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Basolateral and canalicular transport of xenobiotics in the hepatocyte: A review

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Abstract

The molecular and functional characterization of several proteins involved in the uptake and excretion of xenobiotics and endogenous compounds in the hepatocyte has been achieved through intensive research conducted in the past few years. These studies have lead to the identification of specific membrane transporters located in the basolateral and canalicular membrane domains of the hepatocyte. The organic anion-transporting polypeptide (OATP), present in the basolateral membrane of the hepatocyte, is responsible for the translocation of xenobiotics from the sinusoidal space into the hepatocyte. Once inside the cell, unconjugated neutral, anionic and cationic xenobiotics can be secreted into bile by the multidrug-resistance P-glycoprotein 1 (MDR1). Conjugated xenobiotics (e.g. glucuronides and glutathione conjugates) are secreted into bile by the canalicular multispecific organic anion transporter (cMOAT). Other transporters play key physiological roles, including the basolateral uptake of bile salts (sodium-taurocholate cotransporter, NTCP) and the secretion into bile of conjugated and unconjugated bile salts (bile salt export pump, BSEP) and phospholipids (MDR2). Experimental approaches used to investigate the role of the basolateral and canalicular transporters in the hepatocyte have included both *in vivo* and *in vitro* models. Animal models lacking canalicular transporters include the 'hyperbilirubinemic' rats (Groningen-Yellow (GY), Eisai hyperbilirubinemic (EHB) and TR^- rats), which are deficient in the cMOAT protein, and 'knock-out' mice, lacking either the MDR1 or MDR2 transporter. Although no animal models are currently available for the study of basolateral transporters, their function has been conveniently investigated through heterologous expression in *Xenopus laevis* oocytes and also with basolateral membrane vesicles isolated from hepatocytes. The total number of basolateral and canalicular transport proteins present in the hepatocyte is still unknown, but current knowledge indicates that there are at least four present in the basolateral membrane and five in the canalicular domain. The present review focuses on the current knowledge about the most relevant hepatocyte transporters involved in the uptake of foreign and endogenous compounds from the sinusoidal space and in their active secretion into bile. The first part of the review deals with the basolateral (sinusoidal) transport of organic anions, and the major basolateral transporters (e.g. NTCP, OATP) are described here, both in terms of their known biochemistry and physiology. In the second part of the review, the canalicular (apical) transport of organic anions is discussed and the biochemistry and physiological role of MDR1, MDR2, cMOAT and BSEP is described in detail. The concluding remarks point out areas of research that need to be addressed in order to answer important questions that still remain unanswered in this important field of study.

Introduction

Venous blood from the stomach and intestines flows into the portal vein and the liver before reaching the systemic circulation. Therefore, the liver is the first organ exposed to absorbed nutrients, metals, drugs, environmental toxicants and metabolic by-products of bacteria present in the gastrointestinal tract. Efficient scavenging or uptake processes extract these absorbed compounds or elements from the blood for catabol-

ism, storage and/or secretion into bile. The hepatocyte, the parenchymal cell of the liver, comprises about 90% of the cellular volume of the liver and exhibits a clearly defined polarity. The polarity of the hepatocyte is maintained by three different plasma membrane domains that can be recognized both morphologically and functionally. The highly specialized canalicular (or apical) membrane is rich in microvilli and comprises 10–15% of the membrane surface area of the cell; the smooth lateral membrane accounts for 15% of the surface area, and the sinusoidal (or basolateral) membrane, also rich in microvilli, comprises at least 70% of the cell surface (Evans, 1980). It is important to note that although the liver is considered to be the major detoxifying organ, nutrients and xenobiotics are first absorbed across the intestinal epithelial cells. It has become increasingly evident that these cells are capable of metabolizing and conjugating several compounds in a process called 'first pass elimination' or presystemic metabolism (Parkinson, 1996). The present review, however, will focus on the movement of xenobiotics accross the hepatocyte once they have been subjected to this presystemic metabolism.

The first process involved in the hepatic elimination of compounds from the circulating blood is uptake across the basolateral membrane. Many endogenous and xenobiotic compounds are taken up into the hepatocyte by transport systems located in the sinusoidal membrane. Internalized compounds may then be metabolized (primarily by monooxygenases and transferases), and subsequently secreted into bile by primary-active ATP-dependent export pumps located in the canalicular membrane. Bile is a yellow fluid that contains bile acids, glutathione, phospholipids, cholesterol, bilirubin and other organic anions, proteins, metals, ions (bicarbonate, H^+, Cl^-), and xenobiotics. Water, electrolytes, small inert solutes and small proteins can be secreted into bile by the paracellular route (through the intercellular spaces and tight-junctions that connect two hepatocytes), without requiring a carrier protein or a specialized channel (Oude Elferink and Jansen, 1994). The formation of bile is essential not only for the uptake of lipid nutrients from the small intestine but also for the excretion of endogenous and xenobiotic compounds into the lumen of the small intestine. The adequate secretion of bile is also dependent on the functionality of specialized carrier proteins located both in the basolateral and canalicular hepatocyte membrane.

