PREGNANE X RECEPTOR: ITS VERSATILE ROLE BEYOND XENOBIOTIC REGULATION

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ABSTRACT

The pregnane X receptor (PXR, also known as steroid and xenobiotic receptor or SXR, NR1I2) is a ligand-activated transcription factor that belongs to the nuclear receptor superfamily. The enlarged, hydrophobic, and flexible ligand binding domain (LBD) of PXR allows it to bind to diverse substrates and regulate their metabolism, and thus is considered as a key xenosensor. In the past decades, the function of PXR in the regulation of xenobiotic metabolism has been extensively studied. However, in recent years, compelling evidence has been found that PXR can also be implicated in various physiological conditions as well as different diseases. This review provides an update for newly identified agonists and antagonists for PXR, and also highlights the regulatory role of PXR beyond xenobiotic metabolism, *i.e.* in lipid metabolism, glucose metabolism, inflammation, *etc*. Further research to improve the understanding of the non-xenobiotic functions of PXR are still needed for its therapeutic applications.

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Introduction:

Humans and other animals get exposed to various chemicals every day. Regardless of whether or not exposure is intentional or accidental, these chemicals must be metabolized in order to maintain proper functioning of the body. These compounds can be classified as xenobiotics, which are foreign substances found in an organism. In addition, naturally occurring materials, known as endobiotics, can also be classified as xenobiotics when they are present in unusually high levels (Soucek 2011). Xenobiotics are omnipresent and they come in various different forms such as drugs, cosmetics, and household cleaning products, among other things. With the continuously modernizing world, humans have become more exposed to these xenobiotics. This warrants a better understanding of how the body processes these compounds.

Certain signaling molecules activate proteins that regulate transcription of specific genes; such proteins are known as nuclear receptors (Forman and Evans 1995). Xenobiotic receptors, which is a subgroup under the nuclear receptor superfamily, bind a wide array of both endobiotics and xenobiotics and they play a pivotal role in the regulation of drug metabolizing enzymes (Mackowiak and Wang 2016). One of the most extensively studied xenobiotic receptors is the pregnane X receptor (PXR), also known as steroid and xenobiotic receptor (SXR), which was found to induce CYP3A via the binding of pregnenolone 16α-carbonitrile (PCN), giving rise to the name of the said receptor (Kliewer *et al.* 1998).

What makes PXR unique is its ligand binding promiscuity. Nuclear receptors have a DNA-binding domain (DBD) and a ligand-binding domain (LBD). For the most part, the DBDs of nuclear receptors are highly conserved, while more variations can be observed with the LBDs. The characteristic promiscuity of PXR can be attributed to its large and mostly hydrophobic

ligand binding cavity with a few polar residues scattered throughout, as well as a flexible loop that allows the binding of both large and small ligands (Watkins *et al.* 2001). Given this feature of PXR, it is able to bind different substances and regulate their metabolism. This review lists established and recently discovered agonists of PXR.

PXR Structure:

Unlike other members of the nuclear receptor superfamily that are highly specific with their substrates, PXR stands out since it can bind a wide range of ligands. Over time, the structure of PXR, both in its apo form and when it is bound to a ligand, has been studied extensively to elucidate its substrate promiscuity. This feature can be attributed to deviances in its structure from the usual nuclear receptors. PXR has 5 strands of β sheets, as opposed to common nuclear receptors with only 3. In addition, it has a flexible loop in place of alpha helix 6, which can change size to fit big and small ligands (Watkins *et al.* 2001). In its apo form, the PXR binding pocket is 1294 Å. This can shrink to 1280 Å when bound to cholesterol lowering compound SR12813, but it could also expand to fit larger molecules like hyperforin, the active compound in St. John's Wort (Watkins *et al.* 2003).

The PXR LBD is a three-layered helical sandwich and its binding pocket is lined with 20 hydrophobic, 4 polar, and 4 potentially charged amino acids, with the polar residues scattered throughout. Different ligands contact varying numbers of amino acids once in the binding pocket (Watkins *et al.* 2001). For instance, hyperforin contacts 12 residues, T1317 contacts 15, and rifampicin touches 18 (Watkins *et al.* 2003, Chrencik *et al.* 2005, Xue *et al.* 2007). Mutation studies have been done to test the effects of changing the residue lining of the binding pocket to substrate affinity and results vary depending on the ligand. Aside from this, the presence of

coactivators also affects the way ligands bind to PXR. SRC-1 was found to trap SR12813 in a single rigid conformation when bound to PXR, unlike the initial finding where it can have 3 different binding conformations when bound alone to the receptor (Watkins *et al.* 2003).

