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Regulation of Transporters by Nuclear Hormone Receptors: Implications during Inflammation

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Abstract: Membrane transporters play a critical role in the absorption, distribution, and elimination of both endogenous substrates and xenobiotics. Defects in transporter function can lead to altered drug disposition including toxicity or loss of efficacy. Inflammation is one condition during which variable drug response has been demonstrated, and this can be attributed, at least in part, to changes in the expression of transporter genes. Thus, knowledge of the mechanisms behind transporter regulation can significantly contribute to our ability to predict variations in drug disposition among individuals and during inflammatory disease. The discovery of several xenobiotic-activated nuclear hormone receptors during the past decade including the pregnane X receptor, constitutive androstane receptor, and farnesoid X receptor has contributed greatly toward this endeavor. These receptors regulate the expression of transporters such as P-glycoprotein, MRP2, MRP3, BCRP, and OATP2 (Oatp1a1/OATP1B1), all of which undergo altered expression during an inflammatory response. Nuclear receptors may therefore play an important role in mediating this effect. This review presents what is currently known about the role of nuclear receptors in transporter regulation during inflammation. The use of this knowledge toward understanding interindividual variation in drug response and drug interactions during inflammation as well toward the development of therapeutics to treat transporter-related diseases will also be discussed.

Keywords: Drug transporters; inflammation; cytokines; nuclear receptors; PXR

The cellular uptake and efflux of many drugs, xenobiotics, and endogenous compounds throughout the body is dependent on the expression and activity of membrane transport proteins. It is well-recognized that transporters in the liver and gastrointestinal tract, such as those belonging to the ATP-binding cassette (ABC) or solute carrier (SLC) families, play an important role in determining drug and endobiotic absorption and excretion. Other tissues including the kidney, brain, placenta, testis, lung, and peripheral blood mononuclear cells also express these transporters to various extents. Drug interactions, multidrug resistance, and interindividual differences in drug response have been attributed to altered or varied transporter expression. Interestingly, altered transporter expression and drug responses have been observed in diseases associated with an inflammatory response, such as infection,[1,2] renal disease,[3] hypoxia[4] and cancer.[5] Considering the fact that

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endobiotics such as bile acids, hormones, inflammatory mediators, nucleotides, and glutathione are also substrates for these transporters, it is not surprising that defects in the expression and/or activity of these transporters have also been linked with a number of diseases such as Dubin−Johnson syndrome[6] and progressive familial intrahepatic cholestasis.[7] Thus, regulating transporter expression is critical toward the success of drug therapy as well as for maintaining cellular homeostasis.

During the past decade, the discovery and characterization of a number of xenobiotic-activated nuclear hormone receptors has greatly accelerated the understanding of transporter gene regulation. Among them, NR1I2, the pregnane X receptor (PXR; also referred to as SXR in humans but PXR will be used throughout for clarity), the constitutive androstane receptor (CAR), and the farnesoid X receptor (FXR) have emerged as important regulators of transporter expression in response to both xeno- and endobiotics as well as during diseases such as inflammation, cholestasis, and cancer. The extensive involvement of nuclear receptors in transporter regulation is just beginning to be realized, along with their potential as targets in the design of strategies to treat transporter-related diseases.

Although the implications of gene regulation by nuclear receptors in several diseases are emerging, this review will focus on their role in transporter regulation during inflammation. Inflammation is the body's defensive response to injury, tissue damage, infection, or other stress-evoking stimuli in an attempt to remove or isolate the cause of the disturbance and restore homeostasis. It is a complex condition involving a host of metabolic changes which are primarily mediated by the pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon. Inflammation is a pathological condition that leads to other diseases (e.g., cholestasis), is a component of disease (e.g., inflammatory bowel disease), or results from diseases (e.g., cancer). Hence, it is a major factor that can impact the success of drug therapy for such diseases.

Inflammation has long been associated with altered drug disposition. Early studies by Schneider et al.[8] demonstrated increased plasma levels of propranolol and oxypranolol in patients with inflammatory disease versus healthy controls. Likewise, animal models of inflammation demonstrated significant increases in the plasma levels of propranolol and acebutolol.[9,10] These changes could be attributed to decreased drug clearance resulting from changes in the hepatic as well as extra-hepatic expression of drug metabolizing enzymes and transporters.

