Hepatic drug transporters and nuclear receptors: Regulation by therapeutic agents

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Abstract
The canalicular membrane represents the excretory pole of hepatocytes. Bile is an important route of elimination of potentially toxic endo- and xenobiotics (including drugs and toxins), mediated by the major canalicular transporters: multidrug resistance protein 1 (MDR1, ABCB1), also known as P-glycoprotein, multidrug resistance-associated protein 2 (MRP2, ABCC2), and the breast cancer resistance protein (BCRP, ABCG2). Their activities depend on regulation of expression and proper localization at the canalicular membrane, as regulated by transcriptional and post-transcriptional events, respectively. At transcriptional level, specific nuclear receptors (NRs) modulated by ligands, co-activators and co-repressors, mediate the physiological requirements of these transporters. This complex system is also responsible for alterations occurring in specific liver pathologies. We briefly describe the major Class II NRs, pregnane X receptor (PXR) and constitutive androstane receptor (CAR), and their role in regulating expression of multidrug resistance proteins. Several therapeutic agents regulate the expression of relevant drug transporters through activation/inactivation of these NRs. We provide some representative examples of the action of therapeutic agents modulating liver drug transporters, which in addition, involve CAR or PXR as mediators.

Key words: Drug transport; Biliary secretion; ABC proteins; Multidrug resistance proteins; Nuclear receptors; Constitutive androstane receptor; Pregnan X receptor; Therapeutic agents

INTRODUCTION
Hepatocytes are polarized cells and represent 80% of the liver mass. The basolateral and canalicular membranes differ in their composition and functions and are separated by tight junctions that seal off the bile canaliculi. The basolateral membrane is in contact with the sinusoidal blood. The canalicular membrane represents the excretory pole of hepatocytes. Bile formation is largely dependent on active transport of solutes such as bile acids, glutathione and bicarbonate through the canalicular membrane followed by the passive movement of water. Canalicular excretion is the rate-limiting step of bile formation since biliary constituents are secreted into bile against concentration gradients. The canalicular primary bile is further modified by absorptive and secretory processes along the biliary tree. Considerable species specific differences in bile formation exist, including the contribution of ductular bile and bile acid composition. In mammals, bile is essential for solubilization and digestion of dietary lipids.

The sinusoidal uptake and canalicular excretion of most biliary constituents is mediated by several transport systems expressed at the two polar surface domains of liver cells. Basolateral transport systems are responsible for the translocation of molecules across the sinusoidal membrane, whereas active canalicular transport systems are in charge of the biliary excretion. Numerous transport proteins involved in basolateral transport have been identified including the Na+-taurocholate co-transporting...
polypeptide (NTCP, SLC10A1), organic anion transporting polypeptides (OATPs: SLC2 family), multidrug resistance-associated proteins 1, 3 and 4 (MRP1, 3 and 4; ABC1, 3 and 4), and organic anion and cation transporters (OATs, OCTs: SLC22A family). Canalicular transport of osmotically active solutes, contributing to bile formation, is mediated by MRP2 (ABCC2), the bile salt export pump (BSEP, ABCB11), and the organic anion 2 (AE2, SLC4A2), which are involved in biliary excretion of glutathione and glucuronide conjugates, monomeric bile salts, and bicarbonate, respectively. Biliary elimination of drugs is mediated by the multidrug resistance protein 1 (MDR1, ABCB1), also known as P-glycoprotein, MRP2, and the breast cancer resistance protein (BCRP, ABCG2). Though they all belong to the superfamily ABC, in contrast to the majority of its members, BCRP is present as a monomer and consists of only one ATP-binding site and 6 transmembrane regions.

In this review we focus our attention only on the hepatic drug transporters and their regulation by nuclear receptors activated by compounds habitually used as therapeutic agents.