Signaling Pathways:

PXR plays an important role in different signaling pathways that regulate the metabolism of xenobiotics, lipids, and glucose. In addition, PXR is also a key player in the pathways involving inflammation.

PXR in Xenobiotic Metabolism

The role of PXR in drug metabolism has been extensively studied throughout the years. PXR has been established to be a crucial player in the metabolism of xenobiotics. It regulates the expression of phase I and II enzymes, as well as drug transporters. The receptor induces the enzyme cytochrome P450 3A4 (CYP3A4), which is responsible for metabolizing more than half of prescription drugs during Phase I. It has also been discovered that hepatocyte nuclear factor 4 alpha (HNF4a) is involved in this PXR-induced activation of CYP3A4. Knocking down HNF4a reduces the expression of CYP3A (Tirona *et al.* 2003). In addition to HNF4a, SRC1 and PGC1α also interact with PXR to induce CYP3A4 activation. By contrast, Small heterodimer partner (SHP) inhibits the activation of CYP3A4, and PXR activation represses this (Li and Chiang 2006).

When it comes to phase II of drug metabolism, PXR regulates enzymes under the uridine-5'-diphosphate glucuronosyltransferase (UGT), sulfotransferase (SULT), and glutathione S-transferase (GST) families (Tolson and Wang 2010). These enzymes are responsible for making the drugs more hydrophilic thus facilitating their excretion into bile and urine (Xu *et al.*

2005). UGTs conjugate both endobiotics and xenobiotics and they are regulated by ligand activated transcription factors such as PXR (Bock 2010). SULTs, on the other hand, increase solubility in water by forming sulfate conjugates (Bian *et al.* 2007). Lastly, GSTs catalyze the formation of hydrophilic glutathione conjugates (Knight *et al.* 2008). Regulation of these enzymes by PXR is ligand-dependent and the effects could either be activating or inhibiting (Ihunnah *et al.* 2011). These ligand-dependent effects of PXR were also studied by Masuyama and collaborators, who found that certain steroids and endocrine disrupting chemicals (EDCs) preferentially activate one over the other when it comes to CYP3A4 and multiple drug resistance 1 (MDR1), an important drug transporter involved in multidrug resistance (Masuyama *et al.* 2005). In particular, they found that the anticancer agents paclitaxel and cisplatin activated MDR1 more strongly than CYP3A4. In addition to MDR1, PXR activation also induces the expression of another drug transporter, the multidrug resistance-associated protein isoform 2 (Mrp2). An increase in activity of Mrp2 was observed after exposure to known PXR ligands PCN and dexamethasone (Bauer *et al.* 2008). PXR activation has also been shown to increase the expression of organic anion transporting polypeptide 2 (Oatp2). This, however, is also dependent on the PXR ligand. For instance, PCN was found to induce Oatp2 expression, whereas spironolactone and dexamethasone have no effects on Oatp2 expression (Guo *et al.* 2002).

PXR in Lipid Metabolism

With a combination of suppression of β oxidation, upregulation of lipogenesis, and increase in fatty acid uptake, PXR can alter lipid homeostasis (Zhou *et al.* 2006). In a previous study, ChIP assays revealed that treatment with PCN, a known PXR activator, reduced the binding of FoxA2 to the promoters of genes encoding carnitine palmitoyltransferase 1A (CPT1A) and mitochondrial 3-hydroxy-3-methylglutarate CoA synthase 2 (HMGCS2)

(Nakamura *et al.* 2007). Normally, FoxA2 activates these genes which increase the supply of ketone bodies when the body is in a fasting state. When PXR is activated, it binds directly to FoxA2 which then prevents it from binding to Cpt1A and Hmgcs2. This PXR-mediated repression of β-oxidation and ketogenesis could lead to the development of hepatic steatosis due to the accumulation of triglycerides. Another way that PXR exerts such effects is through the inhibition of PPAR α and thiolase, which are genes that also promote β oxidation (Zhou *et al.* 2006).

In addition, PXR also mediates lipid accumulation by upregulating lipogenic enzymes such as stearoyl-Coa desaturase 1 (SCD1) and fatty acid elongase (Zhou *et al.* 2006). PXR activation can also increase the expression of S14, a gene that can be induced by thyroid hormones, and also a key player in de novo lipid synthesis (Moreau *et al.* 2009). Interestingly, another study has found that both activation and knockdown of PXR can induce lipid accumulation by affecting two different lipogenic pathways (Bitter *et al.* 2015). This shows just how complex this receptor can be. In this study, PXR activation upregulated the lipogenic pathway involving the sterol regulatory element-binding protein (SREBP) 1, while PXR knockdown induced lipogenesis by increasing the expression of aldo-keto reductase (AKR) 1B10, which enhances the activity of acetyl-Coa carboxylase (ACC), a rate-limiting enzyme for de novo lipid synthesis (Ma *et al.* 2008). Lastly, PXR activation can also increase fatty acid uptake by upregulating PPARγ, which enhances the activity of CD36, a fatty acid transporter (Zhou *et al.* 2006).

