Published in final edited form as:
Expert Opin Drug Metab Toxicol. 2008 July ; 4(7): 895–908. doi:10.1517/17425255.4.7.895.

The Pregnane X Receptor: From Bench to Bedside

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Abstract

Background—The pregnane X receptor (PXR; NR1I2), a member of the nuclear receptor superfamily, regulates the expression of metabolic enzymes and transporters involved in the response of mammals to their chemical environment.

Objective—To summarize the functions and clinical implications of PXR.

Methods—In the current review, the clinical implications of PXR are discussed, and the use of genetically engineered PXR mouse models is highlighted.

Results/conclusion—Recent advances in mouse models, including *Pxr*-null and *PXR*-humanized mice, provide *in vivo* tools for evaluating the physiological functions of PXR and its role in controlling xenobiotic metabolism and transport. By using the PXR knockout and humanized mouse models, PXR was found to influence drug-drug interactions, hepatic steatosis, and the homeostasis of vitamin D, bile acids and steroid hormones. PXR was also shown to influence inflammatory bowel diseases.

Keywords

clinical implications; nuclear receptor; pregnane X receptor; *PXR*-humanized mouse; *Pxr*-null mouse

1. Introduction to the pregnane X receptor

The pregnane X receptor (PXR; NR1I2), identified in 1998 as a member of the nuclear receptor (NR) superfamily, is expressed in liver and intestine, front line organs involved in the absorption, distribution, metabolism and elimination of xenobiotics and endobiotics, in all mammalian species examined to date [1–4]. PXR, generally regarded as a sensor activated by exogenous and endogenous chemicals, regulates a large number of enzymes and transporters involved in the response of mammals to their chemical environment [5,6]. PXR activation is ligand dependent; following ligand binding, PXR forms a heterodimer with the retinoid X receptor (RXR) that binds to PXR response elements, located in the 5′-flanking regions of PXR target genes, resulting in their transcriptional activation. PXR is mainly associated with the cellular response to xenobiotics, including induction of enzymes involved in drug oxidation and conjugation, as well as induction of xenobiotic and endobiotic transporters [7]. Metabolic

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Declaration of interest
The authors have no conflicts of interest to declare.

enzymes and transporters induced by PXR activation affect the pharmacokinetics of both xenobiotics and endobiotics (Figure 1).

Unlike other NRs such as the steroid receptors that interact selectively with their physiological ligands, PXR ligands are structurally diverse and include prescription drugs, herbal medicines, dietary supplements, environmental pollutants, and endobiotics [8–10]. Indeed, elucidation of the three-dimensional structure of the PXR ligand-binding domain (LBD) revealed that it has a large, spherical ligand binding cavity that allows it to interact with a wide range of hydrophobic chemicals [11,12]. Many PXR ligands have been identified among prescription drugs, and include the antibiotics rifampicin, clotrimazole, and ritonavir; the antineoplastic drugs cyclophosphamide, cyproterone acetate, taxol, tamoxifen, and RU486; the anti-inflammatory agent dexamethasone; the anti-type 2 diabetes drug troglitazone; the anti-hypertensive drugs nifedipine and spironolactone; and the sedatives glutethimide and phenobarbital [9]. Commonly used herbal medicines can also activate PXR, such as St. John's Wort, Gugulipid®, and kava kava [13]. Among dietary supplements, vitamins K_2 and E have been established as weak PXR activators [14,15]. Recently, multiple research groups reported that a number of environmental pollutants are PXR ligands, such as organochlorine pesticides, and polybrominated diphenyl ether flame retardants [16,17]. In addition, some endobiotics were identified as PXR ligands, including certain bile acid precursors, estrogens and progestogens [2,18–20].

PXR activation regulates a large network of genes. In rats treated with pregnenolone 16α-carbonitrile (PCN), a rodent specific PXR ligand, 138 genes were induced, while 82 genes were repressed [21]. Activation of PXR in *PXR*-humanized mice on a *Pxr*-null background revealed that 146 genes were differentially expressed, with 56 upregulated and 90 downregulated [7]. It should be noted, that most of the genes detected in these studies were not validated by direct quantification of the mRNAs and many may not be direct targets of PXR. Thus, the numbers of putative target genes are likely to be overestimated. However, among the PXR upregulated genes found in these studies were those encoding multiple metabolic enzymes and transporters, many of which are known PXR target genes with PXR regulatory elements. The most significantly induced P450s were from the CYP3A and CYP2B subfamilies [1,22]. The human *CYP3A* genes located on chromosome 7q22.1, consist of four members, *CYP3A4*, *CYP3A5*, *CYP3A7*, and *CYP3A43* [23]. Among these, CYP3A4 is among the most abundant of the P450s expressed in liver, and participates in the metabolism of more than 50% of marketed drugs, and some endogenous substrates such as steroids and bile acids [24]. CYP2B, although not as abundant as CYP3A, plays an important role in metabolism of the antidepressant bupropion, the non-nucleoside reverse transcriptase inhibitor efavirenz, and the alkylating antineoplastic drugs ifosfamide and cyclophosphamide. UDP-glucuronosyltransferases and glutathione *S*-transferases are the major phase 2 conjugating enzymes induced following PXR activation [25,26]. Both oxidation and conjugation reactions generally result in the formation of more polar metabolites of the original chemicals. PXR activation also upregulates transporters, such as P-glycoprotein, multidrug resistance-related protein-3, and organic anion transporting polypeptide-2 (OATP2) [27–29]. OATP2 is an influx transporter, while both P-glycoprotein and multidrug resistance-related protein-3 are efflux transporters. P-glycoprotein is an ATP-dependent efflux pump with broad substrate specificity, and serves as a defense mechanism against potentially harmful chemicals and their metabolites. Multidrug resistance-related protein-3 is involved in the transport of biliary and intestinal excretion of organic anions. Due to the role of PXR in regulation of metabolic enzymes and transporters, PXR activation can greatly affect the fate of chemical substances, such as drugs, hormones, nutrients and toxins. The current review summarizes and discusses the role of PXR activation in drug-drug interactions, hepatic steatosis, vitamin D homeostasis, bile acids homeostasis, steroid hormones homeostasis, and inflammatory bowel diseases. The use of genetically modified PXR mouse models is highlighted.

