliary atresia; NTCP, Na^+-dependent Na^+-taurocholate cotransporter; OATP, organic anion transporting protein; MRP, multidrug resistance-associated protein; BSEP, bile salt export pump; MDR, multidrug resistance P-glycoprotein; AE, anion exchanger; * hepatocytes and cholangiocytes; FIC1, P-type ATPase; ↑, increased; ↓, decreased; ↔, unchanged.

study the uptake rate, metabolism, biliary excretion and sinusoidal efflux of a test compound. Liver homogenates and membrane-rich microsomal fractions allow the study of metabolism and membrane composition, transport studies and the isolation of membrane proteins. Fresh isolated hepatocytes from treated animals or in primary culture were extensively used either in transport or metabolic studies. However, preparations of isolated liver cells, either freshly prepared or in culture, lack the polarization of the cell in the tissue. The hepatocyte couplet model,[39,40] which maintains polarization, offer a unique opportunity to study *in vitro* the structural and molecular disturbances underlying experimental models of cholestasis, and the mechanisms of hepatoprotection.

a) Endotoxin treatment

Cholestasis of sepsis is mediated by endotoxins, and by inflammatory LPS-induced cytokines such as tumor necrosis factor alpha (TNF-α) and some interleukins (IL-1b, IL-5).[2,41]

Administration of LPS to rodents induces cholestasis by inhibiting bile salt-dependent and bile salt-independent components of bile flow. The uptake and secretion of bile acids and organic anions are impaired in endotoxin-treated rats[23,25] as well as the biliary excretion of glutathione and bicarbonate.[42]

It was demonstrated that TNF-α also decreases bile salt uptake by the hepatocytes and that pretreatment with anti–TNF-α antibodies blocks the cholestatic effect of LPS.[24] The use of plasma membrane preparations also demonstrated that endotoxin and TNF-α administration to rats caused a reduction in taurocholate transport at both sinusoidal and canalicular membrane domains of rat liver.[25]

The isolated perfused rat liver and liver plasma membrane vesicles were applied to the study of maximal transport of bile acids and organic anions at various times after LPS administration. It was observed that basolateral and canalicular bile acid and organic anion transport were markedly altered in endotoxemia.[17]

Intraperitoneal administration of LPS to rats reduced the expression of Na^+-taurocholate cotransporter polypeptide (Ntcp)-the major bile acid uptake system in the basolateral membrane of rat hepatocyte-at both transcriptional and post-transcriptional levels.[4,26,28]

In addition, LPS and LPS-induced cytokines inhibit the nuclear binding activity of transactivators[4] which have been identified in the Ntcp promoter region.[28,43] In turn, bile salts themselves may affect the transcriptional regulation of membrane and cytosol proteins involved in their transport acting as ligands for nuclear receptors.[44] Ntcp down-regulation in endotoxemic-rat liver (and in other cholestatic animal models) may be attributed to an increased intracellular bile acid concentration on the Ntcp gene promoter.[45] Thus, decreased expression of Ntcp could represent a protective mechanism that prevents further Na^+-dependent bile acid uptake.[46] Expression of sinusoidal membrane transporters belonging to the organic anion transporting proteins (Oatps) family is also reduced in endotoxin-treated rats.[2,25,26] Unlike Ntcp and Oatp, Na^+/K^+-ATPase activity of the basolateral membrane of the hepatocyte is increased in endotoxin-induced cholestasis,[4] as well as the molecular expression of multidrug resistance associated protein 1 (Mrp1) and multidrug resistance P-glycoprotein 1b (Mdr1b), which were also upregulated.[29] These upregulatory mechanisms may be considered beneficial to limit cell injury, facilitating the removal of biliary constituents.[2]

It is recognized that the rate-limiting step in bile formation is the active transport of bile salts and other solutes across the canalicular membrane of the hepatocytes.[3,4] Therefore, impairment of canalicular bile salt transporters and other export pumps may have a primary role in cholestasis due to the resulting intracellular accumulation of bile salts and other possible toxic compounds. In this connection, down-regulation of Mrp2-the canalicular multispecific-organic anion transporter-was demonstrated in endotoxin-induced cholestasis.[29,47]

In addition, endotoxin administration produces a fast redistribution of canalicular Mrp2 to an intracellular compartment.[48] Thus, biliary excretion of bilirubin diglucuronide and other compounds is rapidly impaired after endotoxin treatment.