Furthermore, PXR also plays a role in the synthesis of bile from cholesterol. Hnf4 activates Cyp7a1 and Cyp8b1, which are genes responsible for bile acid synthesis. PGC1 is a

coactivator for various receptors like HNF-4 and PXR. When PXR is activated, it suppresses HNF4 activity by competing for their common coactivator, PGC1. Thus, PXR activation can disrupt cholesterol-bile acid homeostasis, by suppressing the pathway which is responsible for bile acid synthesis (Bhalla *et al.* 2004).

PXR in Glucose Metabolism

Aside from lipid metabolism, PXR also affects glucose metabolism (Kodama *et al.* 2004). Kodama and collaborators found that direct interaction between PXR and FOXO1, an activator of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase 1 (PEPCK1) and glucose-6-phosphatase (G6Pase), is responsible for coregulation of drug and glucose metabolism. FOXO1 activates PXR, so the inhibition of FOXO1 mediated by insulin via the PI3K-Akt pathway can repress PXR induced drug metabolism. Another way that PXR activation regulates gluconeogenesis is through PGC1α. When rifampicin activates PXR, it interacts with PGC1, as previously mentioned, and it suppresses HNF4a. When this occurs, PEPCK is downregulated (Mackowiak et al., 2018). Conversely, the activation of PXR by PCN downregulates FOXO1 and therefore inhibits gluconeogenesis. In another study which was also done by Kodama *et al.*, they highlighted another crosstalk involving PXR; this time, it is with cAMP-response element (CRE)-binding protein (CREB). PXR downregulates gluconeogenesis by repressing the activity of G6Pase. This PXR-induced repression occurs when PXR prevents the binding of CREB to CRE, which in turn inhibits G6Pase transcription (Kodama *et al.* 2007).

Despite downregulating gluconeogenic enzymes as shown by previously mentioned studies, increased postprandial glucose levels in rats treated with PCN and humans taking

rifampin was still observed in a study by Rysa *et al.* (Rysä *et al.* 2013). They attributed this hyperglycemia to decreased expression of hepatic Glut2 mRNA mediated by activation of PXR.

In contrast to studies that show repression of gluconeogenesis by PXR activation, Spruiell et al. found that activation of hPXR in mice upregulates PEPCK and G6Pase when fed with a high-fat diet (HFD) compared to wildtype mice (Spruiell *et al.* 2014). Additionally, they saw an inhibition of UCP1 in white adipose tissue (WAT) and glucokinase in HFD-fed hPXR mice, which resulted in glucose intolerance. A similar upregulation of G6Pase was shown in another study done by Gotoh and Negishi (Gotoh and Negishi 2014). They looked at rifampicin-treated HepG2 cells and found that PXR activation induces gluconeogenesis by activating G6Pase in the presence of serum- and glucocorticoid-regulated kinase 2 (SGK2). However, G6Pase was downregulated by PXR activation without SGK2.

PXR in inflammation

Aside from its canonical role in metabolism, PXR is also involved in the regulation of the inflammatory response. Zhou et al. showed that PXR activation reduces inflammation by repressing NFκB, which is a major regulator of inflammation and immune response. Similarly, NFκB activation inhibits PXR activity, and NFκB repression by IκBαM activates PXR and CYP3A4 (Zhou *et al.* 2006). This relationship between IκBα-induced inhibition of NFκB was further studied by Ye *et al*. (Ye *et al.* 2018). They found that rifampicin activation of PXR upregulated IκBα expression which prevents the nuclear translocation of p50/p65 NFκB heterodimer. When HepG2 cells were treated with lipopolysaccharides (LPS) alone or with rifampicin at the same time, staining revealed an accumulation of p50/p65 in the nucleus. However, when the cells were treated beforehand with rifampicin, this accumulation was not

observed. This suggests that rifampicin-activated PXR repression of LPS-induced inflammation relies on the synthesis of IκBα, instead of direct inhibition.

