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Pregnane X receptor and natural products: beyond drug–drug interactions

Jeff L Staudinger^{†,1}, Xunshan Ding², and Kristin Lichti³

¹Associate Professor, University of Kansas, Department of Pharmacology and Toxicology, 1251 Wescoe Hall Dr, 5044 Malott Hall, Lawrence, Kansas 66045, USA

²Postdoctoral Fellow, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, Texas 75390-9041, USA

³Graduate Student, University of Kansas, Department of Pharmacology and Toxicology, 1251 Wescoe Hall Dr, 5044 Malott Hall, Lawrence, Kansas 66045, USA

Abstract

The pregnane X receptor (PXR, NR1I2) is a member of the nuclear receptor superfamily that is activated by a myriad of compounds and natural products in clinical use. Activation of PXR represents the basis for several clinically important drug–drug interactions. Although PXR activation has undesirable effects in patients on combination therapy, it also mediates the hepatoprotective effects exhibited by some herbal remedies. This review focuses on PXR activation by natural products and the potential therapeutic opportunities presented. In particular, the biological effects of St. John's Wort, guggulipid, kava kava, *Coleus forskolii*, *Hypoxis*, *Sutherlandia*, qing hao, wu wei zi, gan cao and other natural products are discussed. The impact of these natural products on drug metabolism and hepatoprotection is highlighted in the context of activation and antagonism of PXR.

Keywords

drug interaction; herb; nuclear receptor; pregnane X receptor; xenobiotic response

1. Introduction: pregnane X receptor, xenobiotic and endobiotic metabolism

1.1 Pregnane X receptor and xenobiotic metabolism

Nuclear receptors comprise a large superfamily of transcription factors that are characterised by a conserved N-terminal zinc-finger type DNA-binding domain and a C-terminal ligand-binding domain. They are involved in a variety of physiological, developmental and toxicological processes [1]. Pregnane X receptor (PXR, NR1I2) is a member of the nuclear receptor superfamily that was first cloned in 1998 by a research group at Glaxo Wellcome as part of an effort to identify new members of the nuclear receptor superfamily based on homology and the mouse genome sequencing project [2]. Since then, PXR has been identified in various species, including human, monkey, cow, pig, rabbit, rat, mouse, chicken, fish and worms [3–5]. In mammals, PXR is highly expressed in the major organs that are important in xenobiotic biotransformation, including the liver and intestines [2]. Various studies show that activation of PXR in the liver and intestines produces increased expression of a group of genes

that encode proteins involved in the uptake, metabolism and elimination of potentially toxic compounds (Figure 1) [6–10]. It is well established that PXR is a key regulator of xenobiotic-inducible CYP3A gene expression [11,12]. In addition, PXR activation regulates other genes involved in the metabolism of xenobiotic compounds, such as CYP2B, -2C and -24, glutathione *S*-transferases, sulfotransferases and UDP-glucuronosyltransferases (UGTs) [6,13–16]. In rodents, PXR also regulates the expression of the drug transporter genes organic anion-transporting polypeptide 2 (OATP2), multi-drug resistant protein (MDR1) and multi-drug resistant-associated proteins 2 and 3 (MRP2 and -3) [10,17,18]. Therefore, PXR activation has a complex nature. Although it protects cells from toxic insults, it also represents the molecular basis for an important class of drug–drug interactions. For example, if one drug activates PXR, it can be predicted that administration of this drug will promote the elimination of other coadministered drugs that are also metabolised and eliminated by PXR-target gene products, thereby reducing the efficacy of many drug therapies in patients on combination therapy.