In this review, we will first describe what is currently known about the role of nuclear receptors in transporter regulation and their involvement in the regulation of transporters during an acute inflammatory response. We will then discuss and speculate on the implications of nuclear receptor involvement in conditions such as inflammatory bowel disease, cholestasis, and cancer. The impact of nuclear receptors on transporter expression is not limited to their effect in response to drug exposure, but has much further-reaching implications that should be taken into consideration during drug development and therapy.

Changes in Transporter Expression during Inflammation

Studies conducted in several laboratories have shown that an acute inflammatory response induced by the administration of endotoxin (i.e., bacterial lipopolysaccharide) or turpentine down-regulates the expression of numerous transporters in hepatic and extra-hepatic rodent tissues such as intestine, brain, heart, and placenta. These findings are summarized in Tables 1 and 2. For a detailed discussion of the impact of inflammation on drug transporters, we refer the reader to a recent review by Petrovic et al.[11] In addition, *in vivo* administration of the cytokines IL-6, IL-1β, or TNF-α to mice has been shown to alter the expression of several transporters as listed in Table 3. *In vitro* treatments with IL-1β, IL-6, and TNF-α have also revealed differential effects on transporter expression in human and murine hepatoma cell lines as well as primary rat hepatocytes. For instance, IL-6 has been shown to induce *MRP3/Mrp3* mRNA in human hepatoma HuH7 cells but not in human HepG2 or murine Hepa1–6 cells.[12] *In vitro* treatments with IL-6 were shown to decrease *MDR1*/Pgp expression in human HuH7

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Table 1. Effect of Lipopolysaccharide on the Expression of Transporters

transporter	species	tissue	effects of LPS
Mdr1a	mouse	Liver[94]	↓
	rat	Liver[95]	↓
	rat	Intestine[96]	↓
	rat	Brain[2]	↓
	rat	Heart[97]	↓
	rat	Placenta[97]	↓
Mdr1b	mouse	Liver[94]	↓
	rat	Liver[97,2]	↑
		Intestine[96]	↔
	rat	Placenta[97]	↓
	rat	Kidney[97]	↔
MDR2	mouse	Liver[94]	↓
	rat	Liver[95]	↓
MRP2	mouse	Liver[98]	↓
	rat	Liver[99]	↓
	rat	Intestine[96]	↓
MRP3	mouse	Liver[98]	↓
	rat	Intestine[96]	↔
OATP2	mouse	Liver[98]	↓
	rat	Liver[100]	↓
OATP4	mouse	Liver[101]	↓
BSEP	mouse	Liver[98]	↓
NTCP	mouse	Liver[102]	↓
	rat	Liver[103]	↓

Table 2. Impact of Turpentine on Hepatic Transporter Expression in Mice

transporter	species	tissue	effect of turpentine
Mdr1a	mouse	Liver[94]	↓
	rat	Liver[95]	↓
Mdr1b	mouse	Liver[94]	↓
MRP2	mouse	Liver[98]	↓
MRP3	mouse	Liver[98]	↓
OATP2	mouse	Liver[98]	↓
BSEP	mouse	Liver[98]	↓
NTCP	mouse	Liver[102]	↓

cells and *Mdr1a and Mdr1b* expression in primary rat hepatocytes.[12,13]

Changes in transporter expression resulting from inflammation can potentially lead to pharmacokinetic and pharmacodynamic variability in patients. For example, as demonstrated using animal models of acute inflammation, LPS-mediated changes in rodent P-gp expression have been shown to decrease the clearance of doxorubicin[1] and digoxin.[2] Similar changes in drug disposition observed in humans during inflammation (e.g., cyclosporine,[14] amitriptyline[15]) could also likely be attributed to changes in transporter expression. It is therefore important to elucidate the mechanism of inflammation-mediated changes in hepatic gene expression, as this may aid in understanding and predicting drug-disease interactions or potential adverse events, and can provide avenues toward improving therapeutic control of the inflammatory response.