PXR activation has been shown to have a negative regulatory role on proinflammatory mediators and a positive regulatory role on anti-inflammatory mediators during early and late stages of the inflammatory response, respectively. Suppression of LPS-induced expression of inflammatory cytokines such as interleukin 1 β (IL-1β), interleukin 6 (IL6), and tumor necrosis factor alpha (TNFα) was observed in primary cultures of hepatocytes from wildtype mice when PXR was activated. Meanwhile, these inflammatory markers were upregulated in PXR-knockout mice. In contrast to this, PXR activation increased the expression of a certain isoform of IL1 receptor antagonist (IL1-Ra), which negatively regulates IL1 (Sun *et al.* 2015).

In another study, alpha ketoglutarate (AKG) supplementation was shown to modulate PXR activity despite not being a known PXR ligand. The proposed mechanism for this observed PXR induction is through the inhibition of LPS-induced NFκB inflammatory pathway (He *et al.* 2017). This corroborates previous findings that NFκB repression can activate PXR. To further understand the anti-inflammatory effects of PXR, Okamura et al. studied the expression of chemokine-encoding genes and found that PCN-activated PXR only partially suppressed Cxcl2 expression when NFκB was inhibited or mutated (Okamura *et al.* 2020). However, mutation of both NFκB and activator protein 1 (AP-1), a transcription factor involved in the inflammatory pathway, completely repressed Cxcl2 expression. This suggests that AP-1 can also be targeted by PXR to inhibit inflammation.

An imbalance in the gut microbiota can cause intestinal inflammation. Given its role in regulating inflammatory pathways, PXR is also involved in maintaining gut homeostasis. Indole

3-propionic acid (IPA), which is produced by microbes living in the gut, was found to induce PXR and downregulate TNFα. In addition, knocking down PXR (Nr1i2 $^{-/-}$) in enterocytes resulted in an upregulation of toll-like receptor (TLR) pathways. Particularly, they found that TLR4 signaling is an essential causative pathway in the epithelial barrier defects observed in the Nr1i2 $^{-/-}$ mice. When TLR4 is inhibited, expression of inflammatory cytokines is decreased in Nr1i2 deficient mice, whereas these defects in epithelial barrier were corrected in Nr1i2 $^{-/-}$ TLR4 $^{-/-}$ mice (Venkatesh *et al.* 2014). TLR4 has been implicated in the pathogenesis of necrotizing enterocolitis (NEC). PXR was found to regulate the stability of TLR4 mRNA (Huang *et al.* 2018) and it has been shown that PXR activation by lithocholic acid attenuates inflammation in NEC by decreasing the levels of IL6 and TNFα.

Just like how PXR activation decreases IL6 expression, treatment with IL6 can downregulate PXR activity. High levels of cytokines like IL6 decreases the metabolism of drugs, which is a major function of PXR. IL6 treated hepatocytes exhibited a decrease in PXR, followed by a repression of CYP3A4 activity (Yang *et al.* 2010). Another study links this relationship with differentiated embryonic chondrocyte-expressed gene 1 (DEC1), which has been shown to be induced by IL6 (Ning *et al.* 2017). Using primary hepatocytes and HepG2 cells, they showed that IL6-induced expression of DEC1 downregulated PXR activity, along with its target genes like CYP3A4 and MDR1. They also demonstrated the reverse of this process when DEC1 was knocked down. Another study done by Abualsunun and Piquette-Miller demonstrated that PXR repression by IL6 occurs through the STAT3 pathway (Abualsunun and Piquette-Miller 2018). Later on, they found that NFκB is also involved in this process. When NFκB was inhibited, IL6-induced downregulation of PXR activity and those of its target transporters was attenuated (Abualsunun *et al.* 2020).

Role of PXR in Diseases

Given that PXR regulates a wide selection of genes, it comes as no surprise that the receptor is involved in a number of diseases. Its role can either be of a protective or aggravating nature, depending on a lot of different factors such as the organ targeted, the ligand, and the genes involved.

Liver

PXR is highly expressed in the liver, which is not only responsible for detoxification, but is also responsible for regulating glucose homeostasis in the body. The role of PXR in glucose metabolism is not very straightforward, as exhibited in the previous section of this review. PXR activation inhibits gluconeogenesis by repressing the enzymes G6Pase and PEPCK (Kodama *et al.* 2007, Mackowiak *et al.* 2018). Despite this downregulation of gluconeogenesis, hyperglycemia and glucose intolerance are still observed when PXR is activated. Some possible explanations for this include PXR-mediated repression of Glut2 and glucokinase (GCK) due to the activation of the receptor by atorvastatin, which decreases glucose uptake in hepatocytes (Rysä *et al.* 2013, Ling *et al.* 2016). Another way that PXR activation affects Glut2 is by localizing the transporter in the cytosol, which further decreases the uptake of glucose (Hassani-Nezhad-Gashti *et al.* 2018). These results implicate PXR in the pathogenesis of diabetes due to its role in dysregulating glucose metabolism.

