Roles of Xenobiotic Receptors in Vascular Pathophysiology

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The pregnane X receptor (PXR) and constitutive androstane receptor (CAR), 2 closely related and liver-enriched members of the nuclear receptor superfamily, and aryl hydrocarbon receptor (AhR), a nonnuclear receptor transcription factor (TF), are major receptors/TFs regulating the expression of genes for the clearance and detoxification of xenobiotics. They are hence defined as “xenobiotic receptors”. Recent studies have demonstrated that PXR, CAR and AhR also regulate the expression of key proteins involved in endobiotic responses such as the metabolic homeostasis of lipids, glucose, and bile acid, and inflammatory processes. It is suggested that the functions of PXR, CAR and AhR may be closely implicated in the pathogenesis of metabolic vascular diseases, such as hyperlipidemia, atherogenesis, and hypertension. Therefore, manipulation of the activities of these receptors may provide novel strategies for the treatment of vascular diseases. Here, we review the pathophysiological roles of PXR, CAR and AhR in the vascular system. (*Circ J* 2014; **78**: 1520–1530)

Key Words: Aryl hydrocarbon receptor; Constitutive androstane receptor; Pregnane X receptor; Vascular diseases

The circulation system is the major organ exposed to foreign substances, or “xenobiotics”, as well as endogenous chemicals, or endobiotics, during metabolic homeostasis. Human beings are constantly exposed to potential xenobiotics from the outside environment via the gastrointestinal (food, drinking water and beverages) and respiratory (air pollutants, transportation, phthalates, polybrominated diphenyl ethers and short-chain chlorinated paraffins) tracts, and by direct skin contact or other routes (drugs and plastizers) and consumer products (perfluorinated compounds, phthalates, bisphenol A and volatile organic compounds). Endobiotic homeostasis is the balance of production and elimination of endobiotics such as lipids.^{1–3} Metabolizing and eliminating toxic chemicals, including xenobiotics,¹ as well as endogenous chemicals or endobiotics, in the liver, intestine and vessel wall (macrophages, endothelial cells [ECs] and smooth muscle cells) is the first-line defense system, which is conserved in nearly all animals from fruit flies to humans.¹ This first step of the mechanism is called “detoxification”.

The pregnane X receptor (PXR) and constitutive androstane receptor (CAR), 2 members of the superfamily of nuclear receptors (NRs), are well-recognized “xenobiotic sensors” and can be activated by a variety of structurally diverse chemicals. They regulate the expression of drug/xenobiotic metabolizing enzymes transcriptionally to enhance the elimination of toxic byproducts derived from endogenous metabolites and of exogenous chemicals.^{2–6} These metabolic enzymes are divided into 2 groups: phase I and phase II enzymes.^{7,8} Phase I cytochrome P450 (CYP) enzymes, including CYP 1A1, 1A2, 1B1, 2A6, 2B1, 2B6, 2C9, 2C19, 4A1, 4A3, 3A4, 3A7, 7A1 and 8B1, belong to the monooxygenase superfamily and are high-

ly expressed in liver and intestine and mainly function to catalyze the first step of detoxification.^{9,10} Phase II conjugation reactions are catalyzed by a large group of transferases, such as sulfotransferase (SULT), glutathione S-transferases (GSTs), UDP-glucuronosyltransferases (UGT 1A1, 1A6, 2A1, 1A3, 1A4, 2B4, 1A9) and NADPH: quinone oxidoreductase (NQOR), which conjugate polar functional groups onto xenobiotics and endobiotics to produce water-soluble, inactive metabolites suitable for biliary and urinary excretion.¹¹

Another ligand-activated transcription factor (TF), aryl hydrocarbon receptor (AhR), which is not a member of the NRs, also binds a broad spectrum of xenobiotics.¹² AhR is a basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) TF involved in the adaptive and toxic responses of xenobiotics. In contrast to the other bHLH/PAS proteins, AhR is the only member known to bind naturally occurring xenobiotics.¹³ Activation of cytosolic AhR by any one of a variety of environmental pollutants, such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCBs) and dioxins, results in translocation of AhR to the nucleus where it complexes with the AhR nuclear translocator (ARNT).¹⁴ The complex then recognizes and binds specific DNA sequences, dioxin-responsive elements (DREs), within gene promoter regions and modulates subsequent gene-encoding CYPs transcription.^{15,16}