1.2 Pregnane X receptor and endobiotic metabolism

In addition to xenobiotic metabolism, many PXR targets are also involved in endobiotic metabolism. For example, CYP3A hydroxylates bile acids, whereas MRP2 and OATP2 transport bile acids, thereby lowering bile acid levels in serum when they reach toxic levels in the body. Interestingly, at low micromolar concentrations, lithocholic acid, a secondary bile acid produced in the intestines, efficaciously activates PXR [8,9]. PXR activation not only leads to increases in bile acid detoxification through induction of CYP3A, MRP2 and OATP2, but also represses the expression and activity of CYP7A [8,9], which encodes the rate-limiting enzyme in converting cholesterol to bile acids. Therefore, activation of PXR coordinately lowers bile acid levels through increased uptake, metabolism and excretion, and also through decreased production of bile acids. In addition to regulating endogenous bile acids, PXR has been shown to regulate vitamin D, bilirubin and cholesterol homeostasis [14,19,20], although the role of PXR in the regulation of vitamin D-metabolising enzyme CYP24 has recently been called into question [21]. Moreover, members of the UGT superfamily of drug-metabolising enzymes also mediate the glucuronidation of a plethora of endobiotic compounds, including many steroid hormones as well as thyroid hormones. Specifically, in rodents and humans, PXR activation directly regulates the expression and activity of certain UGT1A family members that are critical in maintaining hormone homeostasis [13,22].

2. Pregnane X receptor has promiscuous ligand-binding properties

As a prototypical nuclear receptor, PXR has a DNA-binding domain (DBD) at the N-terminus and a ligand-binding domain (LBD) at the C-terminus. The DBD is responsible for binding to regulatory DNA sequences. The LBD has a dual function: one to bind ligand, the other to provide an interface for protein–protein interactions. X-ray crystallographic studies have revealed that PXR is quite unique when compared with other nuclear receptors in that its LBD facilitates a remarkably large ligand-binding pocket, at least twice as large as other steroid hormone ligand-binding pockets [23]. This feature of the ligand-binding pocket gives PXR the ability to recognise a variety of structurally diverse compounds as well as the ability to recognise a single compound in different orientations. Examples of drugs in clinical use that activate PXR are shown in Figure 2. In spite of the vast amount of PXR agonists discovered so far, only one PXR antagonist has been reported (Table 1). Ecteinascidin-743 (ET-743), a marine-derived natural product, which is discussed in more detail in Section 4, shows potent antagonist activity against PXR [24]. The existence of PXR antagonism provides a potential molecular basis for preventing PXR-mediated adverse drug interactions.

Agonists activate PXR by directly binding to the receptor. This causes a conformational change of PXR that favours the release of corepressor proteins such as the nuclear receptor corepressor (N-CoR) and the silencing mediator for the retinoid and thyroid receptor corepressor (Figure

2) [24,25]. Agonist binding also strengthens the interaction between PXR and coactivators such as steroid receptor coactivator 1 (SRC-1), glucocorticoid receptor-interacting protein 1 (SRC-2/TIF2), SRC-3 and peroxisome proliferator-activated receptor-binding protein (Figure 3) [24,25]. The nuclear receptor coactivator proteins typically have either endogenous histone acetyltransferase (HAT) activity or aid in recruiting other HAT-containing components to an activated nuclear receptor protein [26,27]. By modulating the chromatin structure through HAT activity, the PXR–multiprotein complex turns on the expression of PXR-target genes through specific DNA-enhancer elements located in their promoter regions.

3. Herbal products that activate the pregnane X receptor

3.1 St. John's Wort

St. John's Wort is a widely used over-the-counter antidepressant derived from the flowers of the plant *Hypericum perforatum*. Clinical studies have revealed numerous cases of drug interactions with the use of St. John's Wort. In the majority of these cases, St. John's Wort increases the metabolism and excretion of coadministered medications, such as the immunosuppressant ciclosporin, the HIV drugs indinavir and ritonavir and oral contraceptives, through induction of intestinal MDR1 and hepatic and intestinal CYP3A4 [28]. Based on the finding that PXR is the key regulator of CYP3A4 gene expression, two research groups simultaneously investigated the activity of St. John's Wort against PXR [29,30]. It was found that extracts prepared from St. John's Wort efficaciously activated human PXR. The studies further reveal that hyperforin (Table 1), the antidepressant component in St. John's Wort, is a potent and efficacious PXR agonist that binds to PXR and strengthens the interaction between PXR and the coactivator SRC-1. These findings suggest that it is possible to modify the structure of hyperforin to develop safer antidepressants that lack PXR activity and, therefore, will not cause adverse drug interactions.

A recent report shows that the effects of St. John's Wort on drug metabolism may be more complicated. It has been shown that long-term administration of St. John's Wort is associated with reduced bioavailability of voriconazole. This is expected based on the interaction of St. John's Wort with PXR. Paradoxically, short-term (10 h) administration of St. John's Wort increases serum concentration of voriconazole [31]. This short-term effect of St. John's Wort on voriconazole concentration is possibly mediated by the inhibitory effect of hyperforin on certain CYPs such as CYP3A4 and -2C9 [32].

