# Pharmaceutical use of mouse models humanized for the xenobiotic receptor

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**The regulation of hepatic cytochrome P450 (CYP) enzymes is implicated in both drug metabolism and drug–drug interactions. The CYP genes are induced by numerous xenobiotics, yet the inducibility shows clear species specificity. Recently, the rodent nuclear receptor PXR and its human homolog, SXR or hPXR, have been established as species-specific xenosensors that regulate CYP3A enzymes. By knocking-out the rodent gene and replacing it with the human receptor, a 'humanized' mouse model has been established. Displaying a human drug-response profile, this mouse represents a unique tool to dissect the drug-induced xenobiotic response and should aid the development of safer drugs.**

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▼ Mammals continuously confront numerous foreign chemicals termed 'xenobiotics', including prescription and non-prescription drugs, herbs, toxins and environmental pollutants. Many xenobiotics can manifest adverse side effects based on a variety of parameters, including overall blood levels, frequency of ingestion and presence of other drugs. Regulation of detoxification and clearance of xenobiotics from the body is largely mediated by the transcriptional induction of the hepatic superfamily of cytochrome P450 (CYP) genes. The enzymatic products of these genes catalyze the conversion of xenobiotics to polar derivatives that can be readily cleared from the body [1,2]. Among the CYP products, the 3A and 2B isoenzymes are of particular medicinal significance, participating in the metabolism of 50–60% and 15–20% of clinical drugs, respectively. In addition, they both contribute to the metabolism of herbal medicines and a variety of endogenous steroids, bile acids and lipid metabolites [1].

The combined metabolic versatility of the CYP3A and CYP2B enzymes coupled with the broad, albeit more restricted inducibility of the CYP genes by numerous pharmaceutical compounds constitutes the molecular basis for many clinical drug–drug interactions. Such interactions pose one of the most vexing problems in drug development. These problems arise when P450 inducers, such as glucocorticoids, phenobarbital or rifampicin, are administered concurrently with medicines that are normally metabolized by CYP enzymes [3]. Because CYP enzymes can recognize a large spectrum of pharmaceutical substrates, a CYP gene-inducing compound is potentially capable of affecting the metabolism and clearance of any co-consumed drugs. For example, St John's wort, a popular herbal remedy for depression, has been reported to trigger severe adverse interactions with several clinical drugs. Such drug interactions are likely to be the result of the induction of CYP3A by St John's wort and the subsequent increased metabolism and/or decreased bioavailability of co-metabolized drugs, such as oral contraceptives, the HIV protease inhibitor indinavir and the immunosuppressant cyclosporin [4–7]. In the case of birth control pills, the use of St John's wort enhances drug clearance, increasing contraceptive failure and thus the birth of 'miracle babies'.

Despite the widely appreciated medical relevance and much previous research, the molecular basis for drug induction of CYP3A and CYP2B enzymes remained elusive for many decades. The problem of mechanism was compounded by the fact that the xenobiotic response can be induced by divergent chemical classes of pharmaceutical and natural compounds. Moreover, unlike conserved hormonal responses, *CYP* gene inducibility

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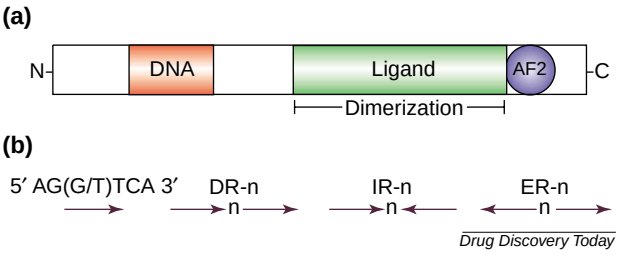


**Figure 1.** Schematic representation of a typical nuclear receptor. Nuclear hormone receptors are ligand-dependent transcription factors. **(a)** The conserved DNA-binding and ligand-binding domains, as well as the transactivation function (AF2), are shown. **(b)** Nuclear receptor response elements are composed of direct repeats (DR-n), inverted repeats (IR-n) and everted repeats (ER-n) of the hexad core sequence AG(G/T)TCA. Specificity for different hormone responses is given by the number of nucleotides (n) spaced between the two core elements.

shows clear species specificity, adding to the mystery of xenobiotic response. For example, the antibiotic rifampicin is a specific CYP3A inducer in humans and rabbits, whereas PCN (pregnenolone-16α-carbonitrile), an anti-glucocorticoid, is a rodent-specific CYP3A inducer (see Ref. [8] and references therein). These observations made it difficult to anticipate the molecular features underlying the response.