Table 3. Impact of Cytokines on Hepatic Transporter Expression in Mice

transporter	cytokine	impact of cytokine on transporter expression
Mdr1a[94]	IL-1β	↓
	IL-6	↔
	TNF-α	↔
Mdr1b[94]	IL-1β	↔
	IL-6	↓
	TNF-α	↑
Mdr2[94]	IL-1β	↓
	IL-6	↓
	TNF-α	↔
MRP2[98]	IL-1β	↓
	IL-6	↓
	TNF-α	↓
MRP3[98]	IL-1β	↔
	IL-6	↔
	TNF-α	↓
OATP2[98]	IL-1β	↓
	IL-6	↓
	TNF-α	↓
BSEP[98]	IL-1β	↓
	IL-6	↓
	TNF-α	↔
NTCP[103,102]	IL-1β	↓
	IL-6	↓, ↔
	TNF-α	↓

The functions, characteristics, and regulation of the transporters which we will discuss below are described in numerous other reviews;[16,17] therefore, we will limit our focus on the regulatory role of nuclear hormone receptors during inflammatory conditions.

Regulation of Transporters by Nuclear Hormone Receptors

P-Glycoprotein (P-gp/ABCB1). The P-glycoprotein efflux transporter is encoded by the *Multidrug resistance 1* (*MDR1*)

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gene in humans and the *Mdr1a* and *Mdr1b* genes in rodents. It is involved in the transport of a plethora of structurally and functionally unrelated xenobiotics, and overexpression leads to drug resistance. There is much evidence that this transporter is regulated by PXR, and positive correlations between *MDR1* and *PXR* mRNA levels have been observed in human peripheral blood mononuclear cells.[18,19] Likewise, *in vitro* studies have implicated rifampicin- and paclitaxel-mediated activation of PXR in the induction of *MDR1* expression in human colon carcinoma cell lines.[20,21] *In vivo* studies have further demonstrated that hepatic *Mdr1a* but not *Mdr1b* mRNA is induced by the PXR activators pregnenolone-16α-carbonitrile (PCN) and RU486 in PXR wild-type but not PXR knockout mice, indicating PXR-dependent regulation of this transporter.[22] Expression of *Mdr1a* mRNA in the murine small intestine was induced by treatment with the PXR activators rifampicin and St. John's wort, but not PCN, and expression in the kidney was not altered.[23] Thus, P-gp and its encoding genes undergo tissue-specific regulation in response to PXR activation, although the mechanism behind these differences remains to be elucidated. Considering that known P-gp inducers such as 2-acetylaminofluorine (2-AAF), paclitaxel, tamoxifen, ritonavir, and saquinavir are also activating ligands of PXR, it is likely that the induction of P-gp by these compounds is mediated by PXR. Overall, PXR appears to be a major contributor to the regulation of P-gp expression in many tissues under diverse conditions. As activation of CAR with artemisinin has also been shown to induce *MDR1* expression in primary human hepatocytes and in cultured human intestinal cells, it appears that CAR may also be involved in P-gp regulation.[24] The involvement of other nuclear receptors has yet to be demonstrated.

Multidrug Resistance Protein 2 (MDR2/ABCB4). Mdr2/MDR2, which is expressed exclusively in the canalicular membrane of hepatocytes, does not appear to be involved in the transport of drugs. Generation of Mdr2 knockout mice revealed that Mdr2 has an important physiological function as a biliary phosphatidylcholine transporter since mice with a disrupted *Mdr2* gene failed to secrete phosphatidylcholine and cholesterol into bile.[25] The nuclear receptor peroxisome proliferator-activated receptor α (PPARα), which plays a key role in cholesterol homeostasis, has been shown to be involved in *Mdr2* regulation.[26]

Bile Salt Export Pump (BSEP/ABCB11). Bsep/BSEP is expressed almost exclusively in the liver and plays an important role in apical bile acid efflux from the liver. Regulation of *Bsep/BSEP* expression has been primarily attributed to the nuclear receptor farnesoid X receptor (FXR).[27,28] Activation of FXR results in *Bsep* induction and *Bsep* expression is significantly lower in FXR knockout mice compared to wild-types.[29] In HepG2 cells, decreased expression of FXR by RNA interference prevented *BSEP* induction by bile salts.[28] Recent studies have also demonstrated involvement of PXR in *Bsep* regulation in that administration of the PXR activators PCN or RU486 results in a 1.5-fold induction of *Bsep* mRNA in PXR wild-type but not knockout mice.[22] Regulation of *Bsep* by PXR may serve as an additional regulatory pathway besides FXR during cholestasis, as both receptors are activated by bile acids.

