The Nuclear Pregnanne X Receptor Regulates Xenobiotic Detoxification

Steven A. Kliewer

Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX

ABSTRACT The pregnane X receptor (PXR), which is a member of the nuclear receptor family of ligand-activated transcription factors, is an integral component of the body’s defense mechanism against toxic xenobiotics. PXR is activated by a broad spectrum of lipophilic xenobiotics including prescription drugs, herbs, pesticides, endocrine disruptors and other environmental contaminants. The promiscuous ligand-binding properties of PXR are facilitated by the large volume and smooth shape of its ligand-binding pocket. PXR binds to DNA as a heterodimer with the 9-cis retinoic acid receptor (RXR) and regulates a large number of genes involved in the detoxification and excretion of toxic substances. Although PXR evolved to protect the body, its activation by various prescription drugs and herbs such as St. John’s wort represents the molecular basis for an important class of drug-drug interactions. Assays that detect PXR activation can now be used to predict and prevent these drug-drug interactions. J. Nutr. 133: 2444S–2447S, 2003.

KEY WORDS: nuclear receptor ▪ xenobiotic ▪ cytochrome P450 ▪ St. John’s wort ▪ drug-drug interaction

Biological organisms are constantly exposed to potentially harmful environmental chemicals called xenobiotics. Lipophilic xenobiotics are particularly problematic because they have the potential to accumulate to toxic concentrations over long periods of time. In this review, we highlight the role of the orphan nuclear pregnane X receptor (PXR) in the detection and detoxification of lipophilic xenobiotics.

Nuclear receptor signaling

The nuclear receptors comprise a superfamily of ligand-activated transcription factors that includes the steroid, retinoid and thyroid hormone receptors (1,2). Members of the family share a common structure that includes a variable amino-terminal domain, a highly conserved central DNA binding domain (DBD) of ~70 amino acids and a carboxy-terminal ligand-binding domain (LBD) of ~250 amino acids. Typically, there are two transcriptional activation domains in a nuclear receptor: the activation function 1 (AF-1), which resides in the N-terminal domain, and the AF-2, which is present in the C-terminal portion of the LBD (1,2).

The ligands for the nuclear receptors are all small and lipophilic in nature, which permits them to diffuse into cells. The binding of a ligand to the LBD results in a conformational change in the AF-2 that disrupts interactions with transcriptional co-repressor proteins such as N-CoR and SMRT and permits interactions with transcriptional coactivator proteins such as the SRC-1 family members (3). The activated nuclear receptor stimulates the expression of target genes by binding to short DNA sequence motifs, termed response elements, located in the regulatory regions of target genes.

There are 48 nuclear receptors in the human genome. These proteins can be loosely grouped into two categories based on their ligand status. The first group comprises the receptors for the classical endocrine hormones, including glucocorticoids, mineralocorticoids, estrogens, androgens, progesterone, thyroid hormone, vitamin D and all-trans retinoic acid. The ligands for these receptors were all identified as important endocrine hormones long before the receptors were discovered. The second group of nuclear receptors are collectively referred to as “orphans” (4). The orphan receptors were cloned based on their high degree of homology to the classic endocrine hormone receptors. However, at the time of their cloning, nothing was known about the physiologic ligands that act through the orphan receptors.

Over the past decade, rapid progress has been made in characterizing the biological functions of orphan receptors and the ligands that activate them. The process of using orphan receptors to hunt for novel physiologic ligands and signaling pathways is referred to as “reverse endocrinology” (5). Of the 36 orphan receptors in the human genome, ligands have been identified for 14 (6). The orphan subfamily is now known to include receptors for fatty acids (the peroxisome proliferator-activated receptors α, β, and γ), bile acids (the farnesoid X...
receptor), oxysterol metabolites of cholesterol (the liver X receptors \( \alpha \) and \( \beta \)), and lipophilic xenobiotics (the pregnane X receptor and constitutive androstane receptor).

