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Orphan nuclear receptor-mediated xenobiotic regulation in drug metabolism

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The regulation of drug-metabolizing enzymes and transporters has an important role in drug metabolism and many human diseases. The genes that encode these enzymes and transporters are inducible by numerous xenobiotics and endobiotics and the inducibility shows clear species specificity. In the past 4–5 years, orphan nuclear receptors such as PXR and CAR have been established as species-specific xeno-sensors that regulate the expression of many detoxifying enzymes and transporters. Their identification represents a major step forward in understanding the pharmacological and genetic control of the expression of drug-metabolizing enzymes and the implication of this regulation in drug metabolism, drug–drug interactions, and human diseases.

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▼ The past 50 years have witnessed a monumental advance in our understanding of pharmacodynamics (what drugs do to the body) and pharmacokinetics (what the body does to drugs). In the realm of pharmacokinetics, dozens of enzymes responsible for drug biotransformation and transporters responsible for the absorption, distribution and excretion of drugs have been identified.

Drug biotransformation (metabolism) is traditionally classified as either phase I or phase II. Phase I metabolism (functionalization) includes oxidation, reduction, hydrolysis and hydration. Enzymes catalyzing these reactions are found in virtually all tissues but especially in the hepato-intestinal axis. Quantitatively, however, the liver is generally considered to be the most important organ involved in drug metabolism. Located in the endoplasmic reticulum of hepatocytes is a family of heme proteins known as cytochrome P450 (P450 or CYP). CYP is the central constituent of the so-called microsomal mixed-function oxidase system. The components of this system catalyze the splitting of molecular oxygen with one atom being inserted into the drug molecule and the other undergoing reduction to water. The human genome encodes 57 CYP proteins so there is a substantial genetic component to the process of drug metabolism [1]. Moreover, the activity of CYP enzymes can be induced or inhibited by a variety of environmental chemicals and drugs, adding to the variability in metabolism of different individuals. The products of phase I metabolism are generally more polar and more readily excreted than the parent compounds and are often substrates for phase II enzymes. Phase II metabolism involves conjugation with endogenous hydrophilic compounds to further increase polarity and water solubility and therefore drug excretion. Phase II metabolism is also subject to genetic and environmental variability. Although hepatic drug metabolism has been traditionally equated with 'detoxification', it is now known that in some cases highly reactive metabolites can be formed that react with crucial cellular macromolecules leading to various forms of toxicity.

Although metabolizing enzymes are important in the process of drug disposition, equally important are a group of transporter proteins that are expressed in various tissues, such as the intestine, brain, liver and kidney, which modulate the absorption, distribution and excretion of many drugs. These transporters are classified as either primary, secondary or tertiary. Primary transporters are driven by energy from ATP hydrolysis, whereas secondary and tertiary active transporters are driven by an exchange of intracellular ions. Like the

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drug metabolizing enzymes, each transporter gene family is composed of a multiplicity of members. These proteins control, among other things, absorption of many drugs from the gastrointestinal tract, exclusion of drugs from the brain (a component of the blood–brain barrier), and the active secretion of drugs and metabolites into the bile and/or urine [2].

Drug metabolizing enzymes and transporters are often involved in clinically significant drug–drug interactions. The mechanism of this interaction often involves drug-induced increases in enzyme or transporter activity (induction). As a consequence, disposition of other drugs that are metabolized or transported by the induced protein will change, possibly resulting in an adverse event. The induction of drug-metabolizing enzymes and transporters is mediated by a group of receptors known as orphan nuclear receptors. Chemical interactions with these receptors and the consequences of these interactions are reviewed here.

PXR and CAR: prototypic xenobiotic orphan nuclear receptors

Orphan nuclear receptors belong to the nuclear receptor (NR) superfamily of transcriptional factors. In most cases, these receptor proteins were identified without knowing their endogenous and/or exogenous ligands, so they were called 'orphan' receptors. Most, if not all, NRs share two essential functional domains that include the N-terminal DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) [3]. The conserved DBD consists of two DNA-binding zinc fingers and the LBD folds to form a hydrophobic pocket into which the ligand binds.