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Multidrug Resistance Associated Protein 1 (MRP1/ABCC1). Mrp1/MRP1 is expressed ubiquitously throughout the body, although expression in the liver is quite low. Like P-gp, MRP1 is involved in the efflux transport of a plethora of drugs and xenobiotics, and plays an important role in multidrug resistance.[30] However, little is known about the regulation of *Mrp1/MRP1* by nuclear receptors. Kauffmann et al. showed that *MRP1* was not induced in the MCF-7 breast cancer cell line following treatment with the PXR activator rifampicin.[31]

Multidrug Resistance Associated Protein 2 (MRP2/ABCC2). Mrp2/MRP2, which is expressed in on the apical surface of several epithelial membranes, has been shown to transport numerous organic anionic drugs and their conjugated metabolites. Response elements for several nuclear receptors have been identified in the promoter region of the *Mrp2* gene. Kast et al.[32] identified a binding site approximately 400 bp upstream from the transcriptional start site of the rat *Mrp2* gene which is involved in the ligand-mediated induction of *Mrp2* by PXR, FXR, and CAR. Indeed, subsequent *in vivo* studies reported induction of *Mrp2* in the liver of PXR wild-type but not knockout mice after administration of PCN, RU486, 2-AAF, or cholic acid.[22,33,34] Similarly, induction of *Mrp2* in the jejunum and ileum of wild-type but not PXR-knockout mice has been reported.[35] The PXR activator rifampicin also induced *MRP2* in HepG2 cells[31] and in primary human hepatocytes.[36] Furthermore, *MRP2* mRNA correlated with PXR expression in peripheral blood mononuclear cells.

Multidrug Resistance Associated Protein 3 (MRP3/ABCC3). Mrp3/MRP3 is a basolateral transporter which is involved in the efflux of many xenobiotics as well as endobiotics such as bile acids. It has been shown that *Mrp3/MRP3* mRNA and functional activity are induced by PXR activators in primary cultured hepatocytes and in the human hepatoma cell lines HuH7 and HepG2.[36,37] Likewise, *in vivo* administration of the PXR activators PCN,[22,38] RU486,[37] or cholic acid[34] results in a pronounced induction of *Mrp3* in the liver of wild-type but not PXR-null mice. Activation of PPAR-α[39] and the vitamin D receptor[40] are also thought to play a role in *Mrp3* regulation. On the other hand, investigations into the role of CAR have yielded conflicting results.[41–43]

Organic Anion Transporting Polypeptide 1 (*Oatp1a1/Slco1a1*). Oatp1a1 (commonly known as OATP1 although recently adopted nomenclature designates this transporter as Oatp1a1) is a basolateral transporter that mediates the cellular uptake of several endogenous (e.g., bile acids, steroids) and exogenous (pravastatin, fexofenadine, digoxin) substrates from plasma.[17] The regulation of *Slco1a1* has not been extensively studied, particularly with regard to nuclear receptor involvement. However, activation of PPARα has been shown to down-regulate *Slco1a1* in mouse liver.[26] The implications of this regulatory mechanism have yet to be elucidated.

Organic Anion Transporting Polypeptide 2 (Oatp1a4/Slco1a4). Like Oatp1a1, this transporter has been commonly referred to as OATP2. Oatp1a4 is an uptake transporter that

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is expressed on the basolateral membrane of hepatocytes as well as other tissues. PXR activation by *in vivo* administration of PCN,[38,44] RU486,[22] 2-AAF,[33] or atorvastatin[45] markedly induces the hepatic expression of Oatp1a4/*Slco1a4* in mice. Binding sites for PXR have been identified in the human *SLCO1B1* (the human ortholog of *Slco1a4*) and rat *Slco1a4* promoters.[46,47] Induction may occur in order to remove compounds from the plasma circulation for detoxification in the liver and subsequent excretion. Alternatively, since it also functions as an anion exchanger, Oatp1a4 induction may serve to increase the removal of endogenous anions from cells. As *Slco1a4* is also induced after administration of phenobarbital[48] or bile acid feeding,[49] CAR-mediated regulatory pathways are also likely involved in *Slco1a4* regulation.[45]