2. Animal models used in the study of PXR

High-throughput *in vitro* PXR activation and binding assays have been used to identify PXR ligands [30,31]. Primary cultures of human hepatocytes have also been employed in studies on PXR activation and PXR target gene regulation, and for prediction of drug-drug interactions [32]. However, the general problem remains one of extrapolating from *in vitro* findings to the clinical situation *in vivo*, and *PXR*-expressing cell lines were limited to the study of overall functions of PXR functions, such as PXR-mediated adverse drug interactions and toxicities. In this regard, animal models provide appropriate *in vivo* tools for evaluating the functions of PXR in a whole animal system. In this regard, animal models provide appropriate *in vivo* tools for evaluating the functions of PXR. Recent advances in animal models for PXR include the *Pxr*-null mouse and the *PXR*-humanized mouse models (Figure 2).

2.1 *Pxr*-null mouse models

Two *Pxr*-null mouse models were generated successfully by using similar strategies of disrupting the mouse *Pxr* gene by homologous recombination [33,34]. The *Pxr*-null mice did not display any overt phenotypic abnormalities and extensive biochemical analysis did not reveal any significant differences in serum cholesterol, triglycerides, glucose, or liver enzyme levels. In addition, *Pxr*-null mice developed and reproduced normally [5,12]. These data suggest that PXR is not essential for mouse development or physiological homeostasis. However, as expected, the *Pxr*-null mice did not respond to PXR ligands; hepatic PXR target genes were strongly induced in wild-type mice treated with PCN, but not induced in *Pxr*-null mice [33,34]. Thus, the *Pxr*-null mouse was validated as a reliable model to identify PXR-dependent signaling pathways (Figure 2).

2.2 *PXR*-humanized mouse models

The major reason for generation of the *PXR*-humanized mouse model is the marked species differences in response to PXR ligands. Drugs such as rifampicin, clotrimazole, and troglitazone activate human PXR but are weak activators of rat and mouse PXR. In contrast, dexamethasone and PCN activate the rodent PXR but are weak activators of human PXR [12]. The molecular basis for species differences in response to PXR ligands is likely to be amino acid difference in the LBD or in regions of the protein that affect the structure of the LBD. Like all the other NRs, PXR has a highly conserved DNA binding domain of ~70 amino acids and a LBD of ~300 amino acids in the C-terminal portion of the protein [1,35]. The PXR orthologs in human, rhesus monkey, rabbit, rat, and mouse, share >94% amino acid identity in the DNA binding domain. However, the amino acid differences in the LBD are more extensive; human PXR shares 82% sequence similarity with rabbit, 77% with mouse, 76% with rat, and 52% with fish in the LBD [10,12,36]. While the specific amino acid residues responsible for the differences in ligand affinity between species have not been elucidated, the structure of the human PXR LBD reveals an expansive ligand-binding pocket that differs between species [37,38].

Generation of a *PXR*-humanized mouse model would provide a solution to the problem of species differences in ligand specificity. To this end, two *PXR*-humanized mouse models were generated successfully, the Alb-hPXR mice and BAC-hPXR mice [33,39]. The Alb-SXR/hPXR mouse model was produced by use of a cDNA, while the BAC-hPXR mouse model was generated with a bacterial artificial chromosome (BAC). These *PXR*-humanized mouse models were developed in the *Pxr*-null mouse background and responded to the human-specific PXR ligand rifampicin; no significant response was found with the rodent-specific PXR ligand PCN [33,39]. The notable differences between these two humanized models is that the human PXR cDNA is driven by heterologous promoter that yields liver-specific expression, while the BAC transgene, contains the complete *PXR* gene is under control of the native human *PXR* promoter.

This distinction is quite important since PXR is also expressed in the gut where it can influence the metabolism and transport of drugs [39,40]. A third model, in which the human PXR protein was fused to a viral VP16 coactivator, was also generated yielding ligand independent gene activation resulting in constitutive activation of PXR target genes [33,41]. The *PXR*-humanized mice serve as models to surmount the species differences in response to PXR ligands (Figure 2).

3. Clinical implications of PXR

PXR activation regulates enzymes and transporters that affect the metabolism and elimination of xenobiotics and endobiotics which could result in altered clinical responses. Indeed, data from preclinical research and *in vivo* studies using the *Pxr*-null and *PXR*-humanized mouse models, together with clinical reports, revealed the role of PXR in drug-drug interactions, triglyceride homeostasis and hepatic steatosis, vitamin D homeostasis, metabolic bone disorders, bile acid homeostasis, steroid hormone homeostasis, and the potential benefit of PXR in inflammatory bowel diseases (Figure 3).

3.1. PXR mediated drug-drug interactions

The most common clinical implication for the activation of PXR is the occurrence of drug-drug interactions (Figure 3 and 4). Multiple-therapy regimens are the major reason for drug-drug interactions, especially involving patients with tuberculosis, cancer, HIV, cardiovascular disease, and diabetes. Drug-drug interactions have become a critical issue in health care, as serious adverse drug reactions in patients frequently require hospitalization, and some result in permanent disability or death [42]. Therefore, understanding the mechanism and predicting drug-drug interactions is an important goal for the improvement of drug safety. The identification of PXR revealed a molecular mechanism for many drug-drug interactions. When two or more drugs are combined, and one is a PXR ligand, and others are the substrates of PXR target gene encoded enzymes or transporters, drug-drug interactions can occur. PXR mediated drug-drug interactions are based on pharmacokinetics and result from the interference of the metabolic clearance of one drug by another co-administered drug. The clinical consequences of PXR mediated drug-drug interactions are generally decreased therapeutic efficacy and, occasionally, increased drug toxicity (Figure 4).