The liver is also the site of bile production, and cholestasis occurs when bile flow from the liver to the gallbladder is impaired (Han *et al.* 2016). PXR can act as both a sensor for bile acids in the form of lithocholic acid (LCA) and a regulator that induces genes to reduce LCA levels (Staudinger *et al.* 2001). PCN-induced activation of PXR has a protective role against

cholestasis due to increased expression of CYP3A11 and MRP3, which hydroxylate and transport bile acids for efflux, respectively (Teng and Piquette-Miller 2007). Being the key regulator of xenobiotic and endobiotic metabolism, PXR can also induce phase I and II enzymes, as well as phase III transporters to regulate bile excretion (Jonker *et al.* 2012). PXR can also block bile acid synthesis, as mentioned in the previous section, by competing with HNFα for PGC1, which downregulates CYP7A1 and CYP8B1 (Bhalla *et al.* 2004).

PXR is also implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). Just like in glucose metabolism, the role of PXR in lipid metabolism is also quite complex. As mentioned previously, lipid accumulation can be induced by both the activation and knockdown of PXR and this occurs via PXR activation-induced upregulation of SREBP1 and PXR knockdown-induced upregulation of AKR1B10, both of which promote lipogenesis that contributes to hepatic steatosis (Bitter *et al.* 2015). It has also been observed that rifampicin-induced PXR activation can increase the expression of solute carrier family 13 member 5 (SLC13A5) in human primary hepatocytes, which transports citrate needed for fatty acid and cholesterol synthesis. This increased expression was accompanied by fat accumulation in the hepatocytes, while SLC13A5 knockdown led to a decrease in lipid levels in HepG2 cells (Li *et al.* 2015). Furthermore, it has been reported that PXR polymorphisms may be responsible for increasing the severity of NAFLD (Sookoian *et al.* 2010)

As the key player in drug metabolism, PXR also increases the body's susceptibility to drug-induced liver injury (DILI). PXR-mediated action of drug-metabolizing enzymes (DMEs) could potentially lead to a buildup of toxic metabolites and endogenous toxicants (Wang *et al.*

2014). In addition, PXR-induced drug-drug interactions could also have adverse effects that could lead to DILI (Wang *et al.* 2020).

Activation of PXR could also induce hepatomegaly. One study reported that this enlargement of the liver is due to an increase in hepatocyte size as well as cell proliferation, which occurs because of the interaction between PXR and yes-associated protein (YAP), leading to a nuclear translocation of the latter (Jiang *et al.* 2019). YAP is a key transcription factor in the Hippo signaling pathway and plays a pivotal role in organ size control and tumor suppression by restricting proliferation and promoting apoptosis (Zhao *et al.* 2010, Low *et al.* 2014). A more recent study, however, showed that lipid accumulation and not hepatocyte proliferation is key to hepatomegaly induced by dexamethasone via the PXR-YAP interaction (Jiao *et al.* 2020).

Intestines

In addition to the liver, PXR is also highly expressed in the intestine. Due to its role in inflammation, PXR has been shown to have protective effects against inflammatory bowel disease (IBD) which could manifest as diseases like ulcerative colitis and necrotizing enterocolitis. PXR activation by PCN has been shown to decrease the expression of NFκB and its target genes in mice with dextran sulfate sodium (DSS)-induced IBD (Shah *et al.* 2007). Treatment with rifaximin, an intestine-specific PXR agonist has also been shown to reduce LPS-induced inflammation by decreasing the levels of IL8, TNFα, and other chemokines and cytokines that are produced in response to TLR4 activation (Mencarelli *et al.* 2011). In another study, PXR activation with rifaximin and 2 other agonists rifampicin and SR12813 led to an enhancement of the wound healing response and intestinal epithelial repair. This happens through the activation of p38 MAP kinase-dependent cell migration (Terc *et al.* 2014).

Another proposed mechanism through which PXR exerts its protective effects in the intestine is via the regulation of the expression of myosin light chain kinase (MLCK) and c-Jun N-terminal kinase 1/2 (JNK1/2), which are involved in signaling pathways that disrupt the barrier of intestinal epithelial cells. u upregulates MLCK and JNK1/2 while PXR activation attenuates this and promotes tight junction integrity (Garg *et al.* 2016).