In 1998, the rodent orphan NR pregnane X receptor, PXR [4], and its human homolog hPXR (also known as steroid and xenobiotic receptor, SXR, or PAR [5–7]), were isolated as candidate xenobiotic receptors postulated to regulate *CYP3A* gene expression. It took this name because pregnenolone and its derivative, pregnenolone 16α-carbonitrile (PCN), can activate PXR. Another orphan receptor, constitutive androstane receptor (CAR), was cloned several years earlier [8] but its identity as a xenobiotic receptor was not appreciated until the discovery that its constitutive activity can be inhibited by selective androstane metabolites [9]. The role of CAR in positive xenobiotic regulation of *CYP2B* genes was first shown in 1998 [10].

Xenobiotic receptors, such as PXR and CAR, regulate gene expression by forming heterodimers with the retinoid X receptor (RXR). The regulation is achieved by binding of the PXR–RXR or CAR–RXR heterodimers to the specific xenobiotic response elements (XREs) present in the promoter regions of drug-metabolizing enzymes and transporters (Figure 1). PXR is activated by a variety of xenobiotics

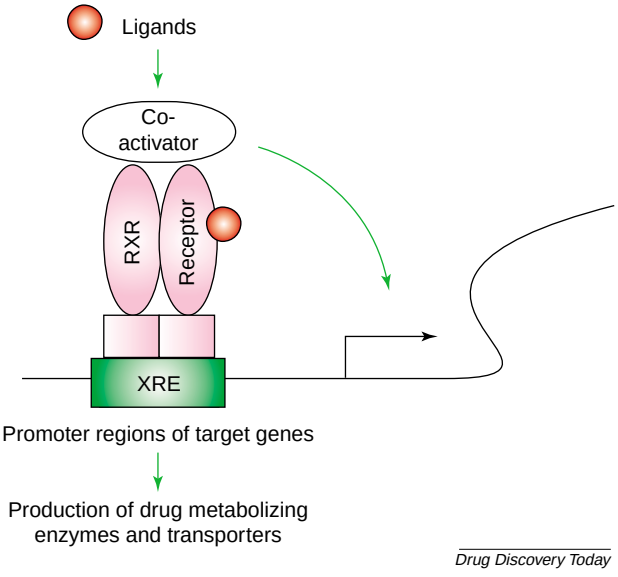

Figure 1. Xenobiotic receptor-mediated regulation of drug-metabolizing enzymes and transporters. Activation of xenobiotic receptors, such as pregnane X receptor (PXR) and constitutive androstane receptor (CAR), induces phase I and II enzymes and drug transporters. This transcriptional activation requires: (1) binding of ligands and recruitment of co-activators; (2) formation of heterodimers with retinoid X receptor (RXR); and (3) binding of the heterodimers to the xenobiotic response elements (XRE) in the target gene promoters.

including drugs known to induce hepatic and intestinal CYP3A activity [4–7]. Although CAR shows relatively high basal activity to transactivate genes without ligand ('constitutive'), its activity can be inhibited by antagonists, such as androstane metabolites [9], and potentiated by agonists, such as phenobarbital (PB) and 1,4-bis[2-(3,5 dichloropyridyloxy)] benzene (TCPOBOP) [10–12].

The respective regulation of CYP3A and CYP2B by PXR and CAR has been firmly established via the generation of mice deficient in PXR and CAR [13–15]. Disruption of the mouse *PXR* locus by homologous recombination abolishes the CYP3A induction in response to PCN and dexamethasone [13,14]. Similarly, CYP2B induction in response to PB and TCPOBOP was completely eliminated in the CAR-null mice [15].