**APICAL DRUG TRANSPORTERS AND NUCLEAR RECEPTORS**

Drug transporters are constitutively expressed in several organs playing an important role in the efflux of xenobiotics and their metabolites; the apical membrane of epithelial secretory tissues, and particularly the liver, being the most relevant sites. Substrates recognized by P-glycoprotein, MRP2 and BCRP represent a wide spectrum of endo- and xenobiotics, including contaminants and therapeutic drugs, either neutral, cationic or anionic, and of hydrophobic or hydrophilic nature. P-glycoprotein is a member of the ABC superfamily of transporters originally described in cancer cell lines, conferring resistance to therapeutic agents. It was the first ABC transporter identified in canalicular membranes of normal hepatocytes. MDR1 functions as an efflux pump for a wide range of amphiphilic, bulky type II cationic drugs together with other hydrophobic compounds, including endogenous and xenogenous metabolites or toxins, steroid hormones, hydrophobic peptides and even glycolipids. MRP2 mediates the biliary elimination of different organic anions, including glutathione-S-conjugates (e.g. of leukotriene C4), glucuronides (e.g. of bilirubin and estrogens), and oxidized glutathione. MRP2 also mediates the canalicular transport of glucuronidated and sulfated bile salts. In addition, MRP2 was found to transfer reduced glutathione, though with very low affinity. MRP1 but not MRP2 was the first member of the superfamily of ABC (ATP Binding Cassette) transporters dependent on ATP hydrolysis, and was initially identified in a human lung cancer cell line. BCRP that was initially found to confer resistance to breast cancer treatment was more recently found to be expressed normally in epithelial tissues and transport sulfated metabolites of drugs with high specificity.
receptor (CAR), and farnesoid X receptor (FXR), form heterodimers with the retinoid X receptor (RXR), prior to interacting with target genes. Since RXR is the obligated partner in the heterodimer formation, its low availability may result in a trans-repressive effect. Receptors with no ligand can exist, and have been found to bind DNA as homodimers. They belong to Class III (e.g. RXR, and the nuclear hepatic factor 4, HNF4). Class IV consists of NRs that act as monomers, like the liver receptor LRH1.

We will focus on class II receptors since they represent the best characterized. More specifically, we will briefly describe those receptors involved in regulating drug transporters, i.e. PXR and CAR. Originally, these NRs were identified as sensors able to respond to a wide variety of environmental xenobiotics to promote detoxification by phase I CYP450 genes. Lehmann et al. showed that hPXR receptor binds to the rifampicin/dexamethasone response element in the CYP3A4 promoter region as a heterodimer with the 9-cis-retinoic acid receptor (RXR). They also reported that hPXR is activated by many CYP3A4 inducers, including several steroids, lovastatin, clotrimazole, rifampicin and phenobarbital. Increasingly at present, data reveals the involvement of NRs in the regulation of Phase I and II enzymes, along with the proteins effluxing their metabolites.

PXR

In 1998, Kliewer et al. identified a new member of the nuclear hormone receptor family activated primarily by pregnanes: PXR (NR1I2). It was principally cloned from mouse liver and later from rabbit, rat and human. PXR is predominantly expressed in liver and intestine, and to a lower extent, in lung and kidney. PXR dimerizes with RXRα immediately after its activation by ligand binding. It was originally believed to be localized mainly at the nucleus, but later it was found that it is present at the cytoplasm, interacting with a protein complex and that, after activation, it translocates to the nucleus to regulate gene transcription. One relevant feature of this receptor is that it recognizes a wide variety of xenobiotics such as ligands, dexamethasone, rifampicin, spironolactone, and pregnenolone 16α-carbonitrile being among the best characterized. It can also bind some specific bile acids such as lithocholic, 3-ketolithocholic, cholic and deoxycholic acids. PXR regulates genes involved in phase I metabolism (e.g. CYP3A) and several genes associated with drug transport such as MDR1, OATP2, MRP2, and MRP3. PXR is remarkably divergent between species, with the rabbit, rat and human receptors sharing only approximately 80% of the amino acid identity in their ligand-binding domains. This feature is reflected by marked pharmacological differences in PXR activation profiles. PXRs from different species are differentially activated by specific compounds, thus correlating well with species-specific induction of CYP3A gene expression. For example, the hypocholesterolemic drug SR122813, the macrolide antibiotic rifampicin and the antidiabetic drug troglitazone are effective activators of the human and rabbit PXR but have modest activity on the rat and mouse PXR. On the contrary, pregnane 16α-carbonitrile is a more potent activator of the rat and mouse than the human and rabbit receptor. In addition, PXR polymorphism has been described and it is assumed to contribute to the observed interindividual variability of gene expression and atypical responses to drugs or altered sensitivity to carcinogens.

CAR

Also known as NR1I3, this NR was identified in 1994 as a receptor interacting with a subset of retinoic acid response elements. It was originally defined as a constitutively activated receptor since it forms a heterodimer with RXR and binds to retinoic acid response element in the absence of ligand. It was demonstrated more recently that CAR activation is a multistep process. The initial step is translocation to the nucleus and interaction with RXRα, a process that can be independent of ligand binding. It is known that CAR participates in regulation of transcription of drug transporter genes such as MRPs (MRP2, 3, and 4) and Oatp2.