**PXR and CYP3A induction**

Members of the cytochrome P450 (CYP) superfamily of heme-dependent monooxygenases often catalyze the first step in the detoxification of lipophilic chemicals (7). These P450 enzymes are remarkable for their broad and overlapping substrate specificities. In humans and other mammals, members of the CYP3A subfamily of P450 enzymes are particularly relevant to xenobiotic metabolism because of their broad substrate specificity and their abundance in the liver and intestine, tissues that are routinely exposed to environmental chemicals (8,9).

The transcription of CYP3A isoymes can be induced by a variety of xenobiotics (8,9). Because many of the chemicals that induce CYP3A expression also are substrates for CYP3A enzymes, this induction provides a mechanism for amplifying the detoxification response during prolonged periods of xenobiotic exposure. Once the xenobioteic challenge is resolved, CYP3A concentrations can return to normal. Induction of CYP3A also represents the basis for an important class of drug-drug interactions (see below).

The orphan nuclear receptor PXR was originally cloned from mouse, but has since been cloned from a wide range of species including mammals, birds and fish (10). PXR was named based on its activation by C21 steroids (pregnanes) including pregnenolone \( 16\alpha\)-carbonitrile (PCN) (Fig. 1), a classic inducer of CYP3A in rats and mice (11). There is now ample evidence that PXR serves as a master transcriptional regulator of CYP3A isoymes (9,12–14):

1) PXR and CYP3A are coexpressed. PXR is highly expressed in the liver and intestine. These are the same tissues in which CYP3A isoymes are most highly expressed and induced in response to xenobiotic exposure.

2) PXR is activated by CYP3A inducers. The discovery that PXR is activated by PCN provided the original link to CYP3A (11). It is now established that PXR is activated by nearly all of the xenobioteics and other chemicals that are known to stimulate CYP3A expression (14). These chemicals include prescription drugs [e.g., rifampicin, dexamethasone, indinavir, paclitaxel (Fig. 1)], pesticides (e.g., trans-nonachlor, chlordane), endocrine disruptors (e.g., phthalic acid, nonylphenol) and other environmental contaminants (e.g., polychlorinated biphenols). Notably, there are marked differences in the pharmacological activation profiles of PXR derived from various species (15). For example, the mouse and rat PXR are activated efficiently by PCN but not rifampicin. Conversely, the human and rabbit PXR are activated efficiently by rifampicin but not PCN. These pharmacological differences in PXR explain the well-documented differences in CYP3A induction between species.

3) PXR binds to CYP3A promoters. Xenobioteic response elements had been defined in the promoters of genes encoding human, rabbit and rat CYP3A isoymes (9). Each of these response elements contains two copies of the nuclear receptor binding site AGTTCA. The two binding sites are either organized as a direct repeat with a spacer of three nucleotides (DR-3) or as an everted repeat with a spacer of six nucleotides (ER-6). PXR binds to each of these response elements as an obligate heterodimer with RXR.

4) CYP3A is dysregulated in PXR-null mice. Recently, two groups have generated mice that lack PXR (16,17). The mice do not have any overt phenotype under standard laboratory conditions. However, their response to xenobioteics is severely compromised. Cyp3a11 is not induced in response to either PCN or dexamethasone in the livers of these PXR-null mice.

**Additional PXR target genes**

Recent gene profiling studies performed with PCN in wild-type and PXR-null mice have revealed that, in addition to CYP3A, PXR regulates a large number of genes involved in xenobiotic detoxification in the liver and intestine (18). Among the genes regulated by PXR are those encoding P450 enzymes, aldehyde dehydrogenases, UDP-glucuronosyltransferases, sulfotransferases, glutathione-S-transferases and various transporters such as multidrug resistance protein 1 and organic anion transporter peptide 2. A similar pattern of gene regulation was observed in experiments performed with primary cultures of human hepatocytes and the PXR ligand rifampicin (18). Thus, activation of PXR coordinately stimulates the expression of a large program of genes involved in the solubilization and excretion of xenobiotics from the body.