The roles of PXR and CAR in bile acid, lipid and glucose homeostasis and inflammation have been elucidated.^{17,18} Because these pathways may be disrupted in vascular diseases and the toxins contribute to vascular damage led us to consider the potential of PXR and CAR as targets for vascular therapy. Meanwhile, numerous studies have demonstrated that PAHs and 2,3,7,8-tetrachlorodibenzo-p-dioxin's (TCDDs), which are

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Figure 1. Schematic representation of the relationship among xenobiotics, xenobiotic sensors (ie, PXR, CAR and AhR) and vascular disease. AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; PXR, pregnane X receptor.

Figure 2. Schematic representation of a typical nuclear receptor and the structure of the aryl hydrocarbon receptor (AhR). (A) Nuclear receptor domain structure. Beginning at the N-terminus, nuclear receptors include the N-terminal domain, DNA binding domain (DBD), Hinge region, ligand binding domain (LBD) and C-terminal domain. (B) Domain architecture of the AhR protein. Text indicates key domain regions: NLS, nuclear localization sequence; NES, nuclear export sequence; bHLH, basic helix-loop-helix domain, PAS, Per-ARNT-Sim domain (A and B repeat regions); TAD, transactivation domain.

Table. Xenobiotic Receptors and Their Activation Compounds (Ligands)

Receptor	Ligands
PXR	Androstanol, coumestrol, PB, TCPOBOP, SR12813, PCN, taxol, lithocholic acid, RU486, steroids (eg, pregnenolone and progesterone), cholesterol metabolites, cafestol from unfiltered coffee, bile acids, and herbal compounds such as hyperforin (active constituent of St. John's Wort), guggulipid, colupulone, isoflavones; drugs such as rifampicin, clotrimazole, ritonavir, metyrapone and cardiovascular drugs (eg, HMG-CoA reductase inhibitors, the 'statins')
CAR	PB, androstanol, clotrimazole, 5 β -pregnane-3,20-dione, retinoic acids, clotrimazole, CPZ, o,p'-DDT, methoxychlor, and tohydrocarbons (eg, 2,3,3',4',5',6-hexachlorobiphenyl, CITCO, hCAR), PCN and TCPOBOP (in mCAR)
AhR	Halogenated aromatic hydrocarbons (eg, polychlorinated dibenzodioxins, dibenzofurans and biphenyls), polycyclic aromatic hydrocarbons (eg, TCDD), derivatives of tryptophan, bilirubin, biliverdin, tryptamine, indole acetic acid, retinoids, tetrapyrroles, and some modified low-density lipoproteins

AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; CITCO, 6-(4-chlorophenyl) imidazo [2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl) oxime; CPZ, chlorpromazine; HMG-CoA, 3-hydroxy-3-methylglutarate-CoA; PB, phenobarbital; PCN, pregnenolone 16 α -carbonitrile; PXR, pregnane X receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene.

the major constituents of cigarette tobacco tar and environmental contaminants, are strongly involved in the pathogenesis of vascular diseases. Because PAH and TCDD-induced toxicities are mediated by the activation of AhR, a direct link between AhR and cardiovascular diseases (CVDs) exists in all probability.¹⁹ This review summarizes recent advances in elucidating the roles of PXR and CAR and AhR in vascular therapy. The relationship among xenobiotics, PXR, CAR, AhR and vascular disease are summarized in **Figure 1**.

Genes and Proteins of PXR, CAR and AhR

PXR, also known as steroid and xenobiotic sensing NR (SXR) or NR subfamily 1, group I, member 2 (NR1I2), is a protein that is encoded by the NR1I2 gene in humans.²⁰ The human PXR gene is located on chromosome 3, locus 3q12-q13.3, and spans approximately 20 kb.^{21,22} CAR, also known as NR subfamily 1, group I, member 3, is a protein that in humans is encoded by the NR1I3 gene. The human CAR gene is located on chromosome 1, locus 1q23.²³ PXR and CAR, like all the members of the NRs, are modular proteins sharing common regions, including the N-terminal DBD, the H region, and the C-terminal LBD (**Figure 2**). AhR is a protein that in humans is encoded by the AHR gene. The human AHR gene is located on chromosome 7: 17.34–17.39 Mb. AhR is a ligand-activated transcriptional factor with the primary function of mediating xenobiotic metabolism through transcriptional activation of Phase I and Phase II drug-metabolizing enzymes, such as CYP1A1, 1A2, 1B1, UGT1A1 and UGT1A6, GSTA2, aldehyde dehydrogenase 3 (ALDH3), or NQOR.^{5,24,25} AhR also share some common target genes with PXR and CAR, such as CYP1A2 and UGT1A1.²⁶ AhR is the only protein in the bHLH/PAS superfamily that requires activation by an exogenous ligand.