Subsequent functional analysis has revealed a much broader role of PXR and CAR in xenobiotic regulation. It became evident that both receptors can function as master regulators in regulating additional phase I and phase II enzymes, as well as drug transporters. The mechanism of this broad regulation is the presence of PXR and CAR response elements in the promoter regions of many of these enzyme and transporter genes ([16]). These include the phase I enzymes CYP2C8/9/19 [17,18] and CYP3A7, phase II enzymes

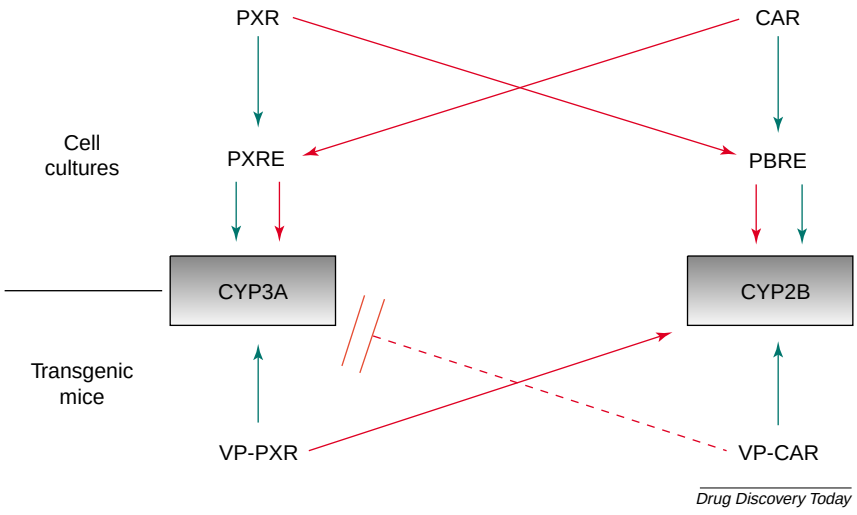

Figure 2. Cross-talk in xenobiotic nuclear receptor-mediated regulation of cytochrome P450 (*CYP*) genes. The reciprocal activation of xenobiotic response genes by pregnane X receptor (PXR) and constitutive androstane receptor (CAR) has been shown in cell cultures. In transgenic mice, activation of PXR induces *CYP3A* and *CYP2B*. In contrast, expression of activated CAR (VP-CAR) in mice induces *CYP2B* but not *CYP3A*. The green and red colored arrows indicate the direct and cross-regulation, respectively. Abbreviations: PXRE, PXR response element; PBRE, phenobarbital response element.

glutathione S-transferases (GSTs) [19], UDP-glucuronosyltransferases (UGTs) [20–22] and sulfotransferases (SULTs) [23,24], the transporters multidrug resistance protein 1 (MDR1) [17,25], MDR2 [26], multidrug resistance-associated protein 2 (MRP2) [27], and the organic anion transporter polypeptide 2 (OATP2) [14]. A broad role of PXR and CAR in xenobiotic regulation was further confirmed by several gene-profiling analyses performed in wild-type, transgenic and knockout mouse models [28,29].

Another unique functional feature of PXR and CAR is the overlap in the genes regulated by these receptors. For instance, PXR can regulate *CYP2B* genes and CAR can regulate *CYP3A* genes. The mechanism of cross-regulation has been shown to be due to shared response elements between receptors, as revealed by receptor-DNA binding analysis and transient transfection and reporter gene assays ([11,30–33], Figure 2). The generation of transgenic mice with hepatic expression of activated receptors enabled the evaluation of potential cross-regulation *in vivo*. The activated VP–PXR and VP–CAR were generated by fusing the VP16 activation domain of the herpes simplex virus to the N-terminal of the receptors. They shared similar DNA-binding specificities with their wild-type counterparts. Genetic activation of PXR *in vivo* caused sustained induction of CYP3A and CYP2B [11,13]. By contrast, in the VP–CAR transgenic mice, although CYP2B was induced as expected, the expression of *CYP3A* was largely unchanged or even slightly suppressed [24] (Figure 2). The lack of CYP3A11 induction in the VP-CAR mice was not due to the unresponsiveness of CYP3A11 in this transgenic line, as the expression of CYP3A11 in the VP–CAR mice remained inducible in response to the CAR ligand TCPOBOP [24].