## Nuclear receptor superfamily and orphan nuclear receptors

The nuclear receptor superfamily includes receptors for hormonal ligands, such as steroids, retinoids, thyroid hormone, vitamin D, prostaglandins and numerous dietary fats. Nuclear receptors are ligand-dependent transcription factors that bind to lipophilic signaling molecules and subsequently control the expression of target genes. These receptors transduce a complex array of extracellular signals into transcriptional responses by coordinate activation of a network of target genes [9].

Sequence analysis and functional studies reveal that the receptors share a common modular structure with a conserved N-terminal DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) (Fig. 1a). The DBD contains two zinc-finger motifs, which mediate interaction with specific DNA sequences known as hormone response elements (HREs). For the nonsteroidal members of the receptor superfamily, the HREs consist of a minimal core hexad consensus sequence, 5′ AG(G/T)TCA 3′, that can be configured into a variety of structured motifs (Fig. 1b). The LBD, in addition to determining ligand specificity, contains a ligand-inducible transactivation function (AF2) and a motif that directs binding with the common heterodimerization partner RXR (retinoid X receptor).

In the absence of a ligand, nuclear receptors are often associated with a nuclear receptor corepressor complex, resulting in inhibition of basal transcription activity of the associated promoter [9,10]. Following hormone treatment, nuclear receptors undergo conformational changes that lead to the release of the corepressor complex and recruitment of coactivators, which function by remodeling chromatin to enable transcriptional activation via a specific HRE. Therefore, nuclear receptors are ligand-dependent and response-element-specific transcription factors. As a consequence, they are perfectly suited to control a set of target genes, both within a single cell and throughout the body, to modulate a coordinated hormonal response.

Orphan nuclear receptors include gene products that are structurally related to nuclear hormone receptors but lack known physiological ligands. Found in almost all animal species examined, orphan receptors represent a diverse and ancient component of the nuclear receptor superfamily. It has become clear that orphan nuclear receptors provide a unique and pivotal resource to uncover new regulatory pathways that affect both health and human diseases [11,12]. In the past 3–4 years, emerging evidence has pointed to a unique role for orphan nuclear receptors in the regulation of drug-metabolizing CYP enzymes by functioning as atypical pleotropic receptors for a remarkable diversity of xenobiotic and endobiotic compounds (Fig. 2).

## Identification of PXR and SXR as xenosensors

The human orphan receptor SXR (steroid and xenobiotic receptor), also known as hPXR and hPAR, and its rodent ortholog PXR (pregnane X receptor) were isolated as candidate xenobiotic receptors (xenosensors) postulated to regulate CYP3A genes [13–16] (for reviews, see Refs [11,17–19]). The hPXR/SXR was identified in a search for a potential human homolog for the xenopus BXR (benzoate X receptor) [20]. BXR is a benzoate receptor that is expressed during early development of *Xenopus laevis*. Benzoates comprise a distinctive molecular class of nuclear receptor ligands and their activity suggests that BXR might control a previously unsuspected and still uncharacterized signaling pathway.

The identification of the human and mouse PXR as xenobiotic receptors was initially suggested by three related observations [13–16]:

- The human and rodent receptors can be activated by a variety of compounds that are known to induce hepatic P450 enzymes, including prescription drugs such as rifampicin and nifedipine, steroid receptor agonists such as dexamethasone and estrogen, steroid antagonists such as PCN and tamoxifen, and bioactive dietary compounds such as phytoestrogens.