3.2 Gugulipid

The gum resin of the *Commiphora mukul* tree has been used for thousands of years in Ayurvedic medicine to treat a variety of ailments, including rheumatoid arthritis, inflammation, obesity and hyperlipidaemia [33]. Gugulipid, an ethyl acetate extract derived from the gum resin of the *Commiphora mukul* tree, has been widely used to treat hyperlipidaemia since its approval in India in 1987. Recently, it has been determined that the stereoisomers *E*- and *Z*-guggulsterone (Table 1) are the active compounds in gugulipid that decrease hepatic cholesterol levels [34,35]. This effect is likely mediated through the antagonism of the nuclear farnesoid X receptor by guggulsterones [36]. As most animal studies and clinical trials have shown that gugulipid significantly reduces serum total cholesterol, low-density lipoprotein and triglyceride levels, and elevates high-density lipoprotein levels, gugulipid is gaining popularity in the Western world, including the US, as an herbal supplement, and is available over the counter at present. However, recent and well-designed studies have reported only small significant decreases in low-density lipoprotein levels with the use of gugulipid, whereas no significant changes in total cholesterol, high-density lipoproteins or triglycerides were reported [37]. Scientific evidence calls into question the use of gugulipid for hyperlipidaemia, with some studies finding cholesterol-lowering effects and others reporting no benefit.

Recent work from Brobst *et al.* shows that guggulsterones are efficacious PXR agonists [38]. Both guggulipid and guggulsterones activate PXR in reporter gene assays. More importantly, both guggulipid and guggulsterone treatments induce the gene expression of CYP3A4 in primary human hepatocytes. Mammalian-two-hybrid and glutathione S-transferase-pull-down assays demonstrate that guggulsterone activates PXR by recruiting the coactivator SRC-1 [38]. These findings raise the issue that, in addition to its desirable effects on lipid disorders, guggulipid may cause serious drug interactions in humans on combination therapy through activation of PXR. The notion that guggulipid produces drug interactions is further supported by the results of a well-controlled human study, in which 1 g of guggulipid administration significantly reduced peak plasma concentrations of either diltiazem or propranolol in patients [40].

In addition to farnesoid X receptor and PXR, studies have shown that guggulsterones modulate the activity of multiple receptors, including the constitutive androstane receptor (CAR), glucocorticoid receptor, progesterone receptor, mineralocorticoid receptor, androgen receptor and estrogen receptor [38,39,41]. These investigations suggest that further studies of guggulipid and guggulsterones are needed to explore the biological functions of this important herbal remedy.

3.3 Kava kava

Kava kava (*Piper methysticum* G. Forster) is an ancient crop of the South Pacific. It has been used for thousands of years in the Pacific as a relaxant in the form of a beverage in social and religious ceremonies. In recent years, it has gained popularity for its effect in treating anxiety. In addition, kava kava is used to treat a wide variety of disorders including insomnia, stress, restlessness, muscle fatigue, gonorrhoea and vaginitis. The active components in kava kava are a group of structurally related lactones, collectively termed kavalactones. The effects of kavalactones are believed to be mediated by receptors in the CNS, such as the GABA receptors. Modulation of voltage-dependent sodium and calcium channels by kavalactones has also been reported [42]. Besides the well-documented effects on the CNS, kavalactones have been shown to modulate the activities of hepatic CYP enzymes. The activities of CYP1A2, -2C9, -2C19, -2D6 and -3A4 are inhibited by kavalactones through a competitive mechanism [43,44]. Interestingly, kava extracts have been found to induce the expression of the CYP3A4 gene, whereas kavalactones repress CYP3A4 enzymatic activity [45]. The induction of CYP3A4 gene expression is mediated through activation of PXR by two kavalactones, desmethoxyyangonin and dihydromethysticin (Table 1). Both kavalactones activate PXR in reporter gene assays, although with less efficacy than the prototypical PXR agonist rifampicin. It remains to be determined whether kavalactones activate PXR through the modulation of PXR–cofactor interactions. In addition, it remains to be determined whether induction of CYP3A4 gene expression by kavalactones is solely dependent on activation of PXR. Nevertheless, because of the dual effects of kava kava on CYP enzyme inhibition and induction of gene expression, it may be predicted that kava kava would affect the metabolism of coadministered medications in a manner similar to St. John's Wort; that is, short-term inhibition and long-term induction. This prediction, however, remains to be confirmed.