Cardiovascular

With its role in lipid metabolism as well as inflammation, PXR is also involved in the pathogenesis of cardiovascular diseases (CVDs). PXR has been shown to upregulate lipogenesis as mentioned in the previous section of this review. Using ApoE $^{-/-}$ mice as experimental model, Zhou and collaborators found that PXR activation can increase very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels (Zhou *et al.* 2009). One of the major risk factors for developing atherosclerosis is dyslipidemia, so PXR activation can increase one's risk of developing CVDs.

PXR activation by endocrine disrupting chemical (EDC) bisphenol A (BPA) has also been shown to induce atherosclerosis independent of plasma lipid levels. Instead, this induction of atherosclerosis is attributed to an increase in the levels of CD36 expression, a fatty acid transporter responsible for the formation of foam cells via lipid uptake in macrophages (Sui *et al.* 2014). To further understand the effects of PXR in different tissues, Sui and colleagues generated LDLR deficient mice with myeloid-specific PXR deficiency. They found that this cell-specific knockout downregulated CD36 expression and led to a decrease in foam cell formation and lipid accumulation in macrophages, although there were no effects in plasma lipid levels (Sui *et al.* 2020).

Once again, the role of PXR is complex and its activation by different ligands can confer protective effects. In another study, PXR activation by statins was shown to have an atheroprotective effect by inhibiting the oxidized LDL- or TNFα-induced activation of NLRP3 inflammasome in endothelial cells (Wang *et al.* 2017). Besides atherosclerosis, PXR has also been shown to affect atherothrombosis by inhibiting SRC-family kinases (SFKs). Activation of PXR in platelets at low doses was observed to inhibit platelet activation, but the consequences of chronic exposure have yet to be explored (Flora *et al.* 2019).

Another cardiovascular condition impacted by PXR is the calcification of aortic valves. A previous study showed that high concentrations of inorganic phosphate, warfarin, a commonly used anticoagulant, can induce calcification in human aortic valve interstitial cells (HAVICs). This process was mediated through the upregulation of bone morphogenetic protein 2 (BMP2) induced alkaline phosphatase (ALP) activation. It has been demonstrated that this warfarin induced calcification occurs in a PXR-dependent manner because inhibition of PXR reduced the observed calcification (Yu *et al.* 2019). In a separate study by the same group, they further investigated if menaquinone-4 (MK-4), a common form of vitamin K12 in animals, can counteract the effects of warfarin, given that the latter is an anticoagulant. Interestingly, it has been found that MK-4 actually enhances the calcification caused by warfarin and it also elicits the same effects on its own via the same pathway as warfarin (Yang *et al.* 2020).

As mentioned previously, PXR is activated by IPA, a metabolite from the intestinal microbiota. The effects of this activation is not limited to the gut, however. IPA-induced activation of PXR has also been shown to decrease vasodilation by downregulating the

expression of nitric oxide synthase (eNOS) and lower nitric oxide in endothelial cells (Pulakazhi Venu *et al.* 2019).

PXR Agonists and Antagonists

With its substrate promiscuity and its role in a plethora of signaling pathways, the amount of substances that regulate the activity of PXR continues to increase as more agonists and antagonists for PXR are discovered. A variety of compounds can modulate PXR including drugs, EDCs, plant extracts, food additives, and more. These substances can either activate or inhibit PXR which can then initiate or suppress a cascade of events; such pathways have been discussed in the earlier sections of this paper. Table 1 outlines new compounds that have been shown to control PXR activity, their physiological impacts, as well as the tissue type studied to obtain the said results. It is important to note the effects of these substances due to their potential effects, either as novel therapeutic agents or as harmful chemicals to be avoided.

Table 1. List of recently studied PXR agonists and antagonists

compound	type	general classification	effect on PXR	effect on PXR target genes	physiological result	tissue/cell	citation
nelfinavir	HIV protease inhibitor	drug	partial agonist, competitive antagonist	downregulate ABCB1 and CYP3A4	resensitize chemoresistant cells	liver	(Burk *et al.* 2021)

gefitinib	tyrosine kinase inhibitor	drug	downregulates	downregulate CYP3A4	reduce chemoresistance	liver	(Abbott *et al.* 2020)
dabrafenib	anti-cancer drug	drug	activates	increase FG19 expression; induce CYP2B6 and CYP3A4	enhance cell proliferation; further studies needed to investigate drug-drug interactions (DDIs)	colon, liver, prostate	(Creusot *et al.* 2020)
quetiapine	antipsychotic	drug	activates	increase NPC1L1 and MTP expression	induce hyperlipidemia	intestine	(Meng *et al.* 2019)
meclizine	antihistamine	drug	activates	inhibit RANKL-induced phosphorylation of ERK and p38	prevents bone loss and suppresses osteoclastogenesis	bone marrow-derived macrophages	(Guo *et al.* 2017)