Bile salt export pump (Bsep) mRNA and proteins levels are also diminished in endotoxin treatment,[4,29,49] explaining the impaired bile salt secretion seen in these animals. However, the reduction in the molecular expression of Bsep produced by endotoxin is less marked than that observed for Mrp2 or Ntcp. This suggests that some bile salt excretory capacity might be preserved in endotoxin-induced cholestasis.[4]

b) Ethinyl estradiol treatment

In rats, the administration of ethinyl estradiol, a synthetic estrogen, diminishes bile flow and produces impairment of transport mechanisms in both basolateral and canalicular hepatocyte membranes.[2,50] In such treated animals, the biliary excretion of bile salts, bilirubin and sulfobromophthalein is reduced as well as that of phospholipids, cholesterol and HCO_3^-.[51,52]

By using stereological methods, it was reported that ethinylestradiol treatment to rats results in a decreased sinusoidal membrane surface density.[53] This was in agreement with impairment of sinusoidal transport systems involved in the uptake of cholephilic compounds reported in ethinylestradiol-treated rats.[54]

As observed for endotoxin treatment, expression and function of Ntcp and Oatp1 are down-regulated in this

form of cholestasis.[47,55] In consistence, reductions in the transport maxima for taurocholate were seen in ethinylestradiol-treated rats[56] although the expression of Bsep was relatively preserved, as occurs in endotoxin treatment.

The effect of ethinylestradiol has been attributed to the endogenous estrogen metabolite estradiol-17β-D- glucuronide.[57] This metabolite is one of a family of glucuronide conjugates of the estrogen D-ring that have been shown to reduce bile flow and bile acid secretion in the rat in a dose-dependent and reversible manner.[57] It was also demonstrated that estradiol-17β-D-glucuronide induces in the rat endocytic internalization of Mrp2, which occurs in parallel with decreased bile flow and Mrp2 transport activity.[58]

Ethinylestradiol and its 17β-D-glucuronide administrations increase tight-junctional permeability in rat liver. This increased paracellular permeability allows for the paracellular regurgitation of bile constituents into the blood.[59-61]

c) Obstructive cholestasis

Obstructive cholestasis is usually the result of physical obstruction of the biliary system at the level of the extrahepatic bile ducts. Therefore, bile duct ligation is thought to affect the domain specific expression of canalicular plasma membrane proteins by impairment of the transcytotic vesicular pathway as well as of the functional integrity of tight junctions.[62] The isolated perfused liver using a bile duct obstructed liver preparation demonstrated that during initial bile duct obstruction, bile acid process is not altered, altough ultrastructural alterations occur early.[63]

Typical of obstructive cholestasis is bile plugging of the interlobular bile ducts, portal expansion, and bile duct proliferation in association with centrilobular cholate injury. These changes derived from increased biliary pressure and to the fact that tight junctions are the only anatomic barrier between bile and portal blood.[64]

Total obstruction

This have been extensively studied using the model of common bile duct ligation in the rat. Under this condition, the hepatocellular excretion of bile constituents is markedly impaired allowing its retention within hepatocytes. Membrane alterations are produced rapidly as observed following the relief of short-term biliary obstruction in rats.[65]

Junctional permeability is increased in bile duct-ligated animals leading to a loss of osmotic driving forces due to the reflux of osmotic active compounds into the interstitium.[66] Tight junction functional permeability is affected more severely by bile duct ligation than by ethinylestradiol treatment which does not affect the transcytotic vesicular pathway.[62]

Typical bile ductular reaction is seen after bile duct obstruction in rats.[67,68] It seems that such typical ductular reaction is the result of multiplication of preexisting bile ducts or may be due to elongation of preexisting bile ductules and ducts induced by increased biliary pressure. Ductular reaction has been associated to proliferation and differentiation of stem cells but its true significance is under discussion.[68,69] Ductular proliferation seems to be modulated by cholinergic system.[70]

Functional studies indicated a marked reduction in sodium-dependent bile salt uptake by hepatocytes shortly after bile duct obstruction in the rat. Fresh isolated hepatocytes from bile duct-ligated rats showed a diminished Na^+-dependent taurocholate uptake consistent with down-regulation of functional Ntcp. Oatp1 is also down-regulated after common bile duct ligation.[55] A reduction in the expression of Ntcp protein was also demonstrated in bile duct ligated mice, which supports the concept that down-regulation of Ntcp in cholestasis limits intracytoplasmatic accumulation of potentially toxic bile acids.[71] In contrast, the expression of Na^+, K^+-ATPase at the basolateral membrane was unchanged in extrahepatic cholestasis.[55]