Ligands and Functions of PXR, CAR and AhR

PXR is activated by a large number of endogenous and exogenous chemicals including androstanol, coumestrol, phenobarbital (PB), 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), SR12813, pregnenolone 16 α -carbonitrile (PCN), taxol, lithocholic acid, mifepristone (RU486), steroids (pregnenolone and progesterone), cholesterol metabolites, cafestol from unfiltered coffee, bile acids, and many other herbal compounds such as hyperforin (active constituent of St. John's Wort), guggulipid, colupulone, and isoflavones.^{22,27} PXR is also activated by widely used pharmaceutical drugs including rifampicin (antibiotic), clotrimazole (antimycotic), ritonavir, metyrapone and cardiovascular drugs such as the HMG-CoA

reductase inhibitors (statins).^{28,29} CAR can be activated by a variety of compounds including PB, androstanol, clotrimazole, 5 β -pregnane-3, 20-dione, retinoic acids, clotrimazole, chlorpromazine (CPZ), o,p'-DDT, methoxychlor, and tohydrocarbons such as 2,3,3',4',5',6-hexachlorobiphenyl, 6-(4-chlorophenyl) imidazo [2,1-b][1,3] thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl) oxime (CITCO, hCAR), PCN and TCPOBOP (in mCAR).^{27,30–33} Although CAR and PXR share some common ligands and also have an overlapping target gene pattern,^{27,34–36} the mode of activation for these 2 receptors is quite different. PXR is located in the nucleus with a low basal activity and is highly activated upon ligand binding.^{22,36} Unlike PXR, CAR resides in the cytoplasm in the non-induced state⁹ and is constitutively active in the absence of ligand and regulated by both agonists and inverse agonists. Ligand binding results in translocation of this protein to the nucleus, where it activates or represses target gene transcription. The constitutive activity of CAR was thought to be related to the ligand-independent recruitment of NR coactivators by CAR.³⁷ PXR and CAR regulate gene expression by forming heterodimers with the retinoid X receptor (RXR, NR1B2).

Above all, PXR can be activated by pregnanes, progesterone, and glucocorticoids, whereas CAR is affected by androstane metabolites, estrogens, and progesterone.^{38,39} For this reason, in addition to functioning as xenobiotic receptors, PXR and CAR are thought to be endobiotic receptors that influence some physiology and diseases.^{5,40} PXR activity is also intricately regulated by phosphorylation, SUMOylation and lysine acetylation. Similar to PXR, CAR's activity is also regulated by phosphorylation.^{41–44}

The synthetic ligands of AhR are some members of the halogenated aromatic hydrocarbons, such as PCBs, polychlorinated dibenzodioxins (PCDDs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).^{45,46} Naturally occurring compounds that have been identified as ligands of AhR include derivatives of tryptophan, bilirubin, biliverdin, tryptamine, indole acetic acid, retinoids, tetrapyrroles, and some modified low-density lipoproteins (LDLs).^{27,47–49} Upon ligand binding, AhR translocates to the nucleus and dimerizes with ARNT. The activated AhR/ARNT heterodimer complex is then capable of either directly or indirectly interacting with DNA by binding to DREs in promoter regions.⁵⁰

In the absence of these ligands, AhR exists primarily in the cytoplasm as an inactive complex with 2 90-kDa heat-shock proteins (HSP90), 1 X-associated protein (XAP), and 1 p23 molecular chaperone protein.⁵¹

Xenobiotic receptors and activation compounds are summarized in **Table**. The signaling pathways of PXR, CAR and

AhR are shown in [Figure 3](#).