Sodium Taurocholate Transporting Polypeptide *(NTCP/Slc10a1).* Ntcp/NTCP is a basolateral bile acid uptake transporter. Regulation has been shown to occur through several bile acid-activated nuclear receptors such as FXR, retinoid X receptor (RXR), and hepatocyte nuclear factors 1α, 3β and 4α. *Ntcp* expression is markedly down-regulated during cholestasis, likely as a feedback mechanism to prevent hepatocellular bile acid levels from reaching toxic levels. The mechanism of *Ntcp* suppression by bile acids is thought to occur by the FXR-dependent activation of small heterodimer partner (SHP), which leads to a decrease in the binding of RXR:RAR to the *Ntcp* promoter.[50,51]

Role of PXR in Transporter Regulation during Inflammation

Acute Inflammation. Studies have pointed to cytokine-mediated modulation of transcription as the major regulatory mechanism responsible for altered gene expression during inflammation. For example, a decrease in the transcription rate of hepatic *Mdr1a* and *Mdr1b* was demonstrated in hepatic nuclear fractions obtained from turpentine-treated rats or IL-6 treated rat hepatocytes.[13] Transcription factors such as signal transducers and activators of transcription (STAT), CCAAT enhancer binding protein (C/EBP; nuclear factor IL-6 (NF-IL6)), activating protein (AP-1), and nuclear factor κ B (NFκB) are activated by cytokine-induced signaling pathways, and these may lead to changes in transporter expression. In light of the more recent findings that nuclear hormone receptors also play a role in the regulation of transporters that are suppressed during an inflammatory response, there is a potential role for these receptors in transporter regulation during the acute phase response.

Early studies demonstrated the involvement of PXR in the regulation of transporter and drug metabolizing enzyme genes during inflammation.[22] While *Mrp2* was substantially suppressed by LPS in $PXR^{+/+}$ mice, this was partially but significantly attenuated in $PXR^{-/-}$ mice, signifying the involvement of PXR in the mechanism of down-regulation. On the other hand, there were no differences between $PXR^{+/+}$ and $PXR^{-/-}$ mice in the extent of suppression of other transporters and *Cyp3a11* following LPS treatment; this lack of difference in *Cyp3a11* was later corroborated by Richardson and Morgan.[52] LPS stimulates the release of several cytokines so it is plausible that several down-regulatory mechanisms are in effect in the LPS-treated animals. Indeed, it is interesting that IL-6 administration was found to significantly down-regulate *Mrp2*, *Bsep* and *Cyp3a11* in $PXR^{+/+}$ mice but not in $PXR^{-/-}$ mice. This suggests that PXR involvement may occur through IL-6 mediated pathways. Of note, administration of IL-6 was also capable of attenuating PXR-mediated induction of these genes in PCN-treated mice, further suggesting that down-regulation of *Mrp2*, *Bsep*, and *Cyp3a11* occurs via a pathway involving PXR. These findings indicate that the down-regulation of transporters by LPS occurs through multiple pathways, one of which is mediated by IL-6 and is PXR-dependent.

Although these studies have established partial involvement of PXR in inflammation-mediated gene suppression, the exact nature of its role remains to be elucidated. It has been noted by several groups that the mRNA and protein

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levels of PXR are reduced by LPS and IL-6,[22,53–55] suggesting a mechanism whereby transporter suppression is, in part, a consequence of the decrease in PXR. Specifically, it has been shown that LPS administration decreases the expression of PXR in the liver, intestine and placenta of rodents.[53,55] Moreover, decreases in PXR expression have been shown to precede the down-regulation of target transporter genes. In rats, the LPS-mediated decrease in nuclear expression of PXR coincides with an increase in cytosolic PXR levels,[56] suggesting that the extrusion of PXR from the nucleus results in decreased nuclear trans-activation of PXR target genes.

What remains to be elucidated is the mechanism behind the suppression of PXR itself during inflammation. Several groups have shown that PXR expression in the fetal liver is dependent on HNF4α.[57–59] Our group[22] and others[51] have found a significant reduction in levels of HNF-4α after administration of LPS, raising the possibility that the loss of PXR resulted from decreased HNF-4α levels. *In vivo* administration of IL-6 but not TNF-α or IL-1β significantly decreases levels of HNF-4α and PXR.[22,60] Thus, it could be speculated that the loss of PXR is subsequent to a reduction in HNF-4α by the release of IL-6 from LPS. As PPARα and FXR have also been shown to be involved in regulation of human PXR expression,[61–64] it is interesting that PPARα and FXR are also down-regulated following LPS treatment.[65,66] Thus, changes in PPARα and FXR expression could contribute to down-regulatory effects.