The molecular and functional characterization of the specific proteins involved in the uptake and excretion of compounds by the hepatocyte has been intensively researched in the the last few years. These studies have demonstrated the critical role that these proteins play on the transport of a wide range of endogenous compounds and xenobiotics and also that their genetic absence is the cause of several human and animal diseases. The present review focuses on the current knowledge about the most relevant hepatocyte transporters involved in the uptake of foreign compounds from the sinusoidal space and in their active secretion into bile.

Basolateral (sinusoidal) transport of organic anions

Organic anions represent a group of chemically heterogeneous compounds which possess a carbon backbone and carry a negative charge at physiological pH; they include a large number of endogenous substances (e.g. bile acids, bilirubin, fatty acids) as well as xenobiotics (anionic drugs, carcinogens, food additives and environmental toxins). Solutes and organic anions can enter the hepatocyte through the sinusoidal membrane both by passive diffusion (no ATP required) and by active transport systems. Functionally, two different active transport mechanisms were initially defined: a Na^+-dependent and a Na^+-independent system (Oude Elferink and Jansen, 1994). However, it later became evident that a distinction between a Na^+-dependent (now known as sodium-taurocholate cotransporting polypeptide, Ntcp) and a Na^+-independent system (now called organic anion transporting polypeptide, oatp) was an oversimplification. Currently, the organic anion transporting polypeptides (oatps) are considered to be a family of multispecific transporters, consisting of at least three members (oatp1, oatp2 and oatp3), two of which are expressed in rat liver: oatp1, and oatp2 (Noé et al., 1997; Abe et al., 1998).

Two bile acid transporters located on the sinusoidal membrane of rat hepatocytes were cloned and expressed in *Xenopus laevis* oocytes: the sodium-taurocholate cotransporter (Ntcp) (Hagenbuch et al., 1991), and the organic-anion-transporting polypeptide (oatp) (Jacquemin et al., 1994) (Figure 1). After their isolation, both transporters were also found in human liver (Hagenbuch and Meier, 1994; Kullak-Ublick et al., 1995). In addition to bile acids, oatp transports several organic anions with different chemical structures (e.g. bile acids, bromosulfophthalein (BSP), conjugated steroid hormones and ochratoxin A) and

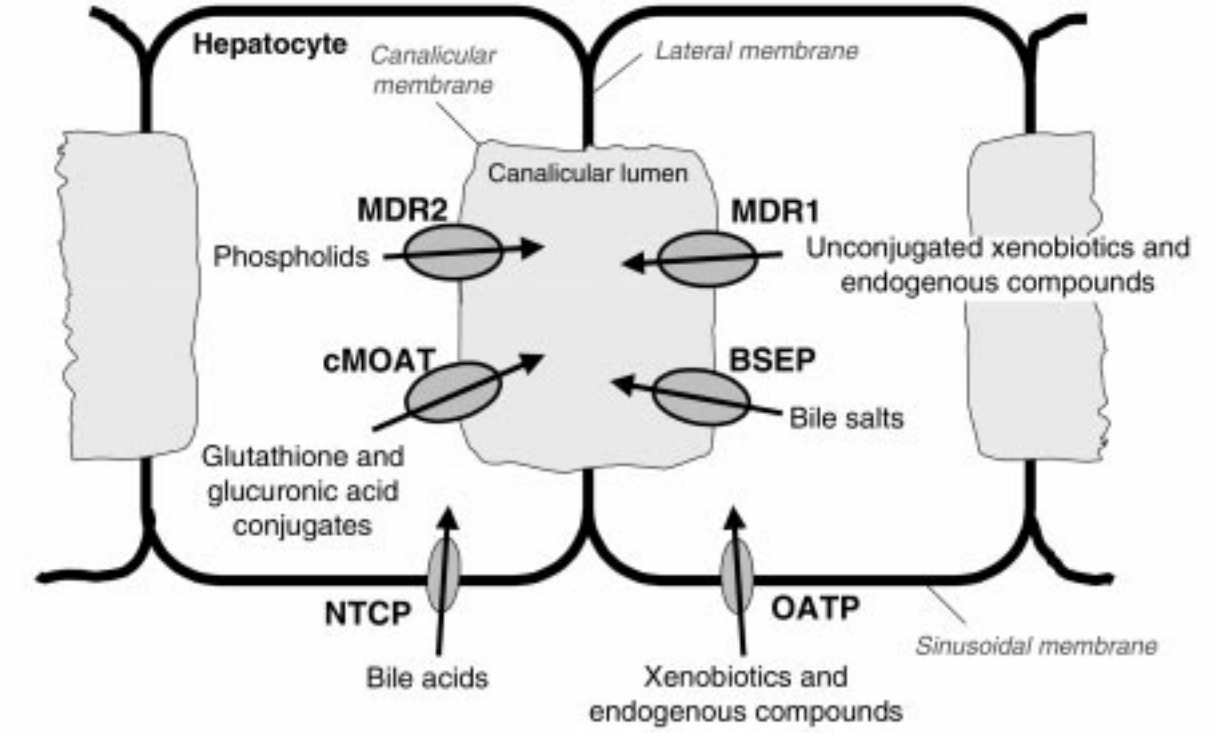

Figure 1. Schematic representation of two adjacent hepatocytes including a bile canaliculus, showing major transport proteins involved in xenobiotic (and bile components) uptake and secretion. NTCP, Na^+-dependent taurocholate transporter; OATP, organic anion transporting polypeptide; BSEP, bile salt export pump; MDR1, multidrug resistance (MDR) P-glycoprotein 1; MDR2, MDR2 P-glycoprotein; cMOAT, canalicular multispecific organic anion transporter.

has therefore been regarded as a multispecific organic anion transporter (Table 1).