3.1.1 Decreased efficacy *via* PXR-enhanced metabolism—Rifampicin, a human PXR ligand used at a high dose and long-term for tuberculosis treatment, is associated with PXR-mediated drug-drug interactions in humans [43]. Rifampicin interacts with over 100 drugs, most notably drugs that are CYP3A substrates, including oral contraceptives, pre-anesthetic midazolam, and anti-HIV protease inhibitors. Drug-drug interactions between rifampicin and oral contraceptives were first reported in the early 1970s. In tuberculosis patients under chemotherapy with rifampicin, a significant high incidence of unwanted pregnancies and menstrual cycle disorders was noted in female patients using oral contraceptives [44]. Drug-drug interactions were also found with midazolam, a fast-acting benzodiazepine used for short minor surgical procedures such as dental extraction. However, midazolam is ineffective in patients treated with rifampicin. Indeed, pharmacokinetic studies on midazolam revealed a ~95% decrease in the area under the curve (AUC) and the peak plasma concentration (C_{max}), ~60% decrease in the half-life ($t_{1/2\beta}$) in healthy volunteers pretreated with rifampicin [45]. Drug-drug interactions between rifamycins and anti-HIV protease inhibitors in tuberculosis and HIV co-infected patients, can result in the loss of HIV suppression. Pretreatment with rifampicin markedly changed the pharmacokinetics of anti-HIV protease inhibitors as revealed by a decrease of the AUC: 82% for amprenavir, 92% for indinavir, 82% for nelfinavir, and 70% for saquinavir [43,46,47]. It is now clear that PXR is involved in these drug-drug interactions; rifampicin activates PXR and upregulates the PXR target gene *CYP3A4*, resulting

in increased metabolic clearance of oral contraceptives, midazolam, and anti-HIV protease inhibitors, leading to decreased efficacy (Figure 4).

St John's Wort is a herbal medicine used for centuries, dating from the early Greeks, for medicinal purposes in the treatment of mental disorders and nerve pain. Today, St John's Wort is widely used for depression, anxiety, and sleep disorders [48]. However, the alarm regarding St John's Wort was raised when a life-threatening adverse drug reaction was reported in two heart transplant patients treated with cyclosporine, one who had self-administered St John's Wort and the other who was prescribed St John's Wort by a psychiatrist [49]. Cyclosporine is an immunosuppressant used in organ transplant patients to reduce the risk of organ rejection. However, in organ transplant patients using St John's Wort, the organ transplant failed. Further studies revealed that St John's Wort contains multiple PXR ligands, most notably hyperforin, which was found to activate PXR with an EC50 at 21 nM [50]. Thus, St John's Wort activates PXR, upregulates CYP3A expression, and accelerates cyclosporine metabolism. Pharmacokinetic studies confirmed the drug-drug interactions between St John's Wort and cyclosporine, through demonstrating a marked decrease of cyclosporine blood levels in patients coadministered St John's Wort [51,52].

3.1.2 Increased toxicity *via* PXR mediated metabolic activation—In most cases, PXR activation is associated with detoxication due to the increased metabolism and elimination of xenobiotics. However, accelerated metabolism might be harmful for some drugs because of the production and accumulation of toxic metabolites (Figure 4). Acetaminophen (APAP), widely used analgesic for relief of fever and headaches, is considered safe for humans at recommended doses. However, acute overdose can result in liver damage and is the leading cause of liver failure in the United States [53]. APAP is metabolized primarily in the liver, where its major metabolites include metabolically inactive sulfate and glucuronide conjugates. The minor metabolic pathway is *via* hepatic P450, which is responsible for the generation of the putative toxic alkylating metabolite *N*-acetyl-*p*-benzo-quinone imine (NAPQI). At recommended doses, NAPQI is quickly detoxicated through conjugation with glutathione to produce a non-toxic derivative. However, under conditions of P450 induction, the risk of APAP toxicity increases due to excess hepatic NAPQI. Pretreatment with the CYP2E1 inducer isoniazid or the CYP1A2 inducer 3-methylcholanthrene increases APAP hepatotoxicity [54, 55]. Enhanced APAP hepatotoxicity by PXR activation was recently reported in mice pretreated with the PXR ligand PCN. Pretreatment with the PXR activator markedly enhanced APAP-induced hepatic injury in wild-type but not in *Pxr*-null mice, suggesting that PXR plays a critical role in APAP-induced hepatic toxicity by inducing CYP3A11 expression and hence increasing bioactivation [56]. However, the role of PXR in APAP hepatotoxicity in humans is not clear.

3.2 Vitamin D homeostasis and PXR mediated metabolic bone disorders

Vitamin D promotes bone formation and mineralization and is essential in skeleton development. Vitamin D deficiency leads to bone softening diseases, such as rickets in children and osteomalacia in adults (Figure 3). In mammals, two major forms of vitamin D exist, vitamin D_2 and vitamin D_3. In humans, vitamin D_3 is more effective than vitamin D_2 [57] while vitamin D_2 is more effective than vitamin D_3 in rats [58]. 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the physiologically active form of vitamin D in humans, is synthesized from vitamin D_3 by hepatic CYP27A1 and CYP2R1, and renal CYP27B1. $1,25(OH)_2D_3$ mediates its biological effects by binding to the vitamin D receptor, and vitamin D receptor activation in the intestine, bone, and kidney leads to the maintenance of calcium and phosphorus levels in the blood and to the maintenance of bone content [59]. Thus, $1,25(OH)_2D_3$ homeostasis is important in controlling bone metabolism. Renal CYP24 is well known to be the major enzyme contributing to the metabolism of $1,25(OH)_2D_3$ to the inactive form 1,24,25-trihydroxyvitamin D_3 (1,24,25