itraconazole	antifungal drug	drug	activates	downregulate CYP3A4	xenobiotic metabolism	liver	(Stepankova *et al.* 2017)
PF-06282999	myeloperoxidase inactivator	drug*	activates	induces CYP3A4	further studies needed to investigate DDIs	liver	(Moscovitz *et al.* 2018)
pyrethroids	insecticides	EDC	activates			liver	(Fujino *et al.* 2019)
propiconazole	fungicide	EDC	activates	upregulate FASN, SCD1, CD36	induce steatosis	liver	(Knebel *et al.* 2019)
tebuconazole	fungicide	EDC	activates	upregulate FASN, SCD1, CD36	induce steatosis	liver	(Knebel *et al.* 2019)
cadmium	metal pollutant	EDC	activates	induces CYP450s	induce oxidative stress and apoptosis	myocardium (swine)	(Zhao *et al.* 2021)

lycium barbarum polysaccharide	constituent of goji berries	plant extract	downregulates	inhibit induction of DMEs	attenuate DEHP-induced liver injury	liver (rat)	(Liu *et al.* 2021)
patchouli alcohol	medicinal oil, fragrance	plant extract	activates	inhibit NFκB	attenuate inflammation, protect against colitis	intestine	(Zhang *et al.* 2020)
garcinoic acid	vitamin E derivative	plant extract	activates	induce CYP3A4 and MDR1	potential therapeutic agent	liver and intestine	(Bartolini *et al.* 2020)
oridonin	compound from Rabdosia rubescens	plant extract	activates	inhibit NFκB	attenuate post-inflammatory irritable bowel syndrome induced by TNBS	intestine	(Shao *et al.* 2020)

sesamin	lignan	plant extract	downregulates	downregulate SCD, FAS, S14	attenuate drug-induced lipogenesis	liver	(Tai *et al.* 2019)
pyrrolizidine alkaloids	plant metabolite	plant extract	activates	induce CYP3A4	induce hepatotoxicity	liver	(Luckert *et al.* 2018)
oridonin	compound from Rabdosia rubescens	plant extract	activates	induce CYP450s	xenobiotic metabolism	liver	(Yi-Wen *et al.* 2018)
tanshinone IIA	extract from rhizome of Salvia miltiorrhiza	plant extract	activates	suppress IL8	protect against oxidative stress	HUVECs	(Zhu *et al.* 2017)
alisol B 23-acetate	active compound from Alisma rhizome	plant extract	activates	induce CYP3A4	potential therapeutic agent	liver	(Kanno *et al.* 2017)
Schisandrol B	active compound from	plant extract	activates	induce CYP3A11,	protect against cholestasis	liver	(Zeng *et al.* 2017)

	Schisandra sphenanthera			UGT1A1, OATP2			
alismanin A	active compound from Alisma rhizome	plant extract	activates			liver	(Wang *et al.* 2017)
alpinetin	flavonoid	plant extract	activates	inhibit NFκB	attenuate colitis	intestine	(Yu *et al.* 2020)
alantolactone	lactone	plant extract	activates	inhibit NFκB	attenuate colitis	intestine	(Ren *et al.* 2019)
epicatechin	flavanol	other	activates	induce CYP3A11; increase myogenin expression	drug metabolism; induce differentiation of C2C12 myoblasts into myotubes	skeletal muscle	(Ortiz-Flores *et al.* 2020)

imazalil	food additive	other	activates	induce CYP3A11	downregulate cell cycle suppressor genes which enhances CAR-dependent hepatocyte proliferation	liver	(Yoshimaru *et al.* 2018)
acetylated cholic acid	bile acid derivative	other	activates	induce CYP3A4, CYP2B6, P-glycoprotein/ MDR1 transporter	potential endogenous therapeutic agent	liver	(Carazo *et al.* 2017)

Parental Exposure and Perinatal Exposure

Throughout the years, it has been shown that parental exposure, particularly maternal exposure, to different environments and compounds can have major effects on their offspring. Intergenerational studies highlight the impacts that such exposures could bring about. In the case of PXR, there is a limited number of studies that aim to answer how parental exposure to PXR

agonists, and consequently, PXR activation, can affect the health of offspring. In addition to parental exposure, perinatal exposure can also have life-long consequences.

One study showed that perinatal exposure to polychlorinated biphenyl (PCB)-153 increases oxidative stress and risk for premature death in PXRKO mice. Chronic exposure of PXRKO mice to PCB-153 starting in utero to until 10 months also led to lower levels of hemoglobin as well as the development of tumors in the intestines (Egusquiza *et al.* 2020). The results of this study highlight the protective effects of PXR when it comes to metabolizing toxicants and ensuring proper xenobiotic metabolism.