Other mechanisms of PXR down-regulation could be responsible during inflammation. Recent reports have demonstrated that NFκB activation, which occurs during inflammation, inhibits the activity of PXR by preventing the PXR-RXR heterodimer from binding to its response elements on target genes.[67,68] Moreover, oxidative stress mechanisms may be involved as coadministration of antioxidants to LPS-treated mice has been reported to partially prevented the down-regulation of *PXR* mRNA.[69,70] It is clear that inflammation-mediated regulation of PXR needs to be further characterized.

Inflammatory Bowel Disease. Much interest in the possible role of P-gp in inflammatory bowel disease (IBD) has arisen as it is believed that P-gp may serve to protect

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the intestine from bacterial toxins in the colon. IBD has been associated with *MDR1* polymorphisms and correlations between P-gp expression and onset of IBD have been shown in patients.[71–74] Elimination of P-gp activity results in the spontaneous development of colitis in Mdr1a knockout mice.[75] On the other hand, numerous inconsistencies in the literature are apparent, and many studies have not detected any correlation between P-gp status and IBD.[76,77]

Since *MDR1* is regulated by human PXR, this has generated interest in the possible role of this nuclear receptor in IBD. Expression of PXR and the target gene *MDR1* were significantly lower in intestinal tissue from patients with IBD as compared to healthy controls.[78] Furthermore, PXR polymorphisms associated with decreased activity have been correlated with increased susceptibility to IBD.[79] It is also plausible that the acute inflammatory response which occurs in active states of IBD would further exacerbate the cycle due to down-regulation of *MDR1* and PXR. Although the link between P-gp and IBD has not been fully elucidated, it is attractive to speculate that PXR could be targeted to treat IBD through induction of P-gp. Prophylactic therapy preventing P-gp down-regulation could avert accumulation of toxins in the colon which is thought to trigger IBD. In addition, treatment of IBD with PXR activators such as hyperforin (the active component of St. John's wort) could induce *MDR1* expression and attenuate further progression. However the impact of administration PXR activators on the *in vivo* expression of *MDR1* in the colon remains to be determined. Further research in this area is required to elucidate the relationship between IBD and the expression of PXR and P-gp.

Cholestasis. One pathological consequence which occurs due to transporter suppression is the onset of cholestasis. A number of the hepatic transporters that have been discussed thus far play important roles in bile acid (BA) uptake or efflux. BAs are endogenous compounds that play a fundamental role in lipid digestion and absorption from the intestine. Although BAs have an important physiological function, they are also highly cytotoxic. When liver function is impaired or the biliary tract is obstructed, bile secretion is reduced, and BA levels in the liver and plasma become elevated, which further exacerbates hepatobiliary damage. Thus, BA levels must be tightly controlled. BA homeostasis is determined to a large extent by the expression and activity of BA transporters in the gut and liver. A disruption in BA movement between the liver and intestine, such as through impaired transport activity due to inflammation-mediated transporter down-regulation, can lead to cholestasis.

Understanding how transporter expression is regulated prior to and during cholestasis may aid in the development of therapeutics against this condition. The fact that nuclear receptors regulate numerous key genes involved in bile acid transport and that PXR plays a role in transporter suppression during inflammation suggests a possibility to employ nuclear receptor modulators to treat cholestasis. Known PXR activators such as rifampicin, and to a lesser extent phenobarbital, have long been used to treat cholestasis.[80] Recent studies have demonstrated the effectiveness of some PXR ligands in attenuating cholestatic liver injury in rodents.[34,81–84] Rifampicin induced the hepatic expression of MRP2 and bile acid metabolizing enzymes in humans, coinciding with

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decreased serum bile acid levels.[85] Similarly, modulators of FXR and CAR have also presented protective effects in animal models of cholestasis, which are accompanied by changes in transporter expression. For example, *Bsep* and *Mdr2* are induced by FXR activation[81,82] whereas *Ntcp* is down-regulated;[51] *Mrp2*, *Mrp3*, *Mrp4*, and *Oatp1a4* are all induced by CAR activation in bile duct-ligated mice.[45] Mrp2 and Mrp3 were up-regulated in cholestatic mice treated with CAR activators.[86] These changes result in increased hepatocyte bile acid efflux and decreased bile acid uptake, respectively, thus decreasing hepatocyte exposure to bile acids. The mechanism behind the anticholestatic effects of PXR, FXR and CAR activators clearly appear to involve changes in transporter expression. However, the efficacy and mechanisms behind their effects requires further exploration.