$(OH)_3D_3$). CYP24 also contributes to the metabolism of $25(OH)D_3$ to 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$), which decreases formation of $1,25(OH)_2D_3$ from $25(OH)D_3$. Recently, CYP24 was identified as a PXR target gene by both *in vivo* and *in vitro* studies, suggesting that drugs such as PXR ligands can modulate CYP24 gene expression and alter the homeostasis of $1,25(OH)_2D_3$ [60]. The PXR target CYP3A4 catalyzes 23- and 24-hydroxylation of $1,25(OH)_2D_3$, resulting in production of the biologically inactive metabolites 1,23*R*,25-trihydroxyvitamin D_3 ($1,23R,25(OH)_3D_3$), 1,23*S*,25-trihydroxyvitamin D_3 ($1,23S,25(OH)_3D_3$), and 1,24*S*,25-trihydroxyvitamin D_3 ($1,24S,25(OH)_3D_3$) [61]. Compared to CYP24, CYP3A4 expression in humans is very abundant especially in liver. However, the affinity and efficiency of CYP3A4 in $1,25(OH)_2D_3$ metabolism are ~10-fold lower than that of CYP24 as revealed by enzyme kinetics studies [61].

Whatever the effect of CYP24 and CYP3A4 on $1,25(OH)_2D_3$ homeostasis, the role of PXR in metabolic bone disorders in humans remains unclear, because: (i) Although a significant proportion of the world's population has been treated with rifampicin over the past 40 years, rifampicin-mediated osteomalacia was not reported to any significant degree [62]; (ii) There was no significant change in plasma $1,25(OH)_2D_3$ levels during short- or long-term treatment with rifampicin [63,64]; (iii) PXR was also proposed to be a factor that increases bone density. Thus, while it is not likely that activation of PXR alters the biological activity of vitamin D, there is a potential for drug-hormone interactions. Vitamin K_2, a critical factor required for blood clotting, was identified as a weak PXR ligand [14]. Indeed, vitamin K_2 supplementation increases bone density *in vivo*, and it is used clinically in the management of osteoporosis. *In vitro*, vitamin K_2 was able to induce bone markers in primary osteocytes isolated from wild-type mouse calvaria but not in cells isolated from *Pxr*-null mice [15]. Further studies indicated that the osteoblastgenic transcription factor *MSX2*, a target gene for PXR, mediated the osteoprotective action of vitamin K_2 [65]. Overall, the role of PXR in vitamin D homeostasis and metabolic bone disorders is not conclusive. For studies on drug-induced osteomalacia, the role of constitutive androstane receptor (CAR) should also be considered, because: (i) CAR ligands can also regulate metabolism and affect vitamin D homeostasis; (ii) CAR ligands, such as phenytoin and phenobarbital, are much more frequently associated with osteomalacia than PXR ligand rifampicin [62].

3.3 Role of PXR in bile acid homeostasis

Bile is produced and secreted by hepatocytes into the gut where it facilitates the digestion and absorption of lipids and vitamins. In addition, bile secretion is an important pathway for the elimination of large hydrophobic endobiotic and xenobiotic metabolites, including many high Mr conjugates. The major components of bile are cholesterol, lecithin, bile pigments, bile acids, and bicarbonate ions. Among the bile components, bile acids are crucial in maintaining bile flow, eliminating of cholesterol from the liver, and emulsify lipids in the gut. Bile acid levels are tightly regulated by multiple NRs, including hepatic nuclear factor 4α(HNF4α), liver X receptor (LXR), farnesoid X receptor, vitamin D receptor, constitutive androstane receptor, and PXR [34,66–68]. Under certain pathological conditions such as cholestasis that results from impaired bile flow, bile acids are potentially toxic. Indeed, serious hepatotoxicity was noted in mice over exposed to lithocholic acid [34,69]. PXR is involved in regulation of the biosynthesis, transport, and metabolism of bile acids (Figure 3). Bile acids are produced in the liver from cholesterol *via* multiple enzyme-dependent steps with the rate limiting step being 7-hydroxylation of cholesterol by cholesterol 7α-hydroxylase (CYP7A1). Since PXR activation downregulates *CYP7A1*, it can impact bile acid synthesis [34]. Further studies suggest that PXR activation promotes PXR interaction with HNF4αand blocks peroxisome proliferator-activated receptor (PPAR) γcoactivator 1αactivation of HNF4α, resulting in inhibition of *CYP7A1* gene transcription [70]. PXR activation also regulates the expression of OATP2, sulfotransferase, and murine Cyp3a11/human CYP3A4, which may promote the

metabolism and transport of bile acids [34]. OATP2 is localized on the basolateral membrane of the hepatocyte and is involved in the cellular uptake of bile acids. Its induction by PXR would presumably increase the uptake of bile acids from the sinusoidal blood into the hepatocyte where the detoxication pathways such as hydroxylation and sulfation could take place by Cyp3a11/CYP3A4 and sulfotransferase, respectively [34].

PXR activation was proposed to decrease the bile acid synthesis *via* downregulation of CYP7A1, and to accelerate bile acid metabolism and elimination through upregulation of metabolic enzymes and transporters [34,70]. Clinically, the human PXR ligand rifampicin has been used in patients with primary biliary cirrhosis for its potential effect on bile acid metabolism. Primary biliary cirrhosis, a disease characterized by inflammatory destruction of the small bile ducts within the liver, presents with cholestasis, jaundice and pruritus. Rifampicin treatment decreased pruritus, and the serum concentration of total and conjugated bile acids was also strikingly reduced [71]. The benefit of rifampicin for primary biliary cirrhosis was proposed as the metabolic regulation, as shown by an increase of antipyrine clearance and the enhanced urinary excretion of 6β-hydroxycortisol [71]. However, the clinical data are controversial on the role of PXR in the biliary system. In humans with or without hepatic cirrhosis, a significant elevation of plasma bile acids was noted after two hours of rifampicin treatment [72]. Cholestatic hepatitis was reported in humans treated with rifampicin alone [73]. At the same time, significant high incidences of hepatotoxicity were reported in primary biliary cirrhosis patients with the treatment of rifampicin [74,75]. Thus, further studies are required to determine the role of PXR in the biliary system, and the high risk of rifampicin-induced hepatotoxicity should be considered for the patients with primary biliary cirrhosis.