PXR, CAR and Vascular Disease

In addition to their conventional roles in xenobiotic metabolism, PXR and CAR have been found in the vasculature where they regulate vascular function, inflammation, bile acid metabolism, lipid and glucose homeostasis.⁵²

PXR and CAR in Drug Metabolism

PXR regulates the genes involved in drug and xenobiotic metabolism, including CYPs, GSTs, multidrug resistance protein 1 (MDR1) and SULTs.¹⁷ The circulation can be a hostile milieu, with increased concentrations of xenobiotics and endobiotics resulting from drugs, chemical agents, smoking, air pollution, nutritional metabolites, and pathogenic microbes or their toxins.⁵³ These circulating endogenous and foreign chemicals can contribute to vascular dysfunction and the development of vascular disease.⁵⁴ Recent studies have demonstrated that PXR is not only expressed in the liver and intestine and its role in detoxification is not liver and intestine specific. PXR and numerous CYPs are also present in human, rat and mouse blood vessels and human and rat aortic endothelial and smooth muscle cells.^{54,55} PXR regulates Phase I drug metabolism (CYP3A4, CYP2B6, and CYP2C8) and MDR1 in human vascular cells. In addition, PXR ligands also induce the expression of GSTM1 to decrease oxidative stress in vascular cells.⁵⁴ Our recent research also indicated that PXR represented a flow-activated detoxification system protecting ECs against damage by xeno-

and endobiotics. Laminar shear stress (LSS), the atheroprotective flow, activated PXR in ECs, whereas oscillatory shear stress, the atheroprone flow, suppressed PXR. LSS-activated PXR protects ECs from apoptosis triggered by doxorubicin via the induction of MDR1 and other detoxification genes. PXR can also stimulate defense mechanisms against oxidative stress, promoting cell survival. Meanwhile, human vascular cells and macrophages express CAR, which links diet to toxicity and immunity by being involved in the bioactivation, detoxification, and transport of various drugs, xenobiotics, endogenous substances and environmental toxins. As such, this means that the body is able to prevent circulating toxins from accessing organs and that the vasculature is capable of protecting itself against damaging insults.

Antimetabolic Inflammation of PXR

Excess nutrients and the ensuing obesity can lead to a status of chronic low-grade inflammation, so-called “metabolic inflammation”. Metabolic inflammation is a coordinated response to harmful stimuli that involves many components of the classical inflammatory response to pathogens and includes systemic increases in circulating inflammatory cytokines and acute phase proteins (eg, C-reactive protein [CRP]), recruitment of leukocytes to inflamed tissues, activation of tissue leukocytes, and generation of reparative tissue responses.⁵⁶ Metabolic inflammation has been widely recognized as playing a critical role in the initiation, propagation, and development of metabolic diseases such as obesity, diabetes, hypertension and atherosclerosis.¹ Recently, we found that PXR can suppress the expres-

sion of proinflammatory adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1, otherwise known as E-selectin) in response to tumor necrosis factor- α (TNF- α) and lipopolysaccharide (LPS) in ECs.⁵³ Overexpression of a constitutively active PXR in rat carotid arteries also potently attenuated proinflammatory responses.⁵³ PXR is also expressed in classical immunocytes including monocytes/macrophages and lymphocytes (both T and B cells) and inhibits inflammation.^{57–59} LPS, interleukin-6 (IL-6) and endotoxin all downregulate the expression of PXR and its target genes.^{60,61} The potent human PXR activator, rifampicin, suppresses the expression of typical nuclear factor κ B (NF- κ B) target genes, such as cyclooxygenase-2 (COX-2), TNF- α , intercellular adhesion molecule 1 (ICAM-1), and IL-1, 2, 6, and 15 in human hepatocytes.⁶² Inflammatory signals also modify PXR at the post-translational level. A recent study showed that the inflammatory response to TNF- α caused SUMOylation of PXR to inhibit the activation of NF- κ B.⁵⁷

PXR and CAR in the Metabolism of Bile Acids

Bile acids are the endproducts of cholesterol utilization and can be extremely toxic if their levels become elevated. Bile acids aid the absorption of dietary fats, via active uptake from the intestines and enterohepatic circulation.⁶³ The relationship between bile acids and vascular disease has been a recent focus of investigation. Sequestering bile acids in the intestinal lumen or preventing their uptake is used therapeutically to lower LDL cholesterol, an independent risk factor for vascular diseases.⁶⁴