A marked reduction of canalicular Mrp2 protein expression was also demonstrated following bile duct obstruction but Bsep expression was relatively preserved as observed during endotoxin and ethinyl estradiol treatments. This explained the ability of the rat liver to continue to excrete bile salts at reduced rates.[47] In contrast, Mdr1b is up-regulated in obstructive cholestasis.[29] Thus, decreased activity of canalicular transporters for bile acids and organic anions in obstructive cholestasis leads to accumulation of potentially toxic compounds in the hepatocytes. However, increased expression of P-glycoproteins could be a secondary response to eliminate some potentially toxic compounds into bile.[71]

Mrp3 expression is also increased after common bile duct ligation.[7] This could provide basolateral efflux of organic anions like bilirubin explaining the appearance of conjugated pigment in plasma and urine in obstructive cholestasis.[2] Therefore, the reciprocal regulation of Mrp2/Mrp3 provides an alternative mechanism for the excretion of toxic bile salts and other Mrp2 substrates during cholestasis.[7] Although Mrp3 expression persists on proliferated bile ducts, evaluation of the functional expression of cholangiocyte transport systems in cholestasis is less complete.[4]

Partial obstruction

A mild incomplete obstruction of the common bile duct of the rat is accompannied by a slight increase of

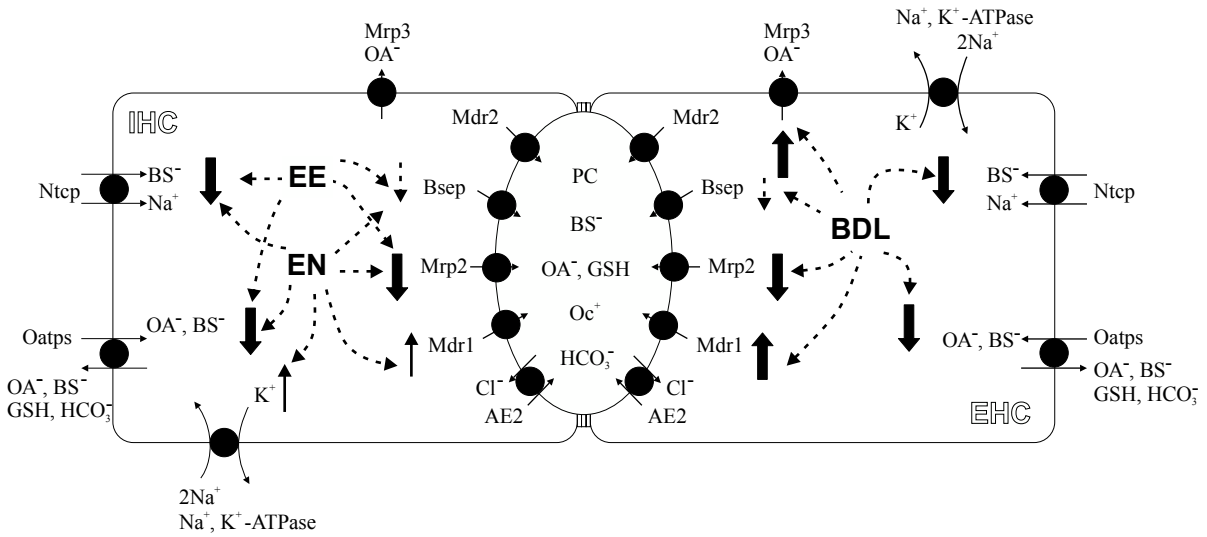

Figure 1. Schematic representation of hepatocyte transport systems in experimental cholestasis.

Circles represent membrane transport systems, with arrows showing direction of transport. IHC, intrahepatic cholestasis; EHC, extrahepatic cholestasis; EN, endotoxin treatment; EE, ethinyl estradiol treatment; BLD, bile duct ligation; Ntcp, Na^+-taurocholate cotransporter polypeptide; Oatps, organic anion transporting polypeptides 1 and 2; Mrp3, basolateral multidrug resistance associated protein 3; Bsep, bile salt export pump; Mdr1 and 2, multidrug resistance P-glycoproteins 1 and 2; Mrp2, canalicular multidrug resistance associated protein 2; AE2, anion exchanger; BS, bile salts; OA, organic anions; OC, organic cations; PC, phosphatidylcholine. Dashed line arrows show transport systems variations (dotted vertical arrow = slight variation; thin vertical arrow = moderate variation; thick vertical arrow = marked variation; downward arrow = down-regulation; upward arrow = up-regulation).

serum bilirubin and mild ductular proliferation, increased volume of portal area and slight portal fibrosis.[35] They were described several changes in that model that suggested an adaptive response of liver.[72] However characterization is incomplete and molecular studies are lacking. This rat model has been applied for obtaining a chronic fibrosing cholangitis.[36]