3.4 PXR mediated hepatic steatosis

The abnormal retention of lipids within the cells results in steatosis, which reflects an impairment of the normal processes of synthesis and breakdown of triglycerides. As the liver is the primary organ of lipid metabolism, steatosis most often occurs in this tissue (Figure 3). While patients with hepatic steatosis have few or no symptoms, infrequently, they may complain of fatigue, malaise and dull right upper quadrant abdominal discomfort. However, the danger of hepatic steatosis is the result of the sequelae, such as liver fibrosis, cirrhosis, and carcinoma. In humans, hepatic steatosis is commonly associated with alcohol abuse or metabolic syndrome (diabetes, hypertension and dyslipidemia), but may also be caused by drugs and certain toxins. Recently, PXR activators were proposed as risk factors for hepatic steatosis. Hepatic lipid accumulation was noted in *PXR*-humanized mice treated with the human PXR activator rifampicin [76]. Expression of an activated PXR in the livers of transgenic mice also resulted in an increased lipid accumulation that was independent of activation of the lipogenic transcriptional factor sterol regulatory element-binding protein-1c but associated with an increased expression of the free fatty acid transporter CD36, and other accessory lipogenic enzymes, such as stearoyl-CoA desaturase-1 and long-chain free fatty acid elongase [76]. It was recently shown that PXR may promote hepatic steatosis by increasing the expression of CD36 directly or indirectly through the PXR-mediated activation of PPARγ [77]. PXR-mediated gene regulation and lipid accumulation are required for the hepatic regenerative response to liver resection, and it was suggested that PXR is essential for normal progression of liver regeneration by modulating lipid homeostasis [78]. T0901317, a high-affinity ligand for both LXR and PXR, induced liver steatosis. Further studies indicated that T0901317-associated hepatic steatosis was mediated by PXR, but not by LXR [79].

The putative role for PXR in hepatic steatosis raised great concern about the safety of drugs that are also PXR ligands, such as the glucocorticoid dexamethasone, the antibiotic rifampicin, and the antimycotic clotrimazole [9]. However, there are very few clinical reports concerning drug-induced hepatic steatosis by PXR ligands. For example, rifampicin, although used by a

large number of tuberculosis patients since 1970s, there are no significant clinical reports of rifampicin-induced hepatic steatosis, fibrosis, cirrhosis, or carcinoma. In addition, liver biopsies were carried out on 100 tuberculosis patients, eight cases after anti-tuberculosis treatment and 92 cases before anti-tuberculosis treatment. There was no evidence indicating rifampicin-induced hepatic steatosis [80]. However, in preclinical studies, fatty livers were noted in rats given a high dose of rifampicin [81,82]. The physiological and pharmacological relevance of this finding to humans is questionable since supra-pharmacological doses were used; rifampicin is a human specific PXR ligand, having virtually no effect on rat PXR at human-equivalent doses [12]. Overall, the role of human PXR in lipid metabolism and hepatic steatosis warrants further investigation.

3.5 PXR and steroid hormone homeostasis

Preclinical studies support the concept of PXR as a potential endocrine disrupting factor that might have broad implications in steroid hormone homeostasis and drug-hormone interactions. Activation of PXR markedly increased plasma concentrations of corticosterone and aldosterone, and their increases were associated with increased expression of adrenal steroidogenic enzymes, including CYP11A1, CYP11B1, CYP11B2, and 3β-hydroxysteroid dehydrogenase [83]. CYP3A4, a PXR target gene, also contributes to the metabolism of steroid hormones. Indeed, recombinant CYP3A4 showed exhibited significant cortisol and testosterone metabolism [84]. Cortisol and testosterone 6β-hydroxylase activities were also used as biomarkers for CYP3A4 induction or inhibition [85,86]. Among the major human hepatic P450s, 6β- and 16α-hydroxylation of progesterone was catalyzed most efficiently by CYP3A4 [87]. In addition, CYP3A4 catalyzes 2-, 4-, and 16-hydroxylation of estradiol [88, 89].

In a *CYP3A4*-transgenic mouse line expressing both human and murine CYP3A, females were found to be deficient in lactation, leading to a markedly lower pup survival. This impaired lactation phenotype was associated with significantly reduced serum estradiol levels in *CYP3A4*-transgenic mice, suggesting that CYP3A4 may play an important role in estradiol homeostasis [90]. This may be of relevance to the treatment of pregnant and lactating women with drugs that are PXR activators. Of note, rifampicin is contraindicated in pregnancy except in the presence of a severe disease untreatable with other drugs, such as tuberculosis, because of teratogenicity found in animal studies and case reports of malformation, death and haemorrhage in infants whose mothers were exposed to rifampicin [91]. The role of PXR in the homeostasis of steroid hormones, especially sex hormones, may provide an important clue as to the mechanism by which rifampicin compromises pregnancy.

3.6 PXR in inflammatory bowel disease

Inflammatory bowel disease (IBD) refers to a chronic inflammatory condition of the digestive tract occurring as one of two major types, ulcerative colitis and Crohn's disease. Ulcerative colitis is limited to the colon while Crohn's disease most commonly affects the small intestine and/or the colon, but can involve any part of the gastrointestinal tract from the mouth to the anus. However, the etiology of IBD is unknown. To date, genetic, infectious, immunologic, and psychological factors have all been implicated in influencing the development of IBD. Recently *PXR* was identified as a gene strongly associated with the susceptibility to IBD in humans [92]. In patients with IBD, decreased expression of PXR and PXR target genes was also noted [93,94].