CAR is found mainly in liver and it is also detected in certain extrahepatic tissues such as the intestine. Pathophysiological conditions such as trauma, sepsis, inflammation or drugs can modify CAR expression. In vivo, CAR is sequestered in the cytoplasm forming a complex with proteins such as heat shock protein 90 (HSP90) and CAR cytoplasmic retention protein (CCRP). In addition, phosphatase 2A (PP2A) is recruited to the HSP90-CCRP-CAR complex. Translocation of CAR to the nucleus, most likely dependent on the activity of PP2A, is followed by association with RXR and binding to the phenobarbital responsive enhancer modules (PBREM). Thus, CAR activation can imply direct binding of an agonist, recruitment of co-activators, dissociation of co-repressors, and the subsequent nuclear translocation and heterodimerization with RXRα, prior to DNA binding and induction of gene expression. COX co-activators so far identified are GRIP1/TIF2, PGC-1, SRC-1, Sp1, ASC-2 and SMC-1. CAR transcriptional activity correlates well with its concentration in the nucleus. The blockage of phenobarbital-mediated induction of CYP2B gene in rodents by okadaic acid, a protein phosphatase inhibitor, has provided an additional indication of the importance of CAR nuclear accumulation in the increase of transcription rate. Some ligands of CAR like androstenediol act as inverse agonists, affecting the protein in such a way that co-repressors instead of co-activators are recruited, and the transcripional activity of the receptor is decreased. Estrogen derivatives display both agonist and antagonist nature by inducing the recruitment of both SRC-1 and NeR after binding to CAR. Alternatively, some CAR activators are not ligands in vitro. Among others, phenobarbital and bilirubin can modulate CAR activity by indirect activation, promoting the nuclear translocation of the receptor without binding to the ligand domain, although the mechanism is not totally understood.
It is widely recognized that enzymes and transporters, these latter systems being involved in the metabolic and disposition of xenobiotics and endogenous substrates. Thus, among other factors, drug exposure can influence the activity of these NRs, affecting the metabolism, toxicity and drug-drug interactions of many xenobiotics or endogenous substances. The following paragraphs describe some representative examples of the action of therapeutic agents modulating drug transporters and involving CAR or PXR as mediators.

Pharmaceutical agents that are agonists of PXR and CAR had been used for treatment of human diseases long before their mechanism of action was clarified. Rifampicin, a human PXR agonist, was found to be effective in the treatment of pruritus in cholestatic disorders. Furthermore, administration of rifampicin to healthy human volunteers significantly induced UDP-glucuronosyltransferase 1A1 (UGT1A1), involved in bilirubin glucuronidation, and MRPII expression, leading to reduction in serum bilirubin levels. Certain traditional Chinese herbs are powerful CAR activators and have been used extensively for management of neonatal jaundice. Phenobarbital, in addition to rifampicin, has been empirically used to treat hyperbilirubinemia due to its inductive properties on UGTs. These compounds are activators of PXR and CAR and the identification of the UGT locus as a direct target for hPXR and hCAR has relevance in both xenobiotic/endobiotic metabolism and disposition in human disease. Simultaneous induction of biotransformation and transport systems by these agents was also effective in increasing the disposition of a variety of carcinogens, as well as estrogen and thyroxin due to its inductive properties on UGTs. These compounds are activators of PXR and CAR and the identification of the UGT locus as a direct target for hPXR and hCAR has relevance in both xenobiotic/endobiotic metabolism and disposition in human disease. Simultaneous induction of biotransformation and transport systems by these agents was also effective in increasing the disposition of a variety of carcinogens, as well as estrogen and thyroxin due to its inductive properties on UGTs. These compounds are activators of PXR and CAR and the identification of the UGT locus as a direct target for hPXR and hCAR has relevance in both xenobiotic/endobiotic metabolism and disposition in human disease. Simultaneous induction of biotransformation and transport systems by these agents was also effective in increasing the disposition of a variety of carcinogens, as well as estrogen and thyroxin.
or PXR, in addition to NrF, are involved in modulation of key drug transporters regulating acacetaminophen toxicity, at toxic or subtoxic doses, needs further exploration.

Whereas a number of drugs targeting different NrFs, which form heterodimers with RXR, have been approved for treatment of metabolic diseases, finding new therapeutic compounds that could modulate drug efflux in a similar way still represents a major challenge. Our increasing understanding of the molecular regulation of transport and detoxification systems, including mediation of NrRs, should help significantly.

CONCLUSION

Major drug transporters in the liver, either at the apical or basolateral level, are extensively regulated by therapeutic agents, and likely involve mediation of NrRs. Targeting NrRs such as CAR and PXR to improve liver diseases, particularly those involving alterations in biliary secretory function, represents a promising perspective. Most of the studies referenced in this current review, which clearly support this possibility, were performed either in rodents or in human cell lines. To what extent the results obtained in these experiments apply to humans is poorly known and needs further exploration.

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