Species specificity of xenobiotic regulation and the generation of 'humanized' mice

Xenobiotic induction of drug-metabolizing enzymes shows striking species specificity. For example, the antibiotic rifampicin (RIF) has been shown to be a CYP3A inducer in humans but not in rodents, whereas pregnenolone-16α-carbonitrile (PCN), an anti-glucocorticoid, is a rodent-specific CYP3A inducer. The species specificity of drug response has added another challenge in understanding the molecular basis of the regulation of drug-metabolizing enzymes and transporters. In the case of mammalian *CYP3A* gene regulation, previous pharmacological studies in primary cultures of hepatocytes suggest that it is not the promoter structure of the *CYP3A* genes that dictates the pattern of CYP3A inducibility but, rather, it must be a species-specific cellular factor(s). Accumulating evidence has established PXR and CAR as examples of these important cellular factors.

Although rodents are the standard laboratory models in the assessment of drug metabolism and toxicity, they probably are not reliable predictors of the human CYP enzyme inducibility due to the species-specificity of xenobiotic response. Using transfection and transgenic approaches, we have demonstrated that the species origin of the PXR receptor, rather than the promoter structure of *CYP3A* genes, dictates the species-specificity of CYP3A inducibility [13]. The species-specific ligand specificity has been thought to be due to the divergence of amino acid sequences in the ligand-binding domains of the human and mouse PXR receptors (Figure 3a). Four residues in the LBD of hPXR were shown to be crucial for interaction with the hPXR-specific ligand SR12813. When each of these residues was mutated to the corresponding hPXR amino acids, the mouse–human hybrid receptor showed a human-like ligand response profile [34]. The hypothesis that the species origin of the receptor is the determining factor for the species specificity of the ligand response led to the generation of 'humanized' mice, in which the mPXR was deleted via

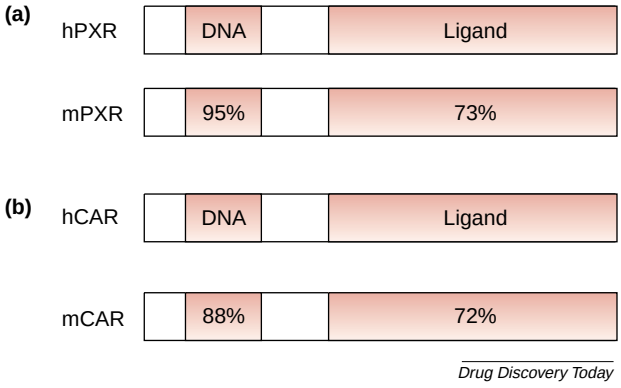

Figure 3. Sequence divergence between the human and rodent xenobiotic receptors. **(a)** Comparison of the human and mouse gene encoding PXR. **(b)** The human and mouse gene encoding CAR. The percentage nucleotide sequence identity in the DNA- and ligand-binding domains is indicated.

homologous recombination and hPXR was introduced into the mouse liver through a liver-specific transgene [13]. These mice exhibited a 'humanized' hepatic xenobiotic response profile, readily responding to the human-specific inducer RIF in a concentration range equivalent to the standard oral dosing regimen in humans [13]. The generation of these mice represents a major step toward generating a humanized rodent toxicological model that is continuously renewable and completely standardized. In addition, a PXR-mediated and mechanism-based transfection- and reporter-gene system has also been shown to be an effective *in vitro* approach to screen for drugs that might be precocious hPXR activators. Although the *in vitro* screen is fast, the availability of hPXR 'humanized' mice offers a unique screening tool to evaluate drug–drug interactions *in vivo*. These humanized mouse models represent important steps in the development of safer human drugs.

The original humanized mice express hPXR exclusively in the liver [13]. The drug-metabolizing enzymes and xenobiotic receptors are also highly expressed in the intestinal tracts, therefore it is conceivable that mouse models with the humanized receptors expressed in the liver and intestine would represent a more complete humanized mouse. This can be achieved using a promoter that can target the expression of hPXR transgene to the liver and intestine. An alternative strategy is to 'knock-in' hPXR in the mouse locus. This would not only direct expression of hPXR both in liver and intestine, but also normalize expression levels and tissue patterns to the endogenous gene.