Drug treatment is the main method for relieving the symptoms of both ulcerative colitis and Crohn's disease. Progress is being made in the development of medications for treating IBD, such as anti-inflammatory drugs and immunosuppressive agents. Thus, identification of the role of PXR in IBD might provide new stratagem for IBD therapeutics. In the dextran sulfate

sodium (DSS)-induced IBD mouse acute colitis model, treatment with the PXR ligand PCN protected against DSS-induced colitis compared with vehicle-treated mice, as defined by body weight loss, diarrhea, rectal bleeding, colon length, and histology. However, this treatment did not decrease the severity of DSS-induced colitis in *Pxr*-null mice indicating a role for PXR in protection against IBD [95]. It has recently been reported that hepatic SCD1 is downregulated in mice with DSS-induced colitis and that this leads to elevated levels of proinflammatory saturated fatty acids and reduced levels of antiinflammatory unsaturated fatty acids [96]. It should be noted that *SCD1* was upregulated in mice by PXR activation [76], and thus PXR activation should be expected to ameliorate the symptoms of DSS-induced colitis in mice having low levels of expression of SCD1 through increased production of unsaturated fatty acids.

Interestingly, budesonide, a glucocorticoid derivative frequently used as an anti-inflammatory drug for IBD, was recently identified as a PXR ligand [97]. Rifaximin, approved by the Food and Drug Administration in 2004 for the treatment of travelers' diarrhea [98] was found to be of potential value in the treatment of chronic gastrointestinal disorders including ulcerative colitis and Crohn's disease. Despite the differences in dose and duration, rifaximin was found to be beneficial in the treatment of active ulcerative colitis, mild-to-moderate Crohn's disease as well as prevention of postoperative recurrence of Crohn's disease [99–101]. The mechanism contributing to the beneficial effects of rifaximin in chronic gastrointestinal disorders are not fully understood. By using *PXR*-humanized, *Pxr*-null, and wild-type mice, rifaximin was identified as a gut-specific human PXR activator [102]. Further human studies are suggested to assess the potential role of PXR activation in therapeutics of IBD.

3.7 Miscellaneous implications

PXR in cancer and chemotherapy: Resistance to chemotherapeutic agents is the major clinical problem and cause of failure in the chemotherapy of human cancer. Understanding the molecular basis of chemoresistance will be valuable for developing more effective chemotherapy. Several molecular targets have been shown to be related to chemoresistance, which include efflux transporters, phase I and II detoxication enzymes and DNA-repair enzymes. Most of these chemoresistance related enzymes, are encoded by PXR target genes [103], such as P-glycoprotein, multidrug resistance proteins, CYP3A, UDP-glucuronosyltransferase and glutathione S-transferases. Some chemotherapeutic agents, such as cyclophosphamide, tamoxifen, and taxol, have been identified as human PXR ligands [8, 9]. Activation of PXR will induce a battery of enzymes and transporters to accelerate metabolism and elimination of chemotherapeutic agents, which may contribute to acquired resistance and multi-drug resistance in chemotherapy. Additionally, PXR and its target genes have been detected in cancerous tissues, including in prostate, breast, endometrium and colon. A localized function of PXR in chemoresistance has been proposed [104–107]. However, the function of PXR in cancerous tissues is not limited to chemoresistance, as a biological function of PXR in human breast carcinoma has been proposed [108]. In human colon cancer cells, the antiapoptotic role of PXR was reported [109].

PXR and antifibrogenesis: PXR was recently proposed as a potential target for antifibrotic therapy. In rats chronically treated with carbon tetrachloride, hepatocyte necrosis and liver fibrogenesis were produced. Interestingly, the extent of fibrosis caused by carbon tetrachloride was significantly inhibited by PCN [110]. The further study investigated the effects of human PXR activators on human hepatic stellate cell transdifferentiation to a profibrogenic phenotype. The expression of PXR was detected in primary cultured hepatic stellate cells. Short-term treatment of hepatic stellate cells with the PXR ligand rifampicin inhibited the expression of fibrosis-related genes. Long-term treatment with rifampicin reduced the proliferation and transdifferentiation of hepatic stellate cells, and all these rifampicin effects were PXR-

dependent. These data suggested that PXR activators inhibit transdifferentiation and proliferation of human hepatic stellate cells, and PXR may therefore be a potential target for antifibrotic therapy [111].

PXR and oxidative stress: The role of PXR in the oxidative stress response has been reported. In this study, a heightened sensitivity to oxidative toxicant paraquat was noted both in *PXR*-humanized mice and wild-type mice upon PXR activation. Consistent with the *in vivo* study, cell lines with activated human PXR were also sensitive to paraquat, and an increased production of reactive oxygen species was observed. These data suggested that PXR activation was a risk factor for oxidative stress caused by an imbalance between the production of reactive oxygen and detoxication of the reactive intermediates [112]. Further studies are indicated on the role of PXR in oxidative stress, because of the importance of oxidative stress in drug toxicity and human disease.

4. Conclusions

The *Pxr*-null and *PXR*-humanized mouse lines serve as valuable *in vivo* models for investigations on PXR. New information such as the target gene network controlled by PXR, and the species differences in response to PXR ligands were obtained using these models. Due to the importance of PXR in the regulation of xenobiotic-metabolizing enzymes and transporters, PXR activation greatly affects the metabolism of drugs, hormones, nutrients and toxins. Based upon data acquired from preclinical studies, especially *in vivo* studies using the aforementioned mouse models, together with clinical reports, it has become clear that PXR is central to drug-drug interactions, and may have a role in vitamin D, bile acid, and steroid hormone homeostasis, hepatic steatosis, and IBD. Among these clinical implications, PXR mediated drug-drug interactions have been confirmed by both preclinical studies and clinical studies, and most cases of PXR mediated drug-drug interactions result in decreased efficacy. All the clinical implications of PXR beyond drug-drug interactions were generally based on preclinical studies, and their importance to humans is not clear; however, these preclinical results provided valuable directions for the future research on PXR.