Sodium-taurocholate cotransporter (NTCP)

Synonyms: NTCP1; rat Ntcp or Ntcp1

Hagenbuch et al. (1991) isolated a cDNA encoding the rat liver Na^+/taurocholate cotransporting system. The cDNA sequence predicted a 39-kDa polypeptide (362 amino acids) with five potential N-linked glycosylation sites and seven putative transmembrane domains; however, translation *in vitro* and in oocytes indicated that the transporter was a 33- to 35-kDa polypeptide, and its molecular mass increased by approximately 6 kDa after partial glycosylation *in vitro* (Hagenbuch et al., 1991). Using a cDNA probe from the cloned rat liver Ntcp, Hagenbuch and Meier (1994) isolated the human NTCP. This transporter is a 349 amino acid-polypeptide, has a calculated molecular mass of 38 kDa, and exhibits 77% amino acid homology with the rat Ntcp. *In vitro* translation experiments indicated that the protein is glycosylated and has a molecular weight similar to the rat Ntcp (Hagenbuch and Meier, 1994).

The NTCP exhibits a very narrow substrate spectrum, prefers taurine conjugates of bile salts predominantly (Horz et al., 1996; Makowski and Pikula, 1997) and it is not involved in the uptake of xenobiotics.

Organic-anion-transporting polypeptide (OATP)

Synonyms: Rat oatp or oatp1

Using the same expression cloning system as Hagenbuch et al. (1991), Jacquemin et al. (1994) isolated a cDNA encoding a rat liver organic anion-transporting polypeptide (oatp, now called oatp1); this transporter was found to be a single polypeptide (670 amino acids) with a glycosylated molecular mass of 71 kDa, unglycosylated molecular mass of 59 kDa, 4 possible N-linked glycosylation sites and 10 putative transmembrane domains. The human liver OATP was cloned by homology with the rat protein and functionally characterized in *Xenopus laevis* oocytes (Kullack-Ublick et al., 1995). The deduced polypeptide had a 67% homology to the rat counterpart and had similar molecular

Table 1. Major hepatocyte membrane transporters involved in xenobiotic uptake and secretion and in bile formation

Transporter and membrane location	Polypeptide length	Molecular mass (kDa)	Glycosylation sites[a]	Transmembrane domains[b]	Substrates
Basolateral					
NTCP (human)	349	–	–	–	Taurine conjugates of bile salts, unconjugated bile salts.
Ntcp (rat)	362	39–41	5	7	
OATP (human)	670	71	8	12	Conjugated and unconjugated bile salts; anionic dyes (BSP, ICG);
Oatp (rat)	670	71	4	10	ochratoxin A; aldosterone, cortisol, dexametasone; ouabain.
Oatp2 (rat)	661	–	5	12	Taurocholate, cholate; estradiol-glucuronide, estrone-3-sulfate; ouabain, digoxin; thyroxine, triiodothyronine.
NLT (rat)	535	58.8	–	–	Salicylate, acetylsalicylate, dicarboxylates, PGE_2, *p*-aminohippurate.
Canalicular					
MDR1 (human) mdr1a/1b (mouse)	1280	170	1	12	Mostly lipophilic cationic compounds: anticancer drugs (e.g. Vinca alkaloids, anthracycline antibiotics); corticoids (e.g. cortisol, aldosterone); central-acting drugs (e.g. domperidone, phenytoin, morphine).
mdr2 (mouse) (or 3) (human) mdr2 (mouse)	1280	170	1	12	Phosphatidyl choline.
BSEP/spgp (rat)	1321	∼160	4	12	Conjugated and unconjugated bile salts: taurochenodeoxycholate, tauroursodeoxycholate, taurocholate, glycocholate, and cholate.
cMOAT/MRP2 (human)	1545	174		17	Endo- and xenobiotic glutathione and glucuronic acid conjugates (e.g. leukotriene C4, dinitrophenyl-glutahione; glucuronidated bilirubin); bile salt sulfates and glucuronides; anionic dyes; reduced glutathione (GSH) and glutathione disulfide (GSSG); several organic anions.
cMoat (rat)	1541	∼200		12	
cMOAT2/MRP3 (human)	1527				Still unknown.
MLP-2/MRP3 (rat)	1523				

[a] Putative glycosylation sites.
[b] Possible transmembrane domains.