Selective obstruction

In this rat model, only the bile ducts draining the median and left hepatic lobes were obstructed, whereas those draining the right and caudate lobes remained patent.[37] Although serum alkaline phosphatase, cholesterol and phospholipid were elevated, serum bilirubin increase was only slight. It was speculated that a significant reserve secretory capacity remained available.[38] Studies on membrane function are lacking.
Figure 1 represents changes in hepatocyte transport systems during intrahepatic and extrahepatic models of experimental cholestasis.

d) Drug-induced cholestasis

Despite species differences, animal models of drug-induced cholestasis reveal effects that may useful for interpretation of deffects produced in humans. Current knowledge regarding the function of hepatocyte- and cholangiocyte-transporting polypeptides involved in drug transport helps to understand how their alteration may result in cholestasis.[7] Rat hepatocytes in primary culture were used to demonstrate that neonatal hepatocytes were equally affected by cholestatic drugs to adult hepatocytes.[73] Cyclosporin A, rifamycin SV, rifampicin, and glibenclamide cis-inhibit Bsep-mediated bile salt transport.[74] It was reported that rifamycin SV may inhibit the biliary excretion of sulfobromophthalein and bilirubin *in vivo* in the rat,[75] and of taurocholic acid in the isolated perfused rat liver.[76] More recently it was demonstrated that both rifamycin SV and rifampicin inhibited Oatp1 and 2 in cultured rat hepatocytes.[77]
Troglitazone was found responsible for the interaction with the hepatobiliary export of bile acids at the level of the canalicular Bsep in rats. Such an interaction might lead to a troglitazone-induced intrahepatic cholestasis in humans as well.[78] However, due to its profound hepatoxicity, this insulin sensitizer has been withdrawn from clinical use.[7] Bsep and Mrp2 may be considered a target for drug-induced cholestasis, and *cis*-inhibition of Bsep and Mrp2 may be both produced by some drugs like cyclosporin A.[7] Despite most studies suggest that Bsep is not a drug transporter, it has been shown that Spgp conveys resistance to taxol, and that murine Bsep transports vinblastine which is considered potentially cholestatic.[7] It was reported that high plasma cholesterol levels are seen in drug-induced cholestasis. This increase was associated with enhanced hepatic cholesterol synthesis.[79]
ANIT (1-naphthylisothiocyanate) is a model toxic compound which causes cholestasis in laboratory ani-

mals. ANIT-treatment induces a transient, fully reversible, intrahepatic cholestasis that results in plasma lipoprotein abnormalities associated to those of human hepatic cholestasis and bile duct-ligated rat. It was suggested that ANIT depletes hepatocytes of GSH through a reversible conjugation process which may play a role in the toxicity of ANIT.[80] Decreased levels of Mdr1 and 2 as a consequence of decreased gene expression or targeting of the protein to the canalicular membrane have been postulated for cholestatic animals including those subjected to ANIT treatment.[2] Moreover, ANIT and common bile duct ligation induced expression of P-gp and Mrp3, whereas expression of Ntcp and Oatp1 was reduced by the same treatments.[81]

Taurolithocholate-induced cholestasis is other widely used model for drug cholestasis, because lithocholic acid is a naturally occurring monohydroxylated bile acid.[82-84]

Other animal models

Naturally occurring-transport mutant (TR^-), Groningen yellow (GY) and Eisai hiperbilirrubinemic (EHBR) rat strains have markedly reduced bile flow due to congenital deficiency in Mrp2 function.[2] However, additional ATP-dependent canalicular conjugated export systems are preserved in mutant EHBR rats.[85] In this connection, it was suggested the presence in these rats of compensatory mechanisms responsible for transport of troglitazone metabolites and bilirubin glucuronides at the basolateral and canalicular sites of hepatocytes.[86] The development of mutant mice with targeted inactivation (knockout) (genetic ablation of Mdr1a gene) and double knockout mice (Mdr1a/Mdr1b-/-) are useful to explore the excretion of xenobiotics and endogenous compounds[2] and to provide major insights into the functions of transport systems in the pathogenesis of cholestasis.

In conclusion the progressive increasing knowledge on hepatocyte and cholangiocyte transport systems, mainly regarding as their functions and molecular regulation, will permit a better understanding of the pathogenesis of cholestasis. As showed in this review different alterations observed in experimental animal models were correlated to some changes proved in humans. Therefore, such models are very useful for improving the interpretation of cholestatic human disease, including both hereditary mutations and acquired defects, and the alterations produced by potentially cholestatic triggering agents.

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