PXR and CAR activation in hepatocytes is protective against hepatotoxicity induced by bile acids.^{65–67} Accumulation of bile acid and bile acid precursors directly leads to PXR activation.^{68,69} PXR regulates the metabolism of bile acids by (1) decreasing the synthesis of bile acid; (2) increasing bile acid catabolism; and (3) promoting the hepatic uptake of bile acids from the blood and their excretion into bile. The PXR-regulated target genes involving these processes include metabolizing enzymes such as CYP3A11, SULT2A, the transporter multidrug resistance-associated protein3 (MRP3), organic anion transporting polypeptide (OATP2), solute carrier organic anion transporter family member 1B1 (SLCO1B1), adenosine triphosphate (ATP)-binding cassette subfamily B 11 (ABCB11), ATP-binding cassette subfamily C2 (ABCC2) and CYP7A1.¹⁷ CAR can downregulate OATP1A1 and upregulate OATP1A4 and MRPs mRNA expressions to promote bile acids efflux from the hepatocytes.^{70–72} CAR also can increase the mRNA expression of efflux transporters (bile salt export pump [BSEP], breast cancer resistance protein [BCRP], MRP2, MRP3, and MDR1) and decrease the levels of uptake transporters (OATP1B3, OAT2, Na+/taurocholate cotransporting polypeptide [NTCP])^{73–76} in human hepatocytes. This CAR role occurs in the absence of the key bile acid sensors, FXR and PXR.^{77,78} Therefore, PXR and CAR may offer a target to effectively reduce bile acids and thus limit LDL cholesterol levels.

PXR and CAR in Lipid and Glucose Homeostasis

A number of clinical observations have shown that many drugs, identified as PXR and/or CAR activators, affect lipid and/or glucose metabolism in patients. The liver plays a critical role in maintaining blood lipid and glucose homeostasis. PXR and CAR can promote hepatic lipogenesis by downregulating genes involved in lipid oxidation such as carnitine palmitoyl-transferase-1A (CPT-1A) and mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase-2 (HMGCS2) and pro-

oxidation proteins, peroxisome proliferator activated receptor (PPAR)- α and thiolase.^{79–82} PXR activation causes hepatic steatosis and induces the expression of CD 36 (a fatty acid translocase involved in long-chain fatty acid [LCFA] transport).^{55,81} PXR also upregulates stearoyl-CoA-desaturase-1 (SCD-1), spot 14 and PPAR- γ .^{69,82} CAR agonist, PB, decreases the enoyl CoA isomerase (ECI) mRNA. In addition, CAR and PXR directly affect lipogenic pathways by activating Insig-1, an endoplasmic reticulum (ER) protein involved in sterol-dependent synthesis of cholesterol.⁸³ PXR agonists do not induce lipogenesis in rat vascular smooth muscle.⁸⁴

Diabetes and prediabetic-elevated plasma glucose levels are risk factors for CVD. Genes involved in gluconeogenesis notably include phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). Activation of PXR in fasting mice decreases serum glucose and suppresses the expression of PEPCK and G6Pase.⁸² PB has been shown to decrease plasma glucose levels and improve insulin sensitivity in diabetic patients.⁸⁵ Activation of CAR represses the expression of PEPCK by competition with hepatocyte nuclear factor 4 α (HNF4 α) for the binding of a DR1 element within the PEPCK gene promoter and the inhibition of HNF4 α transcriptional activity by the sequestration of PPAR- γ coactivator 1 α (PGC1 α).⁸⁶ Forkhead box protein O1 (FoxO1) positively controls the expression of genes involved in gluconeogenesis and it represents the target of insulin's repressive action on the gluconeogenesis pathway. PXR and CAR can repress the transcriptional activity of FoxO1 by preventing its binding to its responsive element IRS in target gene promoters such as PEPCK and G6Pase,¹⁸ implicating PXR and CAR as negative transcriptional regulator of genes involved in glucose metabolism.