characteristics: 670 amino acids, unglycosylated and glycosylated proteins of 59 and 71 kDa, respectively; however, the human polypeptide had 12 putative transmembrane domains and 8 potential glycosylation sites (Kullak-Ublick et al., 1995) (Table 1). In contrast to NTCP, which transports only bile acids, OATP has been shown to be a multispecific transporter. OATP transports not only unconjugated (cholate, glycocholate) and conjugated (taurocholate, taurochenodeoxycholate, tauroursodeoxycholate) bile acids, but also conjugated steroid hormones (estradiol-17-glucuronide, estrone-3-sulfate), several anionic dyes (BSP, indocyanine green) and the nephrotoxic mycotoxin, ochratoxin A (Jacquemin et al., 1994; Kullak-Ublick et al., 1995; Bossuyt et al., 1996a,b; Sekine et al., 1998). OATP, however, does not transport the more

water soluble substrates *p*-aminohippurate, succinate, aspartate and dinitrophenylglutathione (Bossuyt et al., 1996a). Besides organic anions, rat liver oatp1 and human liver OATP are also capable of transporting neutral and cationic compounds (Bossuyt et al., 1996a,b). The substrate spectrum of oatp1 and OATP include the neutral steroids aldosterone, cortisol and dexamethasone, the neutral cardiac glycoside ouabain and the cationic drug APD-ajmalinium. According to Bossuyt et al. (1996b), OATP represents the previously suggested 'multispecific bile acid transporter', that can also account for polyspecific and polyvalent sodium-independent sinusoidal drug uptake in rat liver.

Other basolateral transporters

Organic anion transporter subtype 2 (oatp2)

Noé et al. (1997) isolated a multispecific organic anion transporting polypeptide (oatp2) that is highly expressed in rat brain, liver and kidney. Oatp2 is a 661 amino acid polypeptide with 12 predicted transmembrane domains, five potential glycoyslation sites and six potential protein kinase C phosphorylation sites. Overall oatp2 exhibits an amino acid sequence identity of 77% to oatp1 and of 73% to the human OATP (Noé et al., 1997). The substrate spectrum of oatp2 is similar to that of oatp1. In functional expression studies with *Xenopus laevis* oocytes, oatp2 mediated uptake of the bile acids cholate and taurocholate, the estrogen conjugates estradiol-17-glucuronide and estrone-3-sulfate, the cardiac glycosides ouabain and digoxin (Noé et al., 1997), and the thyroid hormones thyroxine and triiodothyronine (Abe et al., 1998).

Novel liver-specific transport protein (NLT)

A transporter protein preferentially expressed in liver and located on the sinusoidal domain of the plasma membrane was cloned from a rat liver expression library and named novel liver-specific transport protein (NLT); analysis of the deduced amino acid sequence predicted a molecular mass of 58.8 kDa and 12 α-helical transmembrane spanning domains (Simonson et al., 1994). In subsequent studies, Sekine et al. (1998) demonstrated that NLT is a multispecific organic anion transporter in rat liver. The aminoacid sequence of NLT shows 42% identity to that of renal multispecific organic anion transporter (OAT1), and Sekine et al. (1998) proposed that NLT be renamed OAT2 (organic anion transporter 2); when expressed in *Xenopus laevis* oocytes, NLT mediated uptake of organic anions such as salicylate, acetylsalicylate, PGE_2, dicarboxylates, and *p*-aminohippurate. Expression of NLT was detected in the liver and kidney with much greater levels in the liver (Sekine et al., 1998). Horz et al. (1996) found that the loop diuretic bumetanide is not transported by the Ntcp or by the Na^+-independent organic anion transporting polypeptide (oatp1) and provided physiological evidence for the presence of a third organic anion transporter in rat liver cells. However, the molecular characterization of this putative transporter system was not carried out. Sekine et al. (1998) suggested that NLT may be responsible for the hepatocellular uptake of bumetanide, and therefore may constitute the transporter functionally characterized by Horz et al. (1996).

Canalicular (apical) transport of organic anions

Conjugated and unconjugated xenobiotics, as well as endogenous compounds, are actively secreted into bile through the hepatocyte canalicular membrane by specialized proteins (Figure 1). Interestingly, some of these transporters were first isolated from cancer cells resistant to certain drugs and later found to be normally expressed in the hepatocyte. The first of these canalicular transporters to be isolated and characterized was the multidrug-resistance-1 P-glycoprotein (MDR1) (Juliano and Ling, 1976). Later on, a human multidrug resistance-associated protein (MRP) isolated from a lung cancer cell line, was found to be an isoform of the hepatic canalicular multispecific organic-anion transporter (cMOAT). Both MDR1 and cMOAT (together with several other bacterial and eukaryotic transporters) are members of the ABC transporter (ATP-Binding Cassette) protein superfamily; these proteins have two ATP-binding domains and function as transport ATPases hydrolyzing ATP in conjunction with transporting their substrates through cellular or intracellular membranes (Váradi et al., 1998). Members of this superfamily mediate the primary active translocation of all types of compounds across membranes (Oude Elferink et al., 1997). Although comparison of the amino acid sequence of MDR1 and cMOAT shows only a 15% similarity, their drug substrate specificity resemble each other closely (Kusuhara et al., 1998) (Table 1).