CAR, like PXR, also exhibits species-dependent ligand specificity, which might also be explained by the divergence in the LBDs between species (Figure 3b). Neither androstenol nor TCPOBOP, the respective mouse CAR antagonist and agonist, affects human CAR activity. Although moderately potent ligands for hCAR have been reported [35], none exhibited potency comparable with androstenol and TCPOBOP toward mCAR. Nevertheless, humanized CAR mice, analogous to the previously reported humanized hPXR mice, have been created [36]. CAR-null mice were resistant to acetaminophen toxicity but with introduction of hCAR, the sensitivity to acetamenophen toxicity was recovered [36]. hCAR was also shown to mediate xenobiotic induction of bilirubin-clearance enzymes [22].

Beyond PXR and CAR: xenobiotic receptor newcomers

In addition to PXR and CAR, the expression of genes encoding drug-metabolizing enzymes and transporters is also subject to regulation by other nuclear receptors, such as the vitamin D receptor (VDR), farnesoid X receptor (FXR), and the retinoid X receptor (RXR). Recent evidence includes the following.

- Activation of VDR by bile acids or vitamin D3-induced *CYP3A* gene expression [37–39]. The VDR-mediated induction of CYP3A, a bile-acid detoxifying enzyme, could account for the preventive effects of vitamin D on colonic carcinogenesis promoted by high-fat diets or toxic bile acids [38] .
- FXR was also shown to regulate the expression of the gene encoding dehydroepiandrosterone sulfotransferase (*SULT2A9*) [40]. Interestingly, PXR, CAR and FXR regulate *SULT2A9* gene expression by sharing the same IR-0 (inverted repeat without a spacing nucleotide) response element found in the promoter of the rodent *SULT2A* genes.
- A liver-specific deletion of the *RXRα* locus in mice causes decreased basal expression of several *CYP* genes, including *CYP3A* [41], which is consistent with the notion that RXR is the obligatory heterodimerization partner for several xenobiotic receptors.

More recently, hepatic nuclear factor 4α (HNF4α) has been shown to determine PXR- and CAR-mediated xenobiotic induction of *CYP3A4*. The *CYP3A4* promoter activity, even in the presence of PXR or CAR, has been known to be most pronounced in liver-derived cells especially the primary hepatocytes, but minimal or modest in non-hepatic cells, suggesting that a liver-specific factor is required for physiological transcriptional response. HNF4α, a liver-enriched orphan receptor, has been proposed to be one such hepatic factor [42]. A specific *cis*-element was identified in the 5′ regulatory sequences of the *CYP3A4* gene, which confers HNF4α binding and permits PXR- and CAR-mediated gene activation. Consistent with the role of HNF4α in *CYP3A* regulation, mice with conditional liver-specific

deletion of HNF4α, had reduced basal and inducible expression of *CYP3A* [42]. The key role of HNF4α in regulating PXR-mediated xenobiotic induction of liver enzymes in fetal livers was also reported independently by Kamiya *et al.* [43].

Beyond xenobiotics: PXR as an 'endobiotic receptor'

Although PXR has been identified as a 'xenobiotic receptor', emerging evidence has pointed to an equally important role of PXR as an 'endobiotic receptor' – that is, it responds to a wide array of endogenous chemicals. Moreover, the activation of PXR by endogenous ligands has implications in several important physiological and pathological conditions.

One family of endogenous PXR ligands identified shortly after the cloning of PXR are bile acids, the catabolic end products of cholesterol metabolism. Despite some beneficial function, excess accumulation of bile acids, such as the secondary bile acid lithocholic acid (LCA), has been shown to cause cholestasis (impaired bile flow) in experimental animals and has long been suspected of doing the same in humans. Xie *et al.* and Staudinger *et al.* showed that PXR acts as an LCA sensor and plays an essential role in detoxification of cholestatic bile acids [14,44]. Activation of PXR by bile acids or other xenobiotic inducers causes the induction of CYP3A, an enzyme that facilitates the detoxification of bile acids. Pretreatment of wild-type mice, but not the PXR-null mice, with PCN reduced the toxic effects of LCA. Moreover, genetic activation of PXR by expressing the activated PXR in the liver of transgenic mice was sufficient to confer resistance to the hepatotoxicity of LCA [44]. Consistent with the notion that activation of PXR facilitates bile-acid detoxification, increased serum levels of bile acids have been suggested to be a factor in the development of pruritis and studies in humans have shown that PXR activator RIF can be used to treat cholestasis-associated pruritis [45,46].