**Drug-drug interactions**

Although PXR evolved as part of a broader protective response against lipophilic xenobiotics, its activation also represents the basis for an important class of drug-drug interactions. This is because many of the xenobiotics that activate PXR are prescription drugs, including the antibiotic rifampicin, the anti-inflammatory glucocorticoid dexamethasone, the HIV protease inhibitor ritonavir and the cancer drug paclitaxel (9,12–14). Activation of PXR and the subsequent induction of CYP3A and other genes by these drugs can result, in turn, in an accelerated metabolism of other medications. CYP3A4 alone is involved in the metabolism of >50% of all prescription drugs. Thus, drugs that activate PXR have the potential to reduce the clinical efficacy of more than one-half of all other drugs that are coadministered, often with life-threatening consequences. This phenomenon is a serious
problem in the current era of polypharmacy, in which patients are often taking multiple medications.

Ideally, new drugs would not activate PXR. Now that robust in vitro assays are available to detect PXR activity, it is possible to screen candidate drug molecules prospectively and replace those that activate PXR with chemicals that do not (19).

St. John’s wort

St. John’s wort is an herb derived from the flowering plant *Hypericum perforatum* that is widely used to treat mild to moderate depression. In the United States, St. John’s wort is unregulated. In the late 1990s, a series of reports appeared in the medical literature describing interactions between St. John’s wort and various prescription drugs including oral contraceptives, the immunosuppressant cyclosporin, the HIV protease inhibitor indinavir and the anticoagulant warfarin (20, 21). In each case, co-treatment with St. John’s wort resulted in a marked decrease in the effective concentrations of the prescription drug, often with life-threatening consequences.

It was subsequently demonstrated that commercial preparations of St. John’s wort activate PXR in cell-based reporter assays and induce CYP3A4 expression in primary cultures of human hepatocytes (22). St. John’s wort is a complex mixture of about two dozen different chemicals. Further analysis revealed that hyperforin (Fig. 1) is the chemical constituent of St. John’s wort that activates PXR (22, 23). Hyperforin binds directly to PXR with high affinity (Kᵢ ~ 20 nM).

These findings provide a molecular explanation for the clinical reports describing interactions between St. John’s wort and prescription drugs. More importantly, these findings predict that St. John’s wort will interact with all prescription drugs that are metabolized by CYP3A4, which includes more than one-half of all drugs currently in use. This prediction is supported by a recent report that St. John’s wort interacts with the cancer drug irinotecan (24).

PXR structure

Most nuclear receptors interact with their ligand in a highly selective manner. By contrast, PXR serves as a promiscuous receptor for a diverse spectrum of lipophilic substances. How does PXR recognize such a diverse set of chemicals?

Recent X-ray crystallography studies conducted on the PXR LBD both in the presence and absence of ligands provide important insights into the basis for its atypical ligand-binding properties. The overall architecture of the PXR LBD is very similar to that of other nuclear receptors: the LBD is comprised of 12 α-helices that fold to form a hydrophobic pocket in the lower portion of the protein (25). However, there are several features of the PXR LBD that distinguish it from other nuclear receptors. The volume of the PXR ligand-binding pocket is >1300 Å³, which is much larger than that of most other nuclear receptors. The large volume of the pocket is due in part to the presence of two additional strands of β-sheet that are not found in other nuclear receptors. Like other receptors of this class, the ligand-binding pocket is extremely hydrophobic. However, the PXR ligand-binding pocket is unique in terms of its smooth, elliptical shape. This combination of large volume and smooth shape undoubtedly contributes to the unusual ligand-binding properties of PXR. Thus, PXR is ideally suited to function as a broad-specificity sensor of lipophilic xenobiotics.

The identification and characterization of PXR has provided important and unexpected insights into how the body protects itself against the accumulation of toxic lipophilic xenobiotics.

Unlike other nuclear receptors, which are characterized by their high degree of specificity, PXR evolved to be a broad-specificity xenobiotic sensor. Activation of PXR results in the induction of an elaborate network of genes involved in all aspects of xenobiotic detoxification and excretion. Importantly, PXR activation also represents the molecular basis for a prominent class of drug-drug interactions. Our understanding of PXR can be exploited to predict potential interactions between drugs that are currently in use and to design future drugs that will not lead to this class of harmful drug-drug interactions.

LITERATURE CITED