Multi-drug-resistant (MDR) P-glycoproteins

Juliano and Ling (1976) observed that Chinese hamster ovary cells selected for colchicine displayed resistance to a wide range of amphiphilic drugs, and that

the drug resistance phenotype was due to a cell surface glycoprotein which was not present in wild type cells. This glycoprotein was initially thought to affect the permeability of cells, and for this reason Juliano and Ling (1976) designated it the 'P glycoprotein'. Currently, P-glycoproteins (P-gps) are viewed as large cell membrane proteins acting as pumps to extrude drugs from cells at the cost of ATP hydrolysis; P-gps are overexpressed in cancer cells resistant to different hydrophobic drugs, the so-called multidrug-resistant (MDR) cells. P-gp positivity can be evidenced at the time of diagnosis prior to chemotherapy or at relapse after treatment, and has been correlated with treatment failure and poor prognosis in several types of cancer (Fardel et al., 1996). Humans have only one P-gp conferring multidrug resistance (MDR1), whereas mice have two: mdr1a (also called mdr3) and mdr1b (also called mdr1) (Schinkel et al., 1997). Another P-gp present both in humans (MDR3, also called MDR2) and mice (mdr2), is not involved in multi-drug resistance but it is important in the production of bile; this P-gp acts as a flippase (flips compounds from the inner to the outer leaflet of the canalicular membrane) for the extrusion of phospholipids into bile (Oude Elferink et al., 1997). Due to their role as anti-cancer drug extruders, mdr1-type P-gps are important in the intrinsec or acquired resistance against chemotherapy occurring in various cancers (Schinkel et al., 1997). Overexpression of MDR1 appears to be a consistent feature of mammalian cells resistant to multiple anticancer drugs.

Multidrug-resistance-1 P-glycoprotein (MDR1)

Synonyms: P-GLYCOPROTEIN 1; PGY1; GP170; mice mdr1a/1b

As mentioned before, mice have two P-gps involved in multidrug resistance (mdr1a and mrd1b), whereas humans have only one (MDR1). In normal mice, both *mdr1a* and *mdr1b* genes are substantially expressed in the liver and other tissues (Schinkel et al., 1997). Schinkel et al. (1994) generated mice homozygous for a disruption of the *mdr1a* gene (*mdr1a* (–/–) mice) and found that the mice were viable and fertile, appeared phenotypically normal, but had increased sensitivity to the central neurotoxic pesticide ivermectin (100-fold) and to the carcinostatic drug vinblastine (3-fold). Later, the same research group (Schinkel et al., 1997) generated mice genetically deficient in the *mdr1b* gene (*mdr1b* (–/–) mice) and in both the *mdr1a* and *mdr1b* genes (*mdr1a/1b* (–/–) mice). The knock-out mice did not exhibit any abnormalities compared with normal mice since the development, viability, macroscopic and microscopic appearance of major organs, serum clinical chemistry, and hematological and immunohematological parameters were not different in mutant and normal mice.

Mice strains lacking mdr1-type P-gps have been a very useful tool to clarify the function of P-gps *in vivo* and to investigate its substrate specificity. In general, mdr1-type P-gps transport unconjugated endogenous and exogenous compounds, independently of their charge. By comparison of the *mdr1a* (+/+) and (–/–) mice it was observed that the mdr1a P-gp is the major P-gp in the blood-brain barrier and that its absence results in elevated drug levels in many tissues (especially in the brain) and in decreased drug elimination (Schinkel et al., 1994). The spectrum of substrates transported by mdr1-type P-gps is very wide. Using mutant mice strains it was shown that quinidine, ivermectin, and various centrally acting drugs (e.g. domperidone, phenytoin, ondasetron, loperamide, and morphine) are substrates of mdr1-type proteins (Kusuhara et al., 1998); endogenous corticoids like cortisol, corticosterone, and aldosterone (Schinkel et al., 1997), calcium channel inhibitors like verapamil, and calmodulin inhibitors such as trifluoperazine (Garrigos et al., 1997) are also substrates for mdr1-type P-gps. The common characteristics of most of the mdr1 substrates identified so far are: (a) planar structure, (b) high lipophilicity, and (c) neutral or positive charge (Kusuhara et al., 1998). Human MDR1 is the product of the *MDR1* gene. It consists of a 1280 amino acid polypeptide with a molecular mass of about 170 kDa (hence the name GP170), two homologous halves (each containing 6 putative transmembrane segments), and one glycosylation site; MDR1 is not only expressed in the liver but also in other tissues including the adrenal cortex, brush border of renal proximal tubules, and small and large intestine epithelial cells (Kusuhara et al., 1998). In normal human liver, MDR1 was found not only on the canalicular membrane of the hepatocytes but also on the apical surface of epithelial cells in small biliary ductules (Hall et al., 1999). Human MDR1 possibly fulfills the same function(s) of both the mdr1a and mdr1b proteins present in mice (Schinkel et al., 1997). MDR1 substrates include organic cations with high lipophilicity such as *Vinca* alkaloids (vincristine and vinblastine), and anthracycline antibiotics (doxorubicin and doxorubicin-HCl (adriamycin)); however, estradiol-17-glucuronide and methotrexate are

also substrates of MDR1, despite their anionic charge (Kusuhara et al., 1998). The neutral anticancer drug daunomycin (which is also an anthracycline antibiotic) is also a substrate of MDR1 (Benet et al., 1999). Therefore, MDR1 can transport molecules with anionic or cationic charges as well as neutral compounds. This wide substrate spectrum might be related to the fact that MDR1 has several independent binding sites. Garrigos et al. (1997) found at least three separate sites on MDR1 for progesterone, deoxycorticosterone and either vinblastine or verapamil. Garrigos et al. (1997) suggested that the broad substrate specificity for P-gp ATPase modulation and also for its transport function is explained by a multisite model rather than a universal site model.