More recently, the bile acid intermediates formed during cholesterol catabolism have been shown to function as PXR agonists. The sterol 27-hydroxylase (CYP27A1) is an important enzyme in regulating the production of bile acids from cholesterol. In humans, mutations in the *CYP27A1* gene were responsible for the cerebrotendinous xanthomatosis (CTX), a genetic disease manifested by the accumulation of 25-hydroxylated bile alcohols, such as 25-tetrol, several 25-pentol isoforms, and possibly hexols and heptols. The clinical hallmarks of the disease include a marked deposit of sterols in a variety of tissues, a decrease in chenodeoxycholic acid production and associated mental retardation, premature atherosclerosis and tendon and brain xanthomas [47].

Surprisingly, the *CYP27*-null mice did not develop the clinical manifestations of CTX [48,49]. This might be due to a dramatic increase in the expression of *CYP3A* with a resultant increase in the CYP3A-mediated hydroxylation and clearance of bile acid intermediates [50–52]. The increase in CYP3A enzyme production in the *CYP27A*-null mice has been reasoned to be due to the activation of mouse PXR by these bile acid intermediates, among which are three potentially toxic sterols, 7α-hydroxy-4-cholesten-3-one, 5β-cholestan-3α,7α.12α-triol, and 4-cholestan-3-one. Interestingly, these intermediates are more potent inducers toward mPXR than hPXR, which might explain, at least in part, why humans lacking functional CYP27A1 do not display a compensatory increase in CYP3A activity [50,51]. These reports establish the existence of a feed-forward regulatory or salvage pathway, in which potentially toxic bile acid intermediates activate PXR and induce their own metabolism and clearance to avoid accumulation.

In addition to bile acids and their intermediates, Vitamin K_2 has recently been shown to be a hPXR/SXR agonist and able to induce the expression of PXR target genes such as *CYP3A4* [53]. Interestingly, Vitamin K_2 treatment of osteosarcoma cells increased mRNA levels for the osteoblast markers, including bone alkaline phosphatase, osteoprotegerin, osteopontin and matrix Gla protein, suggesting a potential novel role of PXR in bone homeostasis [53].

Implication of xenobiotic regulation in human diseases

The implication of PXR- and CAR-mediated gene regulation in drug metabolism and drug interactions has been recognized since the first cloning of these xenobiotic receptors. Consistent with the notion that these enzymes and transporters are also implicated in the biotransformation and homeostasis of many endogenous chemicals that can influence physiological and pathological processes, accumulating evidence has pointed to a role of orphan receptor-mediated xenobiotic regulation both in normal physiology and in disease states.

Bilirubin clearance and jaundice

Bilirubin is the catabolic byproduct of heme proteins, such as β-globin and CYP enzymes. Accumulation of bilirubin in the blood is potentially hepato- and neuro-toxic. For example, an insufficiency in expression of UGT1A1, a key enzyme for the conjugation of bilirubin in Crigler-Najjar syndrome and Gilbert's disease results in severe hyperbilirubinemia. Deficiency of MDR2, a transporter protein responsible for the hepatic excretion of conjugated bilirubin, leads to Dubin-Johnson syndrome, characterized by the accumulation of glucuronidated bilirubin. Both PXR

