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TOPIC HIGHLIGHT

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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 (NR)s 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; Pregnane X receptor; Therapeutic agents

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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[1]. 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: SLCO family), multidrug resistance-associated proteins 1, 3 and 4 (MRP1, 3 and 4; ABCC1, 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, monoanionic bile salts, and bicarbonate, respectively[1-4]. 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)[5-11]. 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 exogenous metabolites or toxins, steroid hormones, hydrophobic peptides and even glycolipids[12]. 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[2,13]. MRP2 also mediates the canalicular transport of glucuronidated and sulfated bile salts[14]. 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[15]. 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[16-18].

Expression of these canalicular drug transporters are subject to transcriptional and post-transcriptional regulation in response to endogenous and exogenous compounds and different pathologic situations. The short-term changes in transporter activity and expression are generated at post-transcriptional level. An intensive regulation mediated by second messenger and protein-kinases modulates the recruitment of the transporters from intracellular reservoirs to plasmatic membrane or alters the activity by phosphorylation-dephosphorylation and protein-protein interactions. All these factors act in association to regulate the functional state of drug transporters, which were found to be affected to different degrees by liver disease[1,4,19]. Particular interest has been given recently to proper localization of BSEP and MRP2 in the normal situation, which is disrupted in cholestatic disease as a consequence of their internalization and abnormal localization to subapical membrane. This was observed not only in experimental animals such as the rat, but also in humans[20,21].

At transcriptional level, a wide variety of nuclear receptors (NR)s activated 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. The biotransformation systems (phase Ⅰ and Ⅱ reactions) that act in coordination with efflux proteins are also regulated by this same network of NRs[22,23]. Transcriptional regulation of drug transporters by NRs is a complex process involving: (1) ligand binding, (2) the association of NRs with regulatory sites in the genome through their DNA sites, (3) co-regulator recruitment, (4) the regulation of polymerase Ⅱ binding and activity at target promoters, and (5) the ending or attenuation of NR-dependent signaling[24-26].

Co-activators and co-repressors represent key factors in modulation of NR activity. Co-activators are implicated in chromatin relaxation due to their intrinsic histone acetyltransferase or methyltransferase activity. After binding to the activated (agonist-bound) NR, co-activators contribute to the full activation of expression of the target genes. Several co-activators were shown to cooperate with nuclear receptors: the p160 family (SRC-1, TIF-2, GRIP, ACTR), p/CIP and CBP/p300. On the other hand, co-repressors, such as NcoR and SMRT, preferentially bind to inactivated receptors (absence of ligand, antagonist-bound, reverse agonist-bound) and recruit various forms of histone deacetylases, thus leading to chromatin condensation and ultimately, to repression of the target gene expression[27].

As anticipated above, NRs comprise a superfamily of transcription factors activated by ligands which can both activate and repress gene expression. According to their dimerization and DNA binding properties, NRs can be classified into four groups. Class Ⅰ comprises the classical receptors of steroid hormones (estradiol, testosterone, progesterone, cortisol). These form homodimers before binding to response elements in the promoter regions of target genes. NRs belonging to Class Ⅱ, such as pregnane X receptor (PXR), constitutive androstane

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[28].

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[29]. Lehmann *et al*[30] 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[31].

PXR

In 1998, Kliewer *et al*[32] 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[32,33]. 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[34]. 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 litocholic, 3-ketolitocholic, cholic and deoxycholic acids[32-34]. 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*[35-37]. 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 SR12813, 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[33]. 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[38,39].

CAR

Also known as NR1I3, this NR was identified in 1994 as a receptor interacting with a subset of retinoic acid response elements[40]. 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[41]. 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[37,42]. It is known that CAR participates in regulation of transcription of drug transporter genes such as *MRPs* (*MRP2*, *3*, and *4*) and *Oatp2*[23,43,44].

CAR is found mainly in liver and it is also detected in certain extrahepatic tissues such as the intestine[40,45]. Pathophysiological conditions such as trauma, sepsis, inflammation[46] or drugs[47] 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)[48]. In addition, phosphatase 2A (PP2A) is recruited to the HSP90-CCRP-CAR complex[49]. 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[50], prior to DNA binding and induction of gene expression[51]. CAR 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[52]. Some ligands of CAR like androstenol act as inverse agonists, affecting the protein in such a way that co-repressors instead of co-activators are recruited, and the transcriptional activity of the receptor is decreased[53]. Estrogen derivatives display both agonist and antagonist nature by inducing the recruitment of both SRC-1 and NcoR after binding to CAR[54]. 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[49,55].