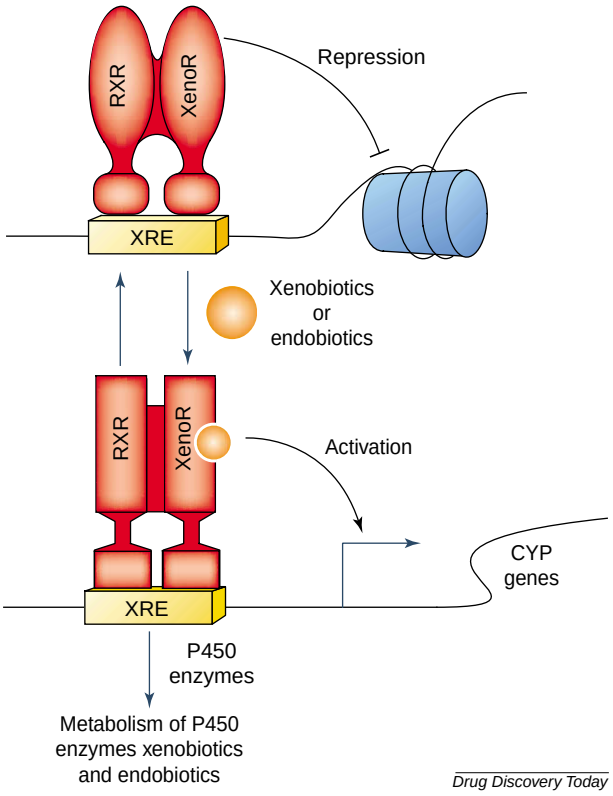


**Figure 2.** Nuclear receptor-mediated *CYP* gene activation. Abbreviations: RXR, retinoid X receptor; XenoR, xenobiotic receptor; XRE, xenobiotic response element.

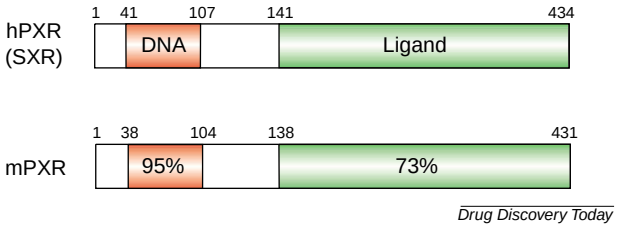


**Figure 3.** Comparison of the human and mouse PXR genes. The percentage nucleotide sequence identity in comparisons of the DNA- and ligand-binding domains of SXR with those of the mouse PXR is indicated. Abbreviations: PXR, pregnane X receptor; SXR, steroid and xenobiotic receptor.

- The receptors are expressed at high levels in the liver and small intestine, two key tissues expressing inducible CYP enzymes that are responsible for steroid and xenobiotic metabolism.
- Receptor-specific response elements are present in the 5′ promoters of genes encoding inducible CYP enzymes, such as the mammalian *CYP3A* genes.

Molecular genetic approaches have been used to unequivocally establish mouse and human PXR as xenosensors mediating the hepatic xenobiotic response. Specifically, it has been shown that targeted disruption of the mouse PXR gene via homologous recombination abolishes induction of CYP3A by dexamethasone or PCN [21,22]. In wild-type animals, PCN pre-treatment is known to alleviate the therapeutic action of a variety of test drugs via its ability to promote rapid inactivation by induction of CYP3A. However, PCN pre-treatment of PXR knockout mice fails to show any protection against paralytic sedatives, such as zoxazolamine and tribromoethanol [23]. This loss of xenoprotection is presumably a result of the failure of PXR knockout mice to mount a hepatic response to activate CYP3A. By contrast, hepatic expression of an activated hPXR/SXR (VPhPXR/VPSXR) transgene causes constitutive activation of *CYP3A* gene expression, resulting in complete protection against these drugs [21]. In addition, the VPhPXR/VPSXR mice show clear resistance to the highly hepatotoxic bile acid LCA (lithocholic acid), which is associated with biliary cholestasis [23].

## Humanized xenobiotic response in mice expressing human nuclear receptor hPXR/SXR

Both hPXR/SXR and mPXR are highly expressed in the liver and small intestine and share many functional properties, in particular, the regulation of *CYP3A* genes. However, these two orthologs are pharmacologically distinct in that strong activators of one receptor are often poor activators of the other. For example, rifampicin is a potent activator for hPXR/SXR but shows only marginal effects on mPXR *in vitro* and little inducibility of CYP3A *in vivo*. By contrast, PCN and dexamethasone are strong inducers of mPXR but act poorly on hPXR/SXR. This species-specific ligand profile is reflected by the sequence divergence in the LBDs of the mouse and human receptors (Fig. 3).