An interesting relationship between P-gp and a major cytochrome P450 human enzyme (CYP3A) has been established in recent years. Cytochrome P450s 3A4 and 5 and P-gp are expressed at high concentrations in both hepatocytes and upper intestinal enterocytes where they limit the bioavailability of many drugs (Hall et al., 1999). Also, these proteins are induced or inhibited by many of the same compounds and demonstrate a broad overlap in substrate and inhibitor specificities, suggesting that they act as a concerted barrier to drug absorption (Benet et al., 1999). However, Kim et al. (1999) investigated the interrelationship between substrates and inhibitors of human CYP3A and P-gp and concluded that the overlap in substrate specificities appears to be fortuitous rather than indicative of a more fundamental relationship.

Multidrug-resistance-3 P-glycoprotein (MDR3)

Synonyms: P-GLYCOPROTEIN 3, PGY3, MDR2; mice mdr2

Human MDR3 (also called MDR2) is a polypeptide with a 77% amino acid homology with the human MDR1; the mice homolog corresponds to mdr2, which exhibits 75% identity with the human MDR1 (Kepler and Arias, 1997). The structure of the human *MDR3* gene is highly similar to those of the human *MDR1* gene and mouse *mdr1b* gene (Lincke et al., 1991) with open reading frames of about 1280 amino acids, and 12 putative membrane spanning domains, 6 in each half of the protein. Since this protein does not confer multidrug resistance to tumor cells (Oude Elferink et al., 1997), the name 'multidrug-resistance' is not appropriate for this transporter. The physiological role of mdr2 was investigated in knock-out mice, lacking the *mdr2* gene (Smith et al., 1993). Homozygous mice (*mdr2* (–/–)) developed a liver disease that appeared to be caused by the complete inability to secrete phospholipid into bile, while heterozygous mice (*mdr2* (+/–)) did not show liver lesions but secreted about 50% of the normal phospholipid level into bile. These findings lead Smith et al. (1993) to suggest that mdr2 P-gp has an essential role in the secretion of phosphatidylcholine into bile. Further studies demonstrated that mdr2 P-gp functions as a flippase, translocating phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane (Oude Elferink et al., 1997). It is interesting to note that while no disease is caused by the absence of the *mdr1a* or *mdr1b* genes, *mdr2* knock-out mice develop severe liver disease characterized by inflammation of the portal tracts, proliferation of the bile ducts, and fibrosis. The absence of mdr2 function clearly has deleterious effects for the cells lining the biliary tree, possibly due to the lack of phophatidylcholine secretion into bile. Phosphatidylcholine plays an important role in bile, including the solubilization of cholesterol (preventing its crystallization in the biliary tract and the formation of cholesterol gallstones) and the inactivation of the detergent action of bile salts (preventing damage to epithelial cells lining the bile duct and the gallbladder) (Oude Elferink et al., 1997). In humans, MDR3 deficiency causes one type of progressive familial intrahepatic cholestasis (type 3), in which cholestasis is accompanied by elevated serum levels of γ-glutamyl-transferase (De Vree et al., 1998).

Canalicular bile-salt-export pump

Synonyms: Bile salt export pump; BSEP; sister of P-glycoprotein; SPGP

Even though the canalicular secretion of bile salts is a vital function of the mammalian liver, the molecular identification of the ATP-dependent carrier protein was elucitated only very recently. In 1995, Childs et al. (1995) identified a novel gene closely related to the P-gps expressed in the pig and other mammalian liver, which they called Sister of P-glycoprotein (*spgp*). Sequence of the gene showed it to be a member of the ABC family, but its function was unknown. Gerloff et al. (1998) cloned the rat *spgp* and expressed it in *Xenopus laevis* oocytes and in vesicles isolated from transfected Sf9 insect cells, and found that the Sister of P-glycoprotein represents the canalicular bile salt export pump (BSEP) of mammalian liver. Spgp is a 1321 amino acid polypeptide with a molecular mass of $\sim$160 kDa, 12 potential transmembrane

segments and 4 potential glycosylation sites; the protein is localized at the canalicular microvilli and at subcanalicular smooth membrane vesicles of rat hepatocytes (Gerloff et al., 1998). BSEP/spgp mediates the transport of bile salts including taurochenodeoxycholate, tauroursodeoxycholate, taurocholate, glycocholate, and cholate (Gerloff et al., 1998). Progressive familial intrahepatic cholestasis type 2 (PFIC2) is characterized by defective canalicular bile salt secretion in the presence of normal serum cholesterol and normal serum levels of γ-glutamyl-transferase. Strautnieks et al. (1998) found that the SPGP transporter is not functional in patients suffering from PFIC2 due to several mutations in the *SPGP* gene.

Canalicular multispecific organic anion transporter (cMOAT)

Synonyms: Multidrug resistance-associated protein 2; MRP2; cMRP

Analogous to the identification of P-gp, the clues about the identity of the canalicular organic anion transport in the hepatocyte came from the cancer research field. The cloning strategy for the rat c*MOAT* gene was based on the hypothesis that the cMOAT was a liver specific homologue of the so-called human 'multidrug resistance-associated protein' (MRP1) discovered by Cole et al. in 1992. MRP1 is a member of the ABC transporter family which confers resistance to several anticancer drugs but is not expressed significantly in the liver (Cole et al., 1992). Paulusma et al. (1996) cloned the rat cMOAT and found it to be a 1541 amino acid transmembrane protein with a molecular mass of 200 kDa, 12 predicted transmembrane regions and an exclusive canalicular/apical localization. The cMOAT protein was found to be 47.6% identical to MRP1 and was highly expressed in liver and to a much lesser extent in kidney, ileum, and duodenum (Paulusma et al., 1996). Rat cMOAT was independently cloned by another group (Büchler et al., 1996), who named this transporter cMRP (canalicular isoform of MRP). The human counterpart of the rat cMOAT was isolated by Taniguchi et al. (1996), who found it to be expressed in liver but undetectable in other tissues. The human cMOAT protein consists of 1545 amino acids, with a predicted molecular mass of 174 kDa, 17 predicted membrane-spanning domains and two ATP binding cassettes (Kajihara et al., 1998).