Exposure to chemicals before birth also affects PXR expression itself. A previous study showed that maternal exposure to lipopolysaccharide downregulated the hepatic expression of PXR in the liver of fetal mice (Xu *et al.* 2005). Along with the decrease in PXR expression, maternal LPS exposure also decreases the hepatic CYP3A11 mRNA expression, which is responsible for oxidative metabolism. These downregulations were found to occur in a dose-dependent manner. This LPS-induced inhibition of PXR and CYP3A11 expression was attenuated by pretreatment with alpha-phenyl-N-t-butylnitrone (PBN), a compound that traps free radicals. This suggests that reactive oxygen species are involved in the LPS-induced downregulation of PXR (Xu *et al.* 2005).

In other cases, PXR activation can be regulated by interactions with other receptors like the androgen receptor (AR). One study looked at the difference in hepatotoxicity between male and female fetal mice and found that this can be attributed to the activation of AR by testosterone produced by the developing testes. In their experiments, they exposed pregnant mice to monocrotaline (MCT) to induce hepatotoxicity. An increase in expression of PXR and CYP3As

was observed in female fetal mice as opposed to the male mice. The activation of AR serves a protective role against hepatotoxicity in developing male fetuses with maternal exposure to MCT (Xiang *et al.* 2020). Another study showed an opposing view to this, wherein prenatal AR activation simulated by administering testosterone to male ovine fetuses increased their risk for dyslipidemia and cholestasis. They also reported an increase in PXR expression in adolescent male mice that were prenatally treated with androgens (Siemienowicz *et al.* 2019). This difference could be due to other factors such as the added MCT treatment for one study and the androgen overexpression in the other, as opposed to just the natural testosterone produced by the developing testes.

In 2018, Sui and colleagues published a paper demonstrating another sex dependent PXR effect (Sui *et al.* 2018). They showed that perinatal exposure to BPA in male huPXR•ApoE$^{-/-}$ mice increased their risk of developing atherosclerosis later in life by upregulating the expression of CD36. No similar finding was observed in female mice. Moreover, the perinatal BPA exposure derived proatherogenic effects was diminished in hPXR deficient mice, indicating the center role of PXR in this intergenerational regulation. Although the authors did not go in depth with the sex differences, they postulated that this could be due to ER signaling.

As mentioned previously, there is not much paternal exposure studies, but one in particular, demonstrated the effects of paternal exposure to nicotine to male offspring health. It has been shown that the gene expression of phase I and II enzymes, as well as phase III transporters, were increased in the hepatocytes of the offspring following paternal nicotine exposure. The expression of PXR and constitutive androstane receptor (CAR) were also increased along with the aforementioned enzymes and transporters (Vallaster *et al.* 2017). This

enhanced xenobiotic metabolism and drug resistance is only observed in male offspring which could potentially mean that this change might be linked to the Y chromosome. Further studies are needed to elucidate the mechanism as to how this paternal exposure elicit changes in the male offspring.

Future Perspectives

The studies related to the environmental influences on human health and wellness have attracted increasing attention in recent years. As the key regulator for xenobiotic metabolism and many other important signaling pathways, PXR plays a pivotal role in linking the external environmental effects and the internal pathophysiological responses. In addition, the marvelous ligand-binding diversity of PXR also makes it a unique receptor which should be continuously investigated since numerous new chemicals are generated in the modern society whereas their potential adverse effects such as endocrine disrupting activities remain largely uncertain (Acerini and Hughes 2006). The newly discovered PXR agonists and antagonists may also provide a novel therapeutic tool for treating diseases.

Much has already been done to understand the contribution of PXR in drug metabolism and other disease pathogenesis in the liver and the intestines, where PXR is highly expressed. Yet the functions of PXR in cardiometabolic diseases remain to be fully elucidated as it is the leading cause of death worldwide and its pathogenesis involves many different cell types/tissues.

Another potential research direction of PXR may be toward its inter- or trans-generational effects in offspring. Up to date, most of the existing studies related to PXR were done to investigate the direct effects in the living organisms during chemical/drug exposure or

disease development (Oladimeji and Chen 2018, Xing *et al.* 2020). Only a few of these studies focused on the effects of maternal exposure and chronic PXR activation to the long-term health of offspring (Xu *et al.* 2005, Sui *et al.* 2018, Xiang *et al.* 2020), leaving the paternal effects of PXR activation nearly a blank field in this research domain. More efforts could be made into this direction in the future.

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