The crystal structure of the hPXR/SXR LBD has recently been solved [24], revealing an overall fold similar to other members of the nuclear receptor superfamily. However, it also revealed several distinct structural features, which appear crucial to its function as a promiscuous xenobiotic receptor that enables binding of a diverse range of chemical structures [24]. The hydrophobic ligand-binding cavity of hPXR/SXR is composed of a large, smooth surface containing only a small number of polar residues, suggesting that activators need not conform to a restricted conformation or orientation. Thus, SR12813, a candidate cholesterol-lowering drug that serves as an hPXR/SXR ligand, was found to bind in three distinct orientations. Effectively, it is being recognized as three different compounds. Based on site-directed mutational analysis, the position and nature of these polar residues were found to be crucial for

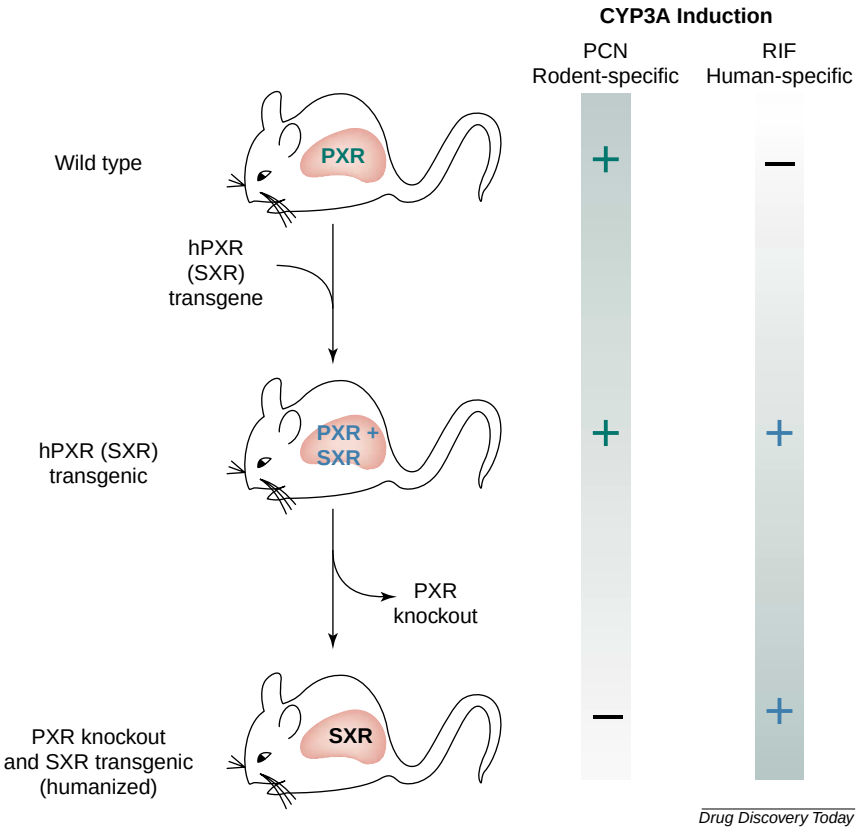


**Figure 4.** Establishment of mouse models with humanized drug response profile. '+' and '–' mean induction and no induction, respectively. Abbreviations: PCN, pregnenolone-16α-carbonitrile; PXR, pregnane X receptor; RIF, rifampicin; SXR, steroid and xenobiotic receptor.

establishing the precise pharmacological activation profile of hPXR/SXR [24]. Indeed, conversion of four amino acids of mouse PXR that correspond to the SR12813-interacting residues in hPXR/SXR produces a hybrid mouse–human PXR that was no longer activated by PCN and was only weakly activated by hPXR/SXR-specific activator SR12813 in reporter assays [24]. The structural and pharmacological differences between hPXR/SXR and mPXR and that of other species might reflect the difference in the diets of rodents and primates and the evolutionary need to respond to a different set of ingested nutrients and xenobiotics.

### *A xeno-sensor mouse*

Based on a hypothesis that the species origin of the receptor is the determining factor for the ligand specificity between species, we have created transgenic mouse models to determine whether the human receptor is sufficient to establish a human response profile. First, hPXR/SXR transgenic mice were generated by expressing the hPXR/SXR in the mouse liver. The liver-specific expression of the transgene was accomplished by using the mouse albumin promoter. Because the resulting mice harbor both mPXR and hPXR/SXR in their livers, the transgenic mice exhibit a chimeric or combined CYP3A response to both the rodent-specific inducer PCN and the human-specific inducer rifampicin (Fig. 4 and Ref. [21]). These results imply that mice only expressing hPXR/SXR could be fully humanized for the xenobiotic response. These animals were created by breeding the hPXR/SXR transgene into the mPXR knockout background. In contrast to the null mice that are devoid of CYP3A induction by steroids, replacement of mPXR with transgenic hPXR/SXR restores xenobiotic regulation with a humanized response profile. Therefore, these experiments provide compelling evidence that PXR functions as a species-specific xenosensor mediating the adaptive hepatic response. This is also one of the rare examples in which replacing a single transcriptional regulator enables conversion of species-specific gene regulation.