5. Expert opinion

It should be emphasized that due to the species differences between rodents and humans, the data generated from preclinical studies should be confirmed in clinical studies (Figure 5). For preclinical studies, the use of *Pxr*-null and *PXR*-humanized mouse models is recommended. Patients treated with drugs that are PXR activators are potential subjects for clinical studies on PXR, such as the tuberculosis patients under chemotherapy with rifampicin. Additional studies are warranted to investigate on the role of PXR in vitamin D homeostasis and metabolic bone disorders, bile acid homeostasis, hepatic steatosis, steroid hormones homeostasis, and IBD. Typically, cell based PXR-reporter assays or primary cultured human hepatocytes can be used to determine if a compound is a PXR agonist. However, the identification of PXR agonists in herbal medicines remains problematic. In addition to PXR agonists, studies on PXR antagonists will also become important, as they might be useful for preventing PXR mediated drug-drug interactions or perturbations of hormone and vitamin homeostasis.

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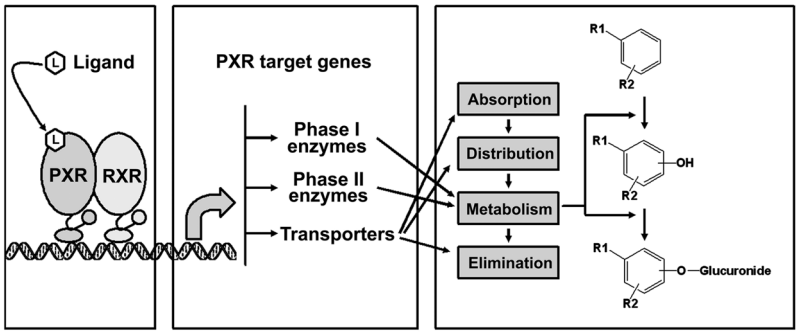

Figure 1. Pregnane X receptor (PXR) activation and its function

PXR activation is ligand dependent. Following ligand binding, PXR forms of a heterodimer with retinoid X receptor (RXR), and subsequently binds to PXR response elements in the 5′-flanking region of the PXR target genes resulting in the transcriptional activation. Among the target genes are those encoding including both phase I and phase II metabolic enzymes, as well as transporters which contribute to absorption, distribution, metabolism and elimination of xenobiotics and endobiotics.

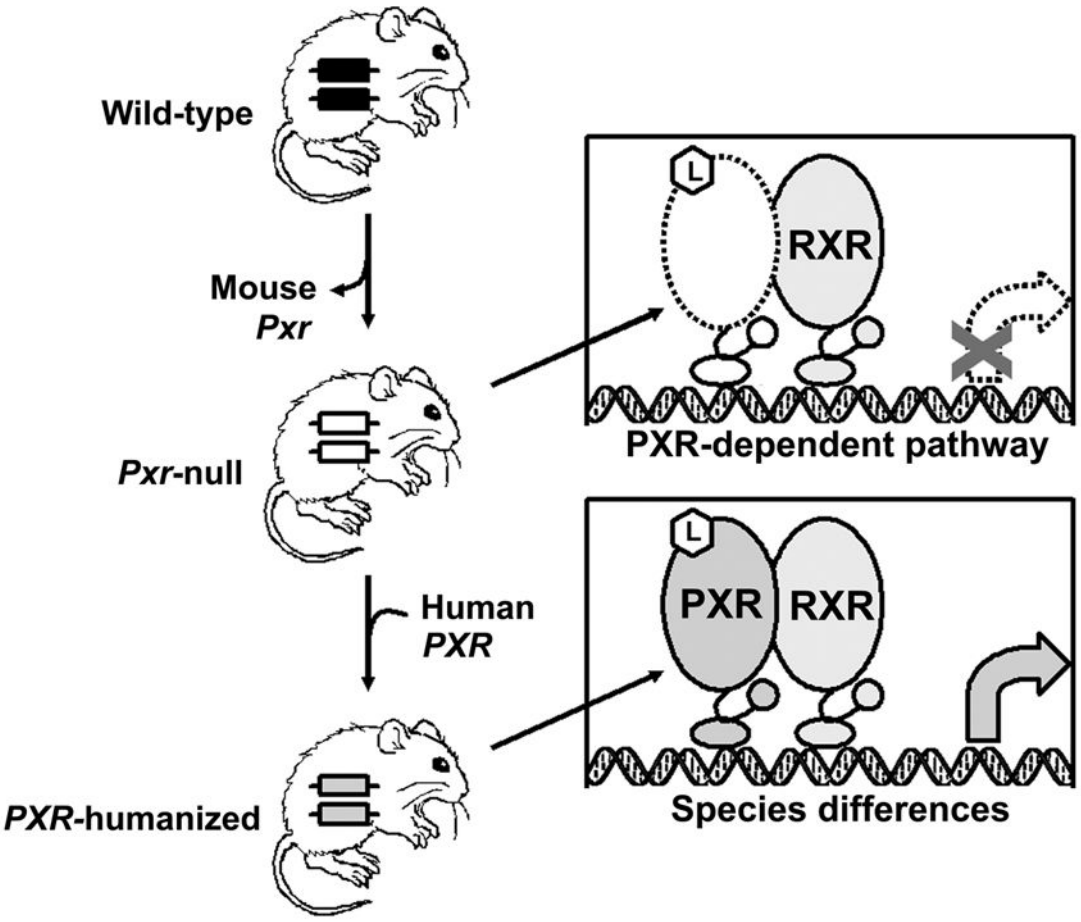

Figure 2. Recent advances in animal models on PXR studies: *Pxr*-null mouse model and *PXR*-humanized mouse model

The *Pxr*-null mouse model is generally used to identify the PXR-dependent signaling pathways and the *PXR*-humanized mouse model is used to overcome the species differences in response to PXR ligands.