Various strains of rats have been shown to be defective in cMOAT as a consequence of heredity and therefore constitute a suitable animal model for the characterization of canalicular organic anion transport. These include the Eisai hyperbilirubinemic (EHB) rat, from the Sprague-Dawley (SD) strain, and the TR^- and Groningen Yellow (GY) strains obtained from the same original Wistar colony (Oude Elferink and Jansen, 1994). These animals have a defect in the hepatobiliary excretion of a broad range of organic anions, including bilirubin glucuronides, glutathione-S-conjugates (e.g. leukotriene C4 and dinitrophenyl-glutathione), 3-OH-glucuronidated and 3-OH-sulfated bile salts, and many others (Paulusma et al., 1996). Büchler et al. (1996) found that cMRP (cMOAT) was not expressed in the liver of the EHB rat and the TR^-/GY mutant. The reason for this lack of expression was given by Paulusma et al. (1996), who found that a one-nucleotide deletion in the cMOAT DNA from TR^-/GY rats results in a frameshift and subsequent introduction of a stop codon. This deletion is responsible for the absence of the gene product from the canalicular membrane and explains the congenital transport deficiency observed in these rats. More recently, Ito et al. (1997) found that the EHB rat cMOAT DNA has a one-nucleotide replacement (GA) at nucleotide 2564, which also results in the introduction of a premature stop codon. Human Dubin-Johnson syndrome is a benign hereditary disease transmitted in an autosomal recesive trait, characterized by conjugated hyperbilirubinemia, an increase in the urinary excretion of coproporphyrin I, lysosomal deposition of black pigment in hepatocytes and prolonged retention of BSP (Paulusma and Oud Elferink, 1997). The syndrome resembles that observed in mutant rats having a defective *cmoat* gene and, in fact, human cMOAT is mutated in patients suffering from Dubin-Johnson syndrome (Kajihara et al., 1998; Paulusma et al., 1997; Wada et al., 1998).

The physiological function of cMOAT has been defined on the basis of its hereditary deficiency in the mutant rat model. Methotrexate ((+) amethopterin, L-MTX) is an anticancer drug, which has two carboxyl groups in its structure, is eliminated mainly by excretion into urine and bile, and undergoes enterohepatic circulation. The biliary excretion of L-MTX was investigated by Masuda et al. (1997) using the mutant EHB rat. After i.v. administration of L-MTX to EHB rats, its plasma dissapearance and biliary excretion was slower than in normal SD rats. ATP-dependence was observed in the uptake of [^{3}H]L-MTX by canalicular membrane vesicles (CMV) prepared from SD rats but not from EHB rats. The ATP-dependent uptake of L-MTX by SD rat CMV showed saturable kinetics

with a K_m of 295 μM. L-MTX competitively inhibited the ATP-dependent uptake of [^{3}H]2,4-dinitrophenyl-S-glutathione, a typical substrate for cMOAT, and the inhibition constant (K_i) of L-MTX was similar to its own K_m. These results suggest that L-MTX is excreted into bile by cMOAT (Masuda et al., 1997), and this study constitutes an example of the experimental approach used currently to investigate the role of cMOAT in the excretion of an organic anion. From the toxicological point of view, cMOAT is perhaps the most important canalicular export pump since many Phase II metabolites are excreted by this transporter. The substrates for cMOAT include glutathione conjugates (e.g. leukotriene C4 and dinitrophenyl-glutathione), glutathione disulfide (GSSG), glucuronide conjugates (including xenobiotic glucuronides and bilirubin glucuronides), and anionic xenobiotics without further metabolism such as BSP, pravastatin and temocaprilat (Makowski and Pikula, 1997; Paulusma et al., 1999). The number of substrates known to be transported by cMOAT is increasing rapidly. Reduced GSH is secreted into bile, where it stimulates bile flow independently of bile salts; therefore, the existence of a GSH transporter had been defined on the basis of functional evidence (Trauner et al., 1998) but its identity was unknown. Recently, Paulusma et al. (1999) found that the cMOAT/MRP2 protein is a low affinity transporter of reduced GSH in rats.