## Advantages of 'humanized' mice in pharmaceutical development

The creation of mouse models with humanized xenobiotic response is of significant practical use in pharmaceutical development. In part, this resides in the reality that rodents are standard components in the assessment of potential toxicity in the development of candidate human drugs. At the same time, they are highly unreliable predictors of the human xenobiotic response underlying drug–drug interactions because of the species-specificity of xenobiotic response. CYP3A induction is believed to influence the therapeutic index for up to one-third of all drugs depending on several factors, such as multiple prescriptions, variable compliance and individual differences in response to the same drug. Thus, the extent to which single drugs, like rifampicin, can up-regulate CYP3A is of pharmacological importance because this activation will not only affect rifampicin metabolism but, in principle, any compounds processed by cytochrome metabolism [25,26].

To date, there has been no reliable system outside of humans to directly and quantitatively assess the drug–drug interactions. Human primary cultured hepatocytes are valuable because, although they come from individuals, they are neither standardized nor easily obtained. Thus,

the generation of Alb–SXR/hPXR transgenic mice represents a major step toward generating a humanized rodent toxicological model that is continuously renewable and completely standardized. These mice readily respond to human inducers, such as rifampicin, in the equivalent range of the standard oral dosing regimen in humans (300–600 mg per 70 kg man) and exhibit similar pharmacokinetics of CYP3A regulation [21,27]. A 'fully' human profile of CYP3A inducibility is obtained in the mPXR null/hPXR (SXR) transgenic mice. This dual loss-of-function and gain-of-function in mouse offers additional advantages for its potential use in pharmacological studies and pharmaceutical development. For example, the humanized hPXR/SXR transgenic mice enable drug metabolism studies, including pharmacokinetics, therapeutic index and toxicity of pharmaceuticals in the exclusive presence of human PXR. The Alb–VPSXR/VPhPXR transgenic mice that constitutively over-express CYP3A provide a unique pharmaceutical model that enables evaluation of these same drug metabolism studies in the presence of enhanced levels of CYP3A enzyme. This enables the *in vivo* generation and measurement of maximal amounts of metabolic intermediates, some of which could be responsible for toxic side effects.

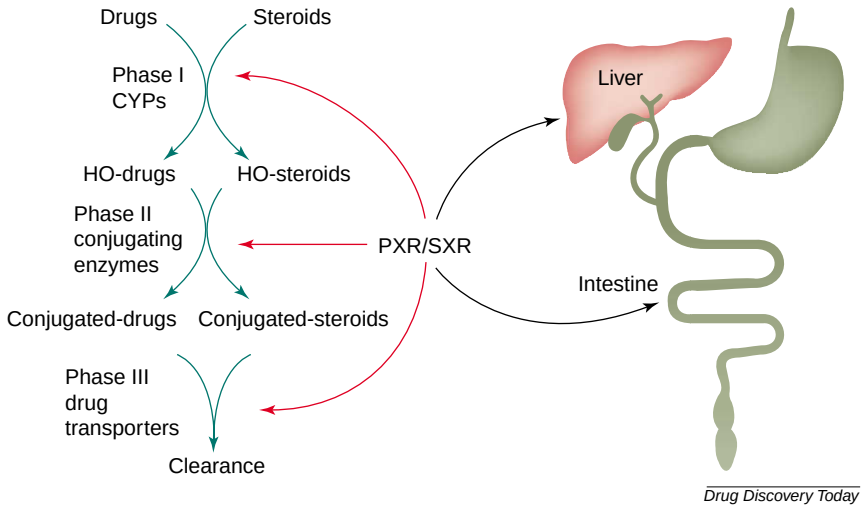


**Figure 5.** Schematic representation of the mammalian xenobiotic response. The steroid and xenobiotic receptor (SXR) might function as a master regulator of xenobiotic response by activating both phase I and II xenobiotic enzymes, as well as the drug transporters, throughout the enterohepatic axis. Abbreviations: HO, hydroxylated; PXR, pregnane X receptor.