and CAR have been shown to induce the expression of *UGT1A1* [20–22] and this has been proposed to explain why the transgenic mice expressing a constitutively active form of hPXR had twice the bilirubin clearance of the wild-type mice [21]. Although it remains to be confirmed in transgenic mice, it is possible that PXR and CAR promote the clearance of bilirubin by increasing the expression of multiple key components in the clearance pathway. In addition to UGT1A1, PXR and CAR have been shown to induce the expression of the genes encoding OATP2, GSTA1 and 2 and MRP2. OATP2 facilitates bilirubin uptake from blood into hepatocytes [54]. GSTA1 and 2 reduce bilirubin back efflux from hepatocytes into blood. Interestingly, Huang *et al.* showed that CAR expression is low in human neonates. This functional deficit might be a factor in neonatal jaundice seen in almost 60% of infants and explain the effectiveness of PB for the treatment of this condition [22].

Detoxification of bile acids

Bile acids are the major products of cholesterol catabolism in the liver. Despite their beneficial role in solubilizing biliary lipids and promoting their absorption, accumulation of bile acids can cause irreversible liver damage, resulting in cholestasis [55]. PXR has been shown to be protective against bile acid hepatotoxicity. Both pharmacological (using PCN) and genetic activation of PXR in mice was sufficient to confer resistance to toxicity by LCA [14,44]. By contrast, mice deficient in PXR showed heightened LCA toxicity. The PXR-mediated protection was originally thought to be due to the induction of CYP3A [44]. Subsequent studies suggest that the induction of hydroxysteroid sulfotransferase (*SULT*), another PXR target gene, might also play a role in this protection [23,56]. More recently, Saini *et al.* [24] reported a novel CAR-mediated and CYP3A-independent pathway of bile acid detoxification. Using transgenic mice bearing conditional expression of the activated CAR, Saini *et al.* demonstrated that activation of CAR is both necessary and sufficient to confer resistance to the hepatotoxicity of LCA [24]. Surprisingly, the CAR-mediated protection is not due to the expected and previously characterized CYP3A pathway but, rather, is associated with a robust induction of *SULT* gene expression and increased LCA sulfation. Interestingly, activation of CAR was also associated with an increased expression of the 3′-phosphoadenosine 5′-phosphosulfate synthetase 2 (PAPSS2), an enzyme responsible for generating the sulfate donor PAPS [24]. However, it is not clear whether or not PAPSS2 is a direct transcriptional target of CAR. Analysis of gene knockout mice revealed that CAR is also indispensable for ligand-dependent activation of SULT and PAPSS2 *in vivo*.