Canalicular multispecific organic anion transporter 2 (cMOAT2/MRP3)

A new cDNA of the ABC superfamily designated cMOAT2/MRP3 was recently isolated; this transporter is mainly expressed in liver and to a lesser extent in colon, small intestine, prostate, and pancreas; the cloned cDNA has an open reading frame coding for 1527 amino acids that is 45% identical to cMOAT1/MRP2 (Uchiumi et al., 1998). The human cMOAT2/MRP3 may function as a membrane transporter in liver, colon and prostate but its role in the transport of specific substrates is still unknown. The human cMOAT2/MRP3 was independently cloned by another group (Kiuchi et al., 1998), who also indicated it to be a 1527 amino acid polypeptide with a 45.9% identity to the human cMOAT1/MRP2, at the amino acid level. The identity of the amino acid sequence of cMOAT2/MRP3 between human and rats was found to be 82.5%, whereas the identity between human cMOAT2/MRP3 and human MRP1 was found to be 56.4% (Kiuchi et al., 1998). One interesting finding reported in this study was that cMOAT2/MRP3 expression is inducible by phenobarbital, a well-known inducer of certain cytochrome P450 enzymes.

Other canalicular transporters: MLP-1 and MLP-2 (rat MRP3)

Two cDNA fragments encoding the carboxyl terminal ATP-binding cassette region were recently amplified by reverse transcription-polymerase chain reaction (RT-PCR) from EHB rat liver (Hirohashi, 1998). These fragments exhibited approximately 70% identity with rat cMOAT and were designated MRP-like proteins (MLP-1 and MLP-2). The full-length cDNA of these proteins code for 1502 and 1523 amino acids, respectively. Northern blot analysis indicated that MLP-1 is expressed mainly in the liver both in normal SD rats and EHB rats, whereas MLP-2 is expressed predominantly in the duodenum, jejunum and colon. Hepatic expression of MLP-2 was only observed in EHB rats but it was induced by ligation of the common bile duct in normal rats. The authors concluded that MLP-1 and MLP-2 might be novel members of the MRP family responsible for the excretion of organic anions and that MLP-2 is an inducible one (Hirohashi et al., 1998). Recent research and sequence alignment analysis indicate that MLP2 is, in fact, the rat homolog of human MRP3 (Kiuchi et al., 1998), and therefore the transporter should be renamed rat cmoat2/MRP3.

Concluding remarks

The molecular and functional characterization of several proteins involved in the excretion of xenobiotics and endogenous compounds in the hepatocyte has been achieved through intensive research conducted in the past few years. These studies have lead to the identification of specific membrane transporters located in the basolateral and canalicular membrane domains of the hepatocyte. The physiological role of the membrane proteins responsible for the basolateral uptake and canalicular secretion of conjugated and unconjugated xenobiotics represent the final step in the metabolism of exogenous compunds, i.e. ‘Phase III’ or excretion. The basolateral membrane transporters, responsible for the uptake of several compounds, have been characterized using the same strategy: heterologous functional expression using *Xenopus leavis* oocytes. Morphologically, these basolateral membrane transporters tend to be smaller in size than the canalicular transporters, and have

less transmembrane segments but more glycosylation sites. Canalicular membrane transporters have been characterized mainly on the basis of their homology with previously identified 'export pumps' present in malignant cells resistant to different anti-cancer drugs. Most canalicular transporters have been cloned using the RT-PCR technique. Several specific substrates transported both by the basolateral and canalicular hepatocyte membrane proteins have been identified and more substrates are identified as more research is being conducted.

The total number of basolateral and canalicular transport proteins is still unknown, but the intensive research being conducted in this field will undoubtedly yield more information regarding the structure and function of each of them. Currently, the nomenclature for the various basolateral and canalicular hepatic transporters is somewhat confusing. For example, some transporters are known by more than 5 different names and one transporter is called 'multidrug resistance-3 P-glycoprotein', even though it is only involved in the transport of phospholipids. This situation is similar to the one that was observed for the nomenclature of cytochrome P450 enzymes (CYP450s), when the different isoenzymes were gradually being identified. It is probable that in the near future a system based on protein/gene homology be developed for all known carrier proteins, analogous to the system developed for CYP450s. At present only human carrier proteins have been classified in such a way (Online Mendelian Inheritance in Man).

Some aspects of the hepatic membrane transport systems remain to be investigated. For example, it is well known that some conjugates formed within the hepatocyte are not excreted into the bile but are excreted in the urine via renal excretion. Urinary excretion of hepatic conjugates implies the existence of 'basolateral export pumps' capable of translocating these conjugates from the hepatocyte into the sinusoidal space; however no information regarding this functionally-defined transporters is available. Another aspect of this field that is still incompletely known is the (co)induction and (co)inhibition of the expression of the different transporters and their physiological relationship with Phase I metabolizing enzymes. The expression of some transporters (e.g. cMOAT2/MRP3 and mdr1) has been shown to be induced by compounds responsible for the induction of some cytochrome P450 enzymes such as phenobarbital. Coinduction of hepatic cMOAT and cytochrome P450 3A4 gene expression was reported in nonhuman primates treated with rifampicin or tamoxifen (Kauffman et al., 1998). Cytochrome P450 and P-gp are induced or inhibited by many of the same compunds (Benet et al., 1999). However, the present knowledge is by far not complete and more research is needed in this area. A complete understanding of the mechanisms of induction/inhibition for membrane transporters and Phase I enzymes is vital since their modulation greatly affect the toxicokinetics of their specific substrates.

Finally, recent studies indicate that myeloid leukemia cells known to be resistant to a number of apoptotic stimuli overexpress MDR1 and cMOAT (Perkins et al., 2000). The interrelationship between apoptosis and the overexpression of extrusion transporters such as MDR1 and cMOAT needs to be fully evaluated.

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