Considering the widespread problem of drug–drug interactions and the inherent unpredictability of this process, coupled with the potential for liver toxicity, there is little obvious benefit in any drug that additionally or spuriously activates hPXR/SXR. Thus, based on these observations, profiling of hPXR/SXR activation could represent a judicious screen during the drug development process for selecting therapeutically active but hPXR/SXR-neutral compounds. From a practical viewpoint, a transfection- and cell-based assay employing hPXR/SXR expression vectors and reporters also represents a quantitative and simple *in vitro* approach in screening for drugs that might be precocious hPXR/SXR activators. Although the *in vitro* screen is fast, the availability of hPXR/SXR transgenic mice offers a unique screening tool to evaluate drug–drug interactions in a 'humanized' *in vivo* system. The humanized mouse models represent important steps in the development of safer drugs.

## Challenges and opportunities in 'nuclear xenobiology'

The initial characterization of the 'humanized' mice relied primarily on the use of classical CYP inducers, such as rifampicin and phenobarbital. A more systemic analysis of the effects of the most common prescription drugs becomes necessary to further validate the use of the mouse models in pharmaceutical development. It is encouraging that – at least in cell culture systems – additional drugs, such as HIV protease inhibitors [28] and the cancer chemotherapeutic drug taxol [29], have been shown to activate hPXR/SXR. Moreover, analogs of both of these classes have been identified that do not activate hPXR/SXR and, as predicted, do not activate drug metabolism or clearance. These observations strongly support the hypothesis that the activation of hPXR/SXR can be used to profile compounds and thus guide chemistry toward agents that have less potential for drug–drug interactions.

### *Beyond CYP enzymes and liver: PXR as a master regulator of the mammalian xenobiotic response*

The metabolism and elimination of xenobiotics are accomplished by the concerted action of the oxidative phase I CYP enzymes, the phase II conjugating enzymes and drug transporters (Fig. 5, and Ref. [30]). The presence of candidate PXR-response elements in genes encoding additional phase I CYP enzymes, such as CYP2A, CYP2C and CYP2E, as well as the phase II UDP-glucuronosyltransferase gene products (UGTs), raises the potential for a broader physiologic function for PXR [13]. Although each of these enzymes is involved in drug metabolism and steroid catabolism [1], whether they are induced by PXR is unclear. If so, this would have widespread implications in understanding the nature and properties of the drug-induced xenobiotic response.

In addition to its ability to mediate CYP3A induction, several PXR agonists have been shown to induce expression of MDR1 (also known as p-glycoprotein) in both primary hepatocytes and colon cancer cells [29]. The *MDR1* gene encodes a drug transporter, ABCB1, which regulates drug efflux. In addition to MDR1, the OATP2 [$Na^+$-independent organic anion transporter 2] and MRP2 (multiple resistance protein 2, also known as ABCC2) [28,31] were also shown to be induced by PXR activators. Even though the molecular mechanism of transporter gene regulation remains to be further established, the current evidence implicates PXR in drug efflux, adding a new dimension to the action of this receptor system. However, whether these additional phase I and II enzymes, as well as the drug transporters, are regulated qualitatively and quantitatively in 'humanized' hPXR/SXR transgenic mice as they are in humans remains to be seen. The VPSXR/VPhPXR transgenic, PXR null, and the combined mPXR null/hPXR (SXR) transgenic mice are invaluable tools to establish the *in vivo* regulation of xenobiotic target genes.

In addition to the liver, the intestine is another key tissue in controlling metabolism and clearance of xenobiotics, as well as the bioavailability of drugs. Both drug metabolizing enzymes and drug transporters are highly expressed in the intestine. In fact, the intestinal P-glycoprotein is a versatile drug efflux pump whose substrate specificity overlaps that of CYP3A enzymes. Interestingly, PXR and constitutive androstane receptor (CAR) are also highly expressed in the intestine, indicating a role for PXR in the regulation of intestinal drug metabolizing enzymes and transporters. Indeed, PXR null mice exhibit loss of CYP3A induction by dexamethasone or PCN in both liver and intestine [21,22]. It is conceivable that mouse models with the humanized receptors expressed in both the liver and the intestine would represent an important step toward generating a more completely humanized mouse. One such strategy is to 'knock-in' the hPXR/SXR in the mouse locus. This would not only direct expression of hPXR/SXR in both liver and intestine but would also normalize expression levels and tissue patterns to the endogenous gene. In summary, we propose that PXR could function as master regulator of the mammalian xenobiotic response by controlling the expression of multiple-phased drug metabolizing and clearance enzymes throughout the enterohepatic axis (Fig. 5).