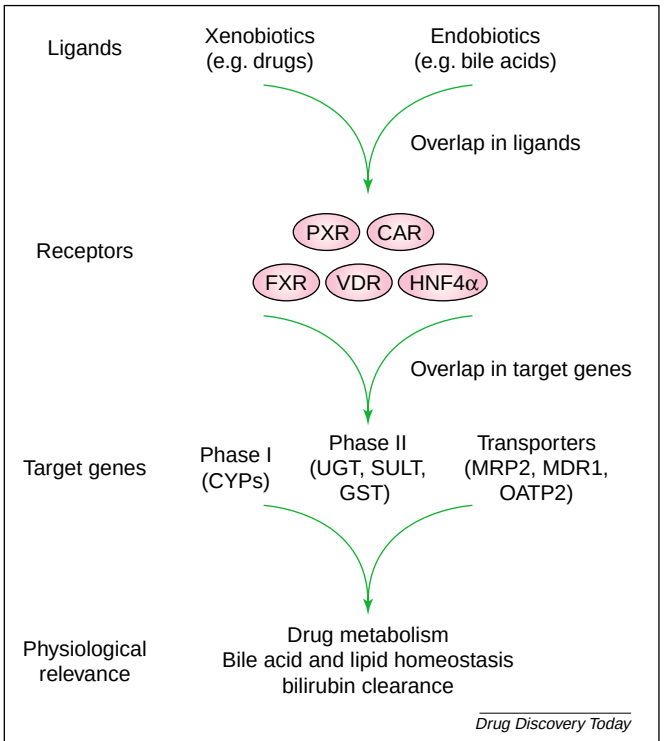

Figure 4. The complexity of mammalian xenobiotic response and its regulation by xenobiotic nuclear receptors. The activation of nuclear receptors by xenobiotic and endobiotic ligands, and subsequent regulation of phase I and phase II enzymes and drug transporters will eventually affect many physiological and pharmacological responses, such as drug metabolism and the homeostasis of bile acids, lipids and bilirubin. Abbreviations: PXR, pregnane X receptor; CAR, constitutive androstane receptor; FXR, farnesoid X receptor; VDR, vitamin D receptor; HNF4α, hepatic nuclear factor 4α; CYPs, cytochrome P450 family; UGT, UDP-glucuronosyltransferases; SULT, sulfotransferase; GST, glutathione S-transferases; MRP2, multidrug resistance-associated protein 2; MDR1, multidrug resistance protein 1; OATP2, organic anion transporter polypeptide 2.

Therefore, CAR has been established to play an essential and unique role in controlling the mammalian sulfation pathways and to facilitate bile-acid detoxification. It is important to note that several other orphan receptors, such as FXR and SHP, also play a crucial role in the homeostasis of bile acids [57–59], but this is beyond the scope of this review.

Summary and perspective

PXR and CAR are two orphan receptors originally identified as ‘xenobiotic receptors’ that regulate CYP gene expression. Subsequent studies have revealed much more complex regulatory pathways governed by these receptors, as summarized in Figure 4.

- Both receptors can function as master regulators to control the expression of phase I and phase II drug-metabolizing

enzymes, as well as members of the drug transporter families.

- Additional nuclear receptors, such as FXR, VDR and HNF4α, have also been shown to participate in the regulatory network.
- There is significant cross-talk among xenobiotic receptors, as manifested by overlap in xenobiotic ligands and target genes. This cross-talk is believed to be the molecular basis for the fail-safe xenobiotic regulatory networks that facilitate host protection.
- Additional functions of these receptors have been identified. A notable function is the establishment of these receptors as 'endobiotic receptors' that respond to a wide array of endogenous chemicals.
- Due to the pleiotropic function of drug-metabolizing enzymes and transporters, the implication of xenobiotic receptor-mediated regulatory pathways has been shown to be far beyond drug metabolism and drug-drug interactions. Additional physiological roles include bile-acid detoxification and bilirubin clearance.

It appears that PXR-controlled xenobiotic regulation is a double-edged sword. One of the remaining challenges is to find out whether the biological actions of PXR make this receptor suitable as a drug target for treatment of human diseases, such as bile acid-associated cholestasis, and for chemoprevention of colon cancers. Both RIF and the herbal remedy St John's Wort have been empirically used to treat cholestatic liver diseases [16]. The relief from cholestasis-associated pruritis and amelioration of cholestasis by RIF was associated with increased 6α-hydroxylation of bile acids, which in turn facilitates glucuronidation by the UGTs at the 6α-hydroxy position. RIF and St John's Wort are both potent agonists of hPXR and *CYP3A* and *UGT* are both PXR target genes, suggesting that the anti-cholestatic effects are mediated by PXR receptor.

Xenobiotic receptors mediate pharmacological and genetic control of the expression of drug-metabolizing enzymes and transporters, therefore the identification of PXR and CAR opens up a new perspective in pharmacogenetics and pharmacogenomics. Pharmacogenetics has traditionally focused on the polymorphism within the coding sequences of genes that encode various enzymes and transporters. Having enhanced our understanding of pharmacogenetics, the cDNA polymorphisms might not explain all of the inter-individual and inter-race variations in enzyme activity. The identification of xenobiotic nuclear receptors leads to several important questions from a pharmacogenomic perspective: (1) are there natural allelic variants of PXR or other xenobiotic receptors that exhibit differential transactivation potency to induce enzymes and transporters? (2) Are there polymorphisms in the promoter regions of target enzyme or transporter genes that might alter the binding affinity of xenobiotic receptors? Recent reports appear to support these notions [60–62]. However, we believe many more comprehensive studies are needed before this pharmacogenomic information can be applied to develop truly 'personalized' medicine.

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