3.4 Coleus forskohlii

In India, practitioners of ayurvedic medicine have used an extract of *Coleus forskohlii* for centuries to treat a variety of disorders including hypothyroidism, hypertension, congestive heart failure, eczema, respiratory disorders and convulsions [46]. *Coleus forskohlii* is gaining popularity in Western society as human studies show that it is effective in treating obesity. Two diterpene compounds, forskolin and 1,9- dideoxyforskolin (Table 1) are found to be the primary constituents in *Coleus forskohlii* and virtually all biological activities of *Coleus forskohlii* have been attributed to forskolin. Forskolin functions by binding to and activating adenylyl cyclase, leading to increased production of cAMP [47]. This feature has also led to

wide use of forskolin as an adenylyl cyclase and cAMP-dependent protein kinase activator in scientific research. More than 17,000 papers using forskolin as a pharmacological tool have been published in scientific literature. Certain cAMP-independent activities of forskolin have been reported. For example, both forskolin and the non-protein kinase-activating diterpene-1,9-dideoxyforskolin induce CYP3A gene expression in primary cultures of rodent hepatocytes [48]. Recent studies have revealed that both forskolin and 1,9-dideoxyforskolin are efficacious PXR agonists [49,50]. Both compounds activate PXR by displacing the corepressor N-CoR and by recruiting coactivators such as SRC-1. The role of PXR in the induction of CYP3A by these two compounds and *Coleus forskohlii* extract is confirmed by using primary mouse PXR-knockout (KO) hepatocytes. It is found that PXR is absolutely required for the induction of CYP3A by 1,9-dideoxyforskolin. Surprisingly, both forskolin and the *Coleus forskohlii* extract still induce CYP3A gene expression in PXR-KO hepatocytes, although to a much lesser extent than in wild-type hepatocytes. The PXR-independent component of the induction of CYP3A gene expression is further confirmed to be cAMP-dependent, as the active cAMP analogue 8-Br-cAMP induces CYP3A gene expression similarly in wild-type and PXR-KO hepatocytes. Interestingly, the work of Staudinger and colleagues also reveals that cAMP and PXR signalling pathways have a synergistic effect on the induction of CYP3A gene expression in primary mouse hepatocytes. The dual nature as both a PXR and adenylyl cyclase activator makes forskolin a 'super-inducer' of CYP3A gene expression in primary mouse hepatocytes. This group's work indicates that *Coleus forskohlii* extract may also interface with PXR agonists and dramatically modulate hepatic CYP enzymes at the transcriptional level. These findings suggest that herbal therapy with *Coleus forskohlii* extract should be approached cautiously in patients on combination therapy, due to the potential for herb–drug interactions.

3.4 Hypoxis and sutherlandia

In Africa, herbal products are often used as primary therapies for HIV and HIV-related symptoms. *Hypoxis* and *Sutherlandia* (Table 1) are two principle herbs used for HIV treatment in sub-Saharan Africa [51]. Although the molecular mechanisms for their therapeutic effects are unknown, both herbs are recommended by the South African Ministry of health for HIV therapy. Despite their popularity in Africa, scientific evidence regarding their therapeutic effectiveness as anti-HIV medications remains poorly documented. Moreover, recent *in vitro* experiments indicate that both herbs inhibit CYP3A4 activity and activate PXR [52]. This is strikingly similar to St. John's Wort and kava kava. Therefore, it is reasonable to predict that *Hypoxis* and *Sutherlandia* may cause serious drug interactions. Given that HIV patients are often on combination therapy, the chance of drug interactions caused by these two herbs is very high and precautions need to be taken.

In the *in vitro* experiments mentioned above, only extracts of the herbs were tested. Therefore, it remains to be determined what components in the herbs affect CYP3A4 and PXR. As herbs contain many different compounds, it is equally important to determine whether the components affecting CYP3A4 and PXR are different from those with therapeutic activities. If they are different, the components with potential therapeutic activities could be purified to treat patients and avoid unwanted side effects caused by CYP3A4 and PXR. In addition, opportunities exist for chemical modification of the active components, so that they retain therapeutic activity, but lack unwanted CYP3A4 and PXR activity.