### *Beyond PXR: a nuclear receptor-mediated metabolic safety net*

In addition to PXR, other nuclear receptors, such as CAR, have also been implicated in drug-induced xenobiotic gene regulation. The identity of CAR as a xenosensor was suggested by two observations: (1) selective androstane metabolites inhibit the constitutive transcriptional activity of CAR [32]; and (2) CAR activates the phenobarbital response element (PBRE) found in promoters of PB-inducible CYP2B genes [33], and this activation was potentiated by xenobiotic compounds, such as phenobarbital and its derivative TCPOBOP [34–38]. The action of CAR as a xenobiotic receptor has been confirmed by a recent study, in which disruption of the mouse CAR locus by homologous recombination resulted in loss of phenobarbital and TCPOBOP activation of the CYP2B10 gene [39]. Interestingly, CAR, like PXR, also exhibits species-dependent ligand specificity. Neither androstenol nor TCPOBOP affects human CAR activity [37] and, strangely, the ligands for human CAR still remain elusive. However, based on the PXR/SXR studies, it is probable that generation of humanized CAR mice by transgenic or knock-in approaches will create a valuable complement to the hPXR/SXR mice. Eventually, the double mPXR/mCAR knockout crossed with double hPXR and hCAR transgenic mice will provide a sophisticated strain of mice to simultaneously monitor the induction of *CYP3A* and *CYP2B* genes and the full genetic network in a simple mouse model. This could be important because emerging evidence has pointed to the existence of receptor crosstalk between these two xenosensors. For example, PXR and CAR were originally thought to independently regulate *CYP3A* and *CYP2B* genes, respectively. Surprisingly, several groups have recently demonstrated that PXR and CAR can cross-regulate each other's target genes [35,40,41] (for a review, see Ref. [17]). Such reciprocal regulation is accomplished via adaptive recognition of DNA response elements, as shown in Fig. 6. Thus, hPXR/SXR can bind the nuclear receptor binding sites found in the PBRE to regulate *CYP2B* gene expression. In a type of functional symmetry, CAR can also activate CYP3A through its previously defined hPXR/SXR response elements. Despite some limitations, the reciprocal regulation of *CYP* genes by multiple xenobiotic receptors not only provides an explanation for the dual activation property of certain drugs, but also reveals the existence of a fail-safe mechanism; that is, a metabolic safety net to provide back-up protection against xenotoxicants.

## Perspective

The identification of nuclear receptors as xenosensors provides a major step in understanding the genetic mechanisms controlling the expression of drug metabolizing enzymes. The established mouse models described above, as well as future humanized animal models, advance our understanding of the molecular complexity of drug-induced xenobiotic response and provides an *in vivo* platform to aid pharmaceutical development.

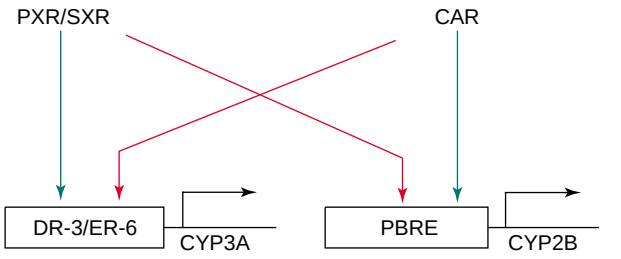


**Figure 6.** Crosstalk between xenobiotic receptors. PXR/SXR and CAR can cross-regulate each other's target genes via reciprocal recognition of DNA response elements. DR-3/ER-6 and phenobarbital response element (PBRE) are nuclear receptor response elements found in the promoters of *CYP3A* and *CYP2B* genes, respectively. Abbreviations: CAR, constitutive androstane receptor; DR, direct repeat; ER, everted repeat; PXR, pregnane X receptor; SXR, steroid and xenobiotic receptor.

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