Cancer-Induced Inflammation and the Impact on Multidrug Resistance. As already discussed, the inflammatory response has a significant impact on transporter expression not only in the liver, but in other tissues as well. This, in turn, can have major pharmacokinetic consequences. It has been demonstrated that cancer patients exhibit decreased hepatic metabolic and transport activity that is thought to be associated with an inflammatory response arising from tumor growth.[87] This can alter the effectiveness and toxicity of chemotherapeutic agents, since many are substrates of drug metabolizing enzymes and drug efflux transporters. Changes in the activity of transporter and enzyme activity could result in altered excretion or distribution to sites of activity and toxicity. On the other hand, decreased expression of multidrug resistance efflux transporters such as P-gp or MRPs in tumors could enhance chemoresponsiveness. However, as many regulatory mechanisms are mutated in cancer cells, the final impact of inflammatory cytokines on tumor expression of multidrug resistance transporters is unknown. Overall, strategies to avoid or minimize resistance are a top priority for development.

Elucidation of PXR regulatory mechanisms may assist this endeavor, as PXR is expressed in many carcinoma cell lines and tumorous tissues, and PXR-mediated transcriptional activation of metabolic enzymes and transport genes contribute to multidrug resistance. For example, PXR mediates the induction of the multidrug resistance genes *MDR1* and *MRP2*, suggesting a possible involvement of PXR in the onset of this phenomenon. Indeed, anticancer drugs such as paclitaxel, discodermolide,[88] and cisplatin[89] are activators of PXR and substrates for P-gp.[90] Resistance to cisplatin in endometrial cancer cells has been correlated with PXR expression.[91] Nevertheless, despite the evidence pointing to a role for PXR in drug resistance, there is still little known regarding chemotherapy response and PXR status in patients. The PXR C-25385T polymorphism, associated with increased PXR and CYP3A4 phenotype, did not significantly affect paclitaxel clearance in ovarian cancer patients.[92] However, there is a need to examine other polymorphisms which translate to more severe changes in PXR and target gene expression. Studies correlating drug resistance with the PXR status of cell lines or tumor-derived tissue are also required.

Nuclear Receptors as Therapeutic Targets

The influence of transporter expression/activity on disease onset and drug response is clearly evident. By designing ways to enhance or suppress transporter function, we may be able to treat or prevent transporter-related diseases and circumvent

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what would normally be failed or hyper-response. Several examples of the potential utility of nuclear receptor activators in the treatment of transporter-related diseases have been presented. PXR activators could minimize inflammation-mediated down-regulation of transporters that are responsible for altered drug disposition and initiation of cholestasis. Cholestatic liver injury could be attenuated by activators of PXR, FXR, or CAR; indeed, there is already clinical evidence for the effectiveness of PXR activators in treating cholestatic conditions.[80,93] PXR activation may also help to maintain P-gp levels and therefore decrease susceptibility to IBD. On the other hand, nuclear receptor inhibition may also be useful in some circumstances. For example, by preventing the induction of efflux transporters following treatment with PXR-activating chemotherapeutic agents or protease inhibitors, resistance may be avoided. The efficacy of these drugs for the treatment of cancer and HIV, respectively, may therefore be greatly enhanced.

Conclusions

Nuclear receptors are key regulators of many hepatic transporters which play diverse roles in xeno- and endobiotic transport. In relation to that, studies by several laboratories provide evidence that nuclear receptors such as PXR constitute an important part of the mechanism of transporter suppression during endotoxemia and IBD and that their role in the regulation of bile acid transporters can be applied toward treating liver injury resulting from cholestasis. These studies show that nuclear receptors are involved in transporter regulation under both healthy and disease conditions.

As transporters are becoming increasingly recognized as major players in the determination of drug absorption and excretion, recent studies suggest that knowledge of how nuclear receptors are involved in transporter regulation will also give us a better understanding of some of the mechanisms governing drug disposition and variation in drug response. The physiological, pathophysiological and pharmacological relevance of nuclear receptor-dependent transporter regulation, particularly during inflammation, should not be underestimated, and is an area of study that should be actively pursued.

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