3.5 Qing hao

Qing hao (*Artemisia annua*) is a perennial herb of the composite flower family that has been used for > 2000 years in China to treat chills and fevers. In the 1970s, Chinese scientists isolated the active compound artemisinin (or qinghaosu) (Table 1) from the herb. Since then, artemisinin and some of its derivatives have been used worldwide as effective antimalarial drugs. The importance of artemisinin and related drugs is highlighted by the fact that they are

the only class of antimalarial drugs for which a resistant malaria-causing parasite has not been found [53]. The primary mechanism for artemisinin drugs has been suggested to be the formation of free radicals from these drugs *in vivo*. The formed free radicals then kill the malaria parasites. Additional mechanisms such as DNA cleavage and polyunsaturated fatty acid degradation may also be involved in the antimalarial activity of this compound.

Monotherapy with artemisinin often results in recrudescence. This is associated with a time-dependent decrease in plasma concentrations of the drug [54,55]. It has been suggested that this might be due to the autoinduction of hepatic enzymes, particularly CYP enzymes. In a recent report, Burk *et al.* found that artemisinin activates PXR and CAR, a close cousin of PXR in the nuclear receptor superfamily, in reporter gene assays [56]. More importantly, they show that artemisinin induces the gene expression of CYP2B6, CYP3A4 and MDR1 in primary human hepatocytes and the human intestinal cell line LS174T. The physiological relevance of these findings remains to be further confirmed as the activation of either PXR or CAR by artemisinin requires much higher concentrations (EC_{50} is $\sim 30 \mu M$ for human PXR; EC_{50} for human CAR was not calculated, but supposedly similar to PXR) than have been reported in humans. These studies also suggest that artemisinin therapy may cause a broader range of drug interactions than recognised at present. Therefore, artemisinin derivatives that are effective for malaria, but do not activate PXR or CAR, may represent safer antimalarial agents.

3.6 Wu Wei Zi and Gan Cao

Wu wei zi is the berries of *Schisandra chinensis* (Chinese Magnoliavine). Wu wei zi means 'five-flavour fruit' in Chinese because it has all the five basic flavours: salty, sweet, sour, pungent (spicy) and bitter. In traditional Chinese medicine, wu wei zi is used to treat many ailments, such as infections, coughing and thirst. Notably, hepatoprotective effects are clinically documented. The primary hepatoprotective and immunomodulating constituents are the lignans, schisandrin, deoxyschisandrin, gomisins and pregomisin [101]. Recently, it was reported that wu wei zi extracts, as well as several compounds found in wu wei zi including Schisandrol B and Schisandrin A and B (Table 1), are potent and efficacious PXR agonists in reporter gene assays [57]. Moreover, they efficaciously induce the PXR target genes CYP3A4 and -2C9 in primary hepatocytes. Gan cao (*Glycyrrhiza uralensis* Fisch), another traditional Chinese medicine that also has anti-inflammatory and hepatoprotective effects, is also found to activate PXR. However, it is not determined whether gan cao can also induce PXR target genes in primary human hepatocytes. Nonetheless, both wu wei zi and gan cao promote drug metabolism *in vivo*. This is illustrated by the increased metabolism of warfarin following either wu wei zi or gan cao treatment in rats.

Contrary to most other cases in which PXR activation is undesirable, the activation of PXR by these two herbs may account for the beneficial effects. Several studies have shown that PXR activation prevents lithocholic acid-induced liver toxicity [9,58]. The beneficial use of PXR is exemplified by St. John's Wort and by rifampicin, which is the prototypical human PXR agonist and has long been used to treat cholestasis in humans [59,60]. Additional studies show that PXR activation promotes bilirubin detoxification in mice [19]. Taken together, these studies again highlight the dual nature of PXR activation: the promotion of drug metabolism, leading to serious potential drug interactions and therapeutic failure, and the activation of detoxifying systems to protect our bodies from toxic insults.