MODULATION OF DRUG TRANSPORTERS BY THERAPEUTIC AGENTS: ROLE OF NUCLEAR RECEPTORS

Synthetic drugs, natural products, endogenous substances, and environmental toxicants are chemicals known to modulate the activity of major Class Ⅱ nuclear receptors, CAR and PXR[56,57]. It is widely recognized that CAR and PXR are major determinants in the regulation of an extensive spectrum of genes involved in the metabolism and disposition of xeno- and endobiotics[37,58-60]. 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[61,62]. Furthermore, administration of rifampicin to healthy human volunteers significantly induced UDP-glucuronosyltransferase 1A1 (UGT1A1), involved in bilirubin glucuronidation, and MRP2 expression, leading to reduction in serum bilirubin levels[63]. Certain traditional Chinese herbs are powerful CAR activators and have been used extensively for management of neonatal jaundice[64]. Phenobarbital, in addition to rifampicin, has been empirically used to treat hyperbilirubinemia[65,66] 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 same agents was also effective in increasing the disposition of a variety of carcinogens, as well as estrogen and thyroxin[67]. MRP2 is one of the best characterized drug transporters to act in coordination with biotransformation systems to increase drug elimination[22]. This is in part due to its universal capacity to respond to NR activators, which in turn activate a wide spectrum of phase I and Ⅱ reactions. Indeed, Kast *et al*[36] have reported that *MRP2/Mrp2* genes are modulated by PXR, FXR and CAR in human and rodents. Interestingly, these three distinct nuclear receptor signaling pathways converge on a common response element in the 5'-flanking region of these same genes.

Glucocorticoids are also well known inducers of several biotransformation and transport systems. In acute cholestasis, as well as in chronic cholestatic disorders such as primary biliary cirrhosis, the beneficial effects of steroids could be attributed not only to their anti-inflammatory and immune-modulatory actions but also to the effects mediated by alterations in biotransformation enzymes and transporters, these latter systems being regulated by NRs[68]. CAR seems to act as a primary glucocorticoid receptor (GR)-response gene, since the CAR gene promoter harbors a GR response element[69]. In addition, glucocorticoids such as dexamethasone induce CAR nuclear translocation. Glucocorticoids also induce PXR expression and nuclear translocation and thus induce target genes expression like *CYP3A4*, *BSEP* and *MRP2*. These latter findings explain the improvement of liver cholestatic diseases such as that induced experimentally by endotoxin administration[70].

As was demonstrated for the steroids pregnenolone 16α-carbonitrile, 5 β-pregnane-3, 20-dione and dexamethasone[71], spironolactone, widely used as a diuretic, also binds to PXR[72]. Rats treated with spironolactone, exhibit up-regulation of Mrp2 and P-gp in liver[73,74] along with increased phase Ⅱ biotransformation reactions[75,76]. Data on increased expression of Mrp2 (protein and mRNA) are consistent with transcriptional regulation of the target genes and with spironolactone-PXR interaction. The potentiality of spironolactone to counteract alterations in biliary secretory function emerges from studies demonstrating that this steroid was able to prevent the decrease in bile flow and biliary secretion of Mrp2 substrates induced by the cholestatic ethynylestradiol[77]. It is interesting to note that spironolactone also leads to up-regulation of PXR mRNA and protein levels (ML Ruiz, SSM Villanueva, MG Luquita, AD Mottino, and VA Catania, unpublished results), reinforcing a role for this nuclear receptor as a modulator of the action of spironolactone. This finding also suggests that an adaptive response to prolonged treatment with therapeutic drugs may result from changes in expression of the NR gene, and consequently from its availability for binding to the respective ligands. Clearly, the binding of an agonist or antagonist to NRs can directly translate physiological and pathophysiological requirements into alterations of gene expression[1,78,79]. These effects can be additionally modulated by transcriptional or post-transcriptional regulation of the transcription factor itself[80,81].

Acetaminophen is a widely used therapeutic drug which can produce hepatotoxicity when administered at high doses. CAR is a key regulator of acetaminophen metabolism and hepatotoxicity. CAR activators, as well as high doses of acetaminophen, induce expression of key drug metabolizing enzymes in wild-type but not in *Car*-/- mice, and administration of the inverse agonist ligand androstanol after treatment with acetaminophen blocks hepatotoxicity in wild-type but not in *Car*-/- mice[82]. In addition, *Car*-/- mice are resistant to acetaminophen hepatotoxicity. In contrast, mice deficient in Nrf2 are highly susceptible to acetaminophen hepatotoxicity and were unable to increase the hepatic basolateral drug transporters Mrp3 and Mrp4, as detected in wild type animals[83]. These transporters may represent an attractive target to reduce acetaminophen hepatotoxicity. Indeed, pretreatment of rats with acetaminophen was shown to increase Mrp3 expression, and thereby induced a shift from biliary to urinary elimination of acetaminophen glucuronide; the subsequent decreased enterohepatic recirculation was postulated to decrease exposure of the liver to acetaminophen and thereby protect against hepatoxicity[84]. Whether CAR

or PXR, in addition to NrF, are involved in modulation of key drug transporters regulating acetaminophen toxicity, at toxic or subtoxic doses, needs further exploration.

Whereas a number of drugs targeting different NRs, which form heterodimers with RXR, have been approved for treatment of metabolic diseases[85], 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 NRs, 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 NRs. Targeting NRs 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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