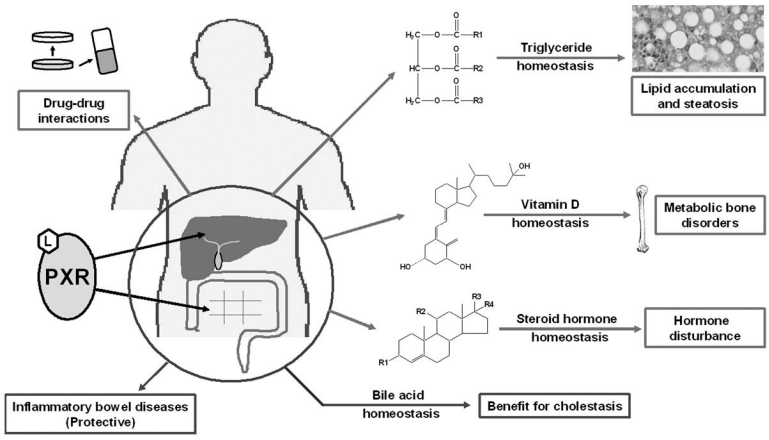

Figure 3. Clinical implications of PXR

PXR is highly expressed in human liver and intestine. PXR activation results in multiple clinical responses. PXR was proposed as a risk factor contributing to drug-drug interactions, hepatic steatosis, metabolic bone disorders, and perturbation of steroid hormone homeostasis. In contrast, PXR activation might be protective for inflammatory bowel diseases, and protective for cholestasis *via* the regulation of bile acids homeostasis.

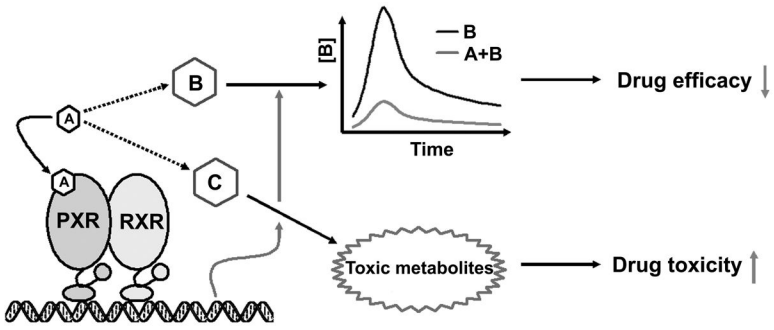

Figure 4. PXR mediated drug-drug interactions

PXR mediated drug-drug interactions are based on pharmacokinetics and result from the interference in the metabolic clearance of one drug by another co-administered drug. For example, drug A is co-administered with drug B or C, and drug A is a PXR ligand that activates PXR and enhances the metabolic clearance of drug B or C. For the drug-drug interactions between drug A and B, the efficacy of drug B decreases due to the lowered area under the curve and the peak plasma concentration. For the drug-drug interactions between drug A and C, the toxicity of drug C increases because of the accumulation of toxic metabolites.

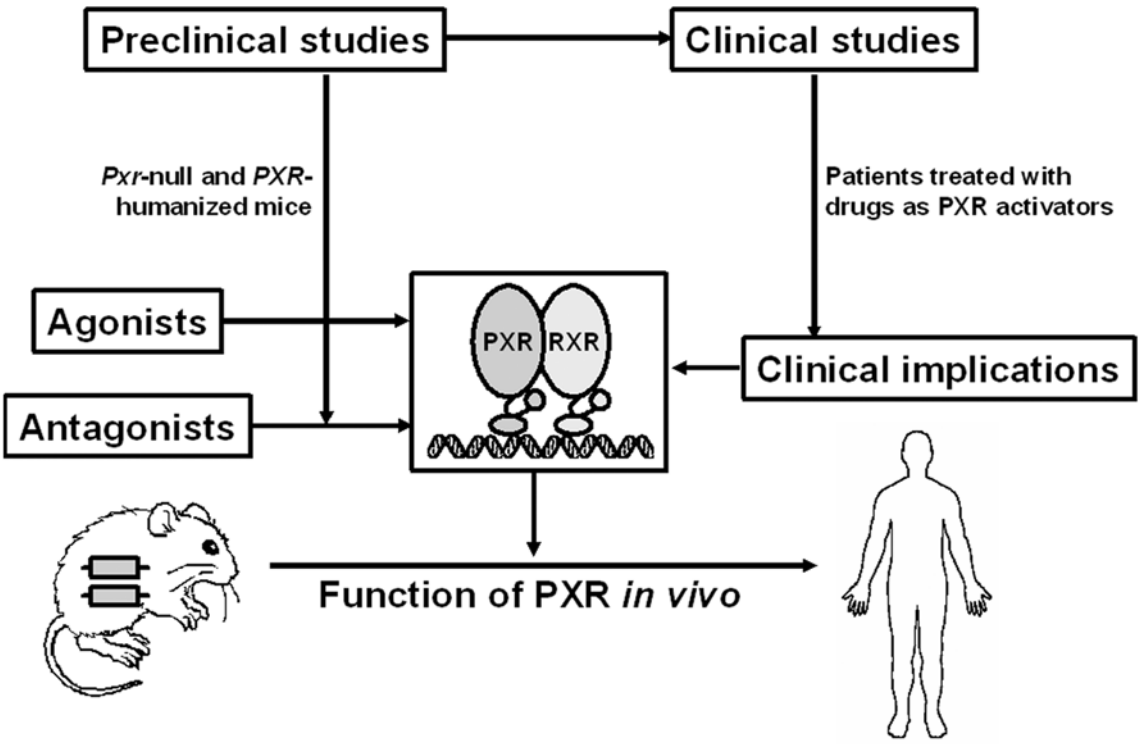

Figure 5. Brief summary of the studies on PXR

The overall goals of preclinical and clinical studies on PXR are to illustrate the function of human PXR *in vivo*, and to predict the risk of PXR agonists and antagonists to which humans are exposed. Preclinical studies on PXR by using *Pxr*-null and *PXR*-humanized mouse models provided valuable information on PXR. However, the preclinical results should be translated to clinical studies because of the species differences between rodents and humans. Clinical studies on PXR are suggested in patients treated with drugs as PXR activators.