3.7 Others

Paclitaxel (Table 1), a widely used antineoplastic agent isolated from the bark of the Pacific yew in the ancient forests of the northwestern US, is an efficacious human PXR agonist [24]. Paclitaxel also activates mouse PXR, although less efficaciously [49]. Docetaxel, a relative of paclitaxel, lacks PXR agonist activity [24]. Activation of PXR by paclitaxel has been suggested

to account for the autoinduction of drug metabolism. Consistent with this notion, docetaxel shows better pharmacokinetic properties than paclitaxel [61,62].

Zearalenone (Table 1) is a mycoestrogen found in the fungi *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium equiseti* and *Fusarium crookwellense*. These fungi commonly exist in agricultural products. It was recently found that zearalenone selectively activates human PXR in reporter gene assays [25]. Moreover, this compound induces PXR target gene expression in two model systems in a human PXR-dependent manner: i) in HepG2 cells transduced with an adenovirus that encodes human PXR; and ii) in PXR-KO primary mouse hepatocytes transduced with a human PXR-expressing adenovirus. Although it remains to be determined whether these findings are relevant in humans, they raise the possibility that the consequences of PXR activation may extend from drug–drug interactions and herb–drug interactions to food–drug interactions.

Other natural products that have been shown to activate human PXR include quercetin, vitamin E and hydroxylated vitamin D₃ [63,64]. However, because these compounds are not well characterised as PXR agonists, they are not discussed in detail here.

4. Antagonism of pregnane X receptor by ecteinascidin-743

ET-743 is a natural compound found in the marine tunicate *Ectenascidia turbinata* (Table 1). Clinical trials have shown that ET-743 treatment has superior activity against sarcoma over conventional therapies with doxorubicin and ifosfamide [65]. ET-743 binds to the minor groove of DNA and blocks the interactions between certain transcription factors and the DNA sequence. This has been proposed as the major mechanism for its antitumour activity. Additional studies indicate that the activity of ET-743 also involves DNA repair machinery because ET-743 shows less cytotoxicity in cell lines deficient in nucleotide excision repair. This unique mechanism of action makes it possible to combine ET-743 with other anticancer drugs to produce a synergistic or additive effect.

Besides its superior antitumour activity, ET-743 also efficaciously inhibits the expression of CYP3A4 and MDR1 genes in tissue cultures [24,66]. The inhibition of CYP3A4 and MDR1 gene expression by ET-743 is likely to be mediated through its antagonism of PXR. In reporter gene assays ET-743 efficaciously represses PXR activation by agonist ligands [24]. The IC₅₀ value for ET-743 against PXR is ~ 3 nM. As overexpression of MDR1 is very problematic in cancer therapy, the ability to inhibit PXR through the use of ET-743 provides a potential means to repress MDR1 expression and thereby potentially enhance cancer chemotherapy.

The toxicity of ET-743 has been reported in both animal studies and human clinical trials. In most cases, it causes liver toxicity, including liver degeneration and patchy focal necrosis of bile duct epithelial cells, although the mechanism for this is not clear [65]. Interestingly, dexamethasone, which has been found to activate PXR at high concentrations, appears to decrease this liver toxicity [67]. Given that PXR activation often protects against liver toxicity, it will be interesting to determine whether the liver toxicity of ET-743 is in fact mediated through its antagonism of PXR, and whether a more potent and efficacious PXR agonist, such as rifampicin, would be more effective at repressing the liver toxicity caused by ET-743.

5. Conclusion

The work cited above clearly shows that the activity of PXR is modulated by a wide range of naturally occurring products. This further emphasises the xenobiotic-sensing nature of this nuclear receptor. Moreover, although most natural products cause potentially unwanted drug interactions through activation of PXR, some of them, such as wu wei zi and gan cao, may exert their beneficial hepatoprotective effects through its activation. ET-743 is a potent and

efficacious PXR antagonist. Although antagonism of PXR by this compound may provide a potential means to enhance drug therapy, it may also account for the liver toxicity induced by this compound. Given that PXR activation protects against certain liver toxicity, it will be interesting to investigate whether PXR agonists will reverse ET-743-induced hepatic toxicity. As mentioned previously, dexamethasone has been reported to reduce ET-743-induced liver toxicity. Therefore, it will be interesting to design ET-743 analogues that retain antitumour activity, but lack PXR activity, and to determine if this will reduce liver toxicity. Finally, several important questions remain: i) what is the mechanism whereby ET-743 antagonises PXR; ii) does ET-743 bind directly to PXR and modulate PXR-cofactor interactions; iii) can this effect be translated into humans *in vivo*; and iv) how does ET-743 cause hepatic toxicity?

6. Expert opinion

Among all the natural compounds discussed herein, only hyperforin has been confirmed to directly bind to PXR with a direct ligand-binding assay. The binding of forskolin and guggulsterone to PXR has been tested with an indirect coactivator receptor ligand-binding assay. No direct or indirect ligand-binding assay has been performed on any of the other compounds discussed. As CAR is activated by certain compounds including bilirubin and phenobarbital through a binding-independent and possibly phosphorylation/dephosphorylation-dependent mechanism [68,69], it will be interesting to investigate whether some of these natural compounds also modulate the activity of PXR through indirect mechanisms such as phosphorylation.

Because herbal products are mostly available over the counter, and because of the notion that herbs are 'all natural' and have 'no deleterious side effect', less caution has been taken with respect to their safety issues. However, the work cited above unquestionably supports the concept that herbs are not necessarily safe. Therefore, to predict and prevent potentially serious herb-drug interactions, systematic screening of natural products for their activity against PXR is needed.

Acknowledgments

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Figure 1. Coordinate regulation of xenobiotic response genes by PXR

Once activated by ligands, PXR turns on the expression of a number of hepatic genes involved in a xenobiotic response. Those include the sinusoidal transporters OATP2 and MRP3, the biotransforming CYPs, UGTs, sulfotransferases and GSTs, and the canalicular transporter Mrp2. In addition, PXR has been shown to regulate the MDR1, which is highly expressed in the intestines.

GST: Glutathione *S*-transferase; MDR: Multi-drug resistance protein; MRP: Multi-drug resistance-associated protein; OATP: Organic anion transporting protein; PXR: Pregnane X receptor; RXR: Retinoid X receptor; SULT: Sulfotransferase; UGT: UDP-glucuronosyltransferase.

Figure 2. Pregnane X receptor ligands are structurally diverse

A variety of structurally diverse compounds have been shown to activate either human or rodent pregnane X receptor.

Figure 3. Ligand-dependent activation of PXR

Unliganded PXR binds to the regulatory regions of target genes as a heterodimer with another nuclear receptor RXR and actively transrepresses target gene expression by recruiting HDAC-containing multi-protein complexes to the proximity of the target gene promoters. Agonist binding causes a conformational change of PXR that favours the exchange of HDAC-containing multi-protein complexes for histone acetyl transferase-containing multi-protein complexes and leads to transactivation of target gene expression.

HDAC: Histone deacetylase; PXR: Pregnane X receptor; RXR: Retinoid X receptor.

Table 1

Natural products that modulate the activity of human PXR.

Sources	Ingredients with PXR activity	PXR activator/antagonist	EC ₅₀ /IC ₅₀	Ref.
St. John's Wort	Hyperforin	Activator (human)	23 nM	[29,30]
Gugulipid	<i>E</i> -guggulsterone	Activator (rodent and human)	ND	[38]
	<i>Z</i> -guggulsterone	Activator (rodent and human)	2.4 μ M	
Kava kava	Desmethoxyyangonin	Activator (human)	ND	
	Dihydromethysticin	Activator (human)	ND	
<i>Coleus forskohlii</i>	Forskolin	Activator (rodent and human)	~ 400 nM	[49,50]
	1,9-Dideoxyforskolin	Activator (rodent and human)	ND	
<i>Hypoxis</i>	ND	Activator (human)	ND	
<i>Sutherlandia</i>	ND	Activator (human)	ND	
Qing hao	Artemisinin (qinghaosu)	Activator (human)	~ 30 μ M	[56]
Wu wei zi	Schisandrol B	Activator (human)	~ 2 μ M	[57]
	Schisandrin A	Activator (human)	~ 2 μ M	[57]
	Schisandrin B	Activator (human)	~ 2 μ M	[57]
Gan cao	ND	Activator (rodent)	ND	
Pacific yew	Paclitaxel	Activator (rodent and human)	~ 5 μ M	[24,49]
Various fungi	Zearalenone	Activator (rodent and human)	~ 1.5 μ M	[25]
<i>Ectenascidia turbinata</i>	Ecteinascidin-743	Antagonist (human)	~ 3 nM	[24]

ND: Not determined; PXR: Pregnane X receptor.