AhR and Vascular Disease

The AhR protein is expressed in most tissues, with the highest mRNA and protein levels found in the lung, liver, kidney, and placenta, and lower levels in the heart and endothelium.^{87–89} Activation of AhR signaling is recognized as the body's primary molecular defense following environmental toxicant exposure.^{90–92} Exposures to AhR ligands can lead to some major metabolic diseases including vascular disease and cancer.^{93–97}

AhR and Atherosclerosis

Atherosclerosis is considered a chronic inflammatory disease of the vessel wall characterized by chemokine-driven mononuclear cell recruitment into the subendothelial space, where the mononuclear cells differentiate into macrophages.⁴⁵ Macrophages and ECs play key roles in atherogenesis by releasing proinflammatory cytokines and forming foam cells in subendothelial lesions.⁹⁸ Exposure to some AhR ligands (eg, dioxins, TCDD, PAH) causes inflammatory responses in macrophages and may lead to the formation of foam cells^{93,98} and is associated with progression of atherosclerosis.⁹⁹

Exposure of ApoE^{-/-} mice to TCDD time-dependently accelerated the progression of atherosclerosis. TCDD promotes foam cell formation and induces the expression of inflammatory mediators including NF- κ B, COX-2, IL-1 β , TNF- α , IL-8 and metalloproteinase 12 (MMP-12).⁹³ Urban dust also activates AhR and increases the production of CRP and IL-6 in human macrophages.^{98,100} TCDD-induced inflammation can be further enhanced by a high-fat diet in mice and deteriorates the formation of complex atheromas. Conversely, the AhR antagonist, TCDD, significantly suppresses the expressions of COX-2 and CYP1A1 induced by these particles and their organic extracts.⁹⁸ Recently, the IL-8 receptor (IL8R), also known

as CXCR2 (a G protein-coupled receptor), was implicated as a contributory factor and is considered to play a pivotal role in inflammatory diseases⁵¹ and ultimately, production of the CXCR2 target gene VEGF mediates the atherogenic activity of environmental pollutants through the induction of a vascular inflammatory response by activating the AhR-signaling pathway.^{101,102}

Besides the function of macrophages, vascular endothelial dysfunction is the initiation step in atherosclerogenesis, which is characterized by increased adhesiveness caused by the presentation of cellular adhesion molecules, such as ICAM-1 and VCAM-1.^{103,104} By regulating AP-1, MEK and p38-MAPK, benzo[a]pyrene (BP) is able to increase ICAM-1 protein only after pretreatment with an AhR agonist, β -naphthoflavone (β -NF).¹⁰⁵ Indoxyl sulfate (IS), an endogenous agonist for AhR, induces monocyte chemoattractant protein-1 (MCP-1) expression through reactive oxygen species production in human umbilical vein ECs and contributes to the development of atherosclerosis.¹⁰⁶ These studies strongly support the hypothesis that AhR may be a therapeutic target for downregulation of vascular inflammatory responses such as atherosclerosis.¹⁶

AhR and Hypertension

A recent study conducted by researchers at Boston University found that increased exposure to air pollution makes humans more prone to developing hypertension.¹⁰⁷ AhR and AhR-regulated phase I/II genes in the endothelium are critically involved in blood pressure regulation and are required to maintain normal basal levels of blood pressure.¹⁰⁸ The AhR agonist, TCDD, induces high blood pressure and AhR-mediated CYP overexpression.^{109–113} AhR null mice have significantly elevated mean arterial pressures (MAP) as well as increased circulating angiotensin II (AngII) and plasma endothelin-1 (ET-1) levels.¹¹⁴ Hypotensive AhR^{-/-} mice exhibit a significantly higher level of endothelial nitric oxide synthase (eNOS) and enhanced vascular nitric oxide (NO) production.¹¹⁵ TCDD exposure of ECs increases the production of ROS, and decreases acetylcholine-stimulated NO production by inducing CYP1A1 and CYP1B1.¹¹⁶ AhR could serve as a target in the treatment of high blood pressure and other NO-dependent

vascular diseases.

Recently, it was reported that AhR signaling played an important role in regulatory T cells. AhR participates in Th17 cell differentiation through regulating Stat1 activation.¹¹⁷ T cells also play important roles in hypertension. Mice lacking T cells have blunted hypertension during AngII infusion. Hypertension increased the T-lymphocyte production of TNF- α , and inhibited TNF- α could prevent the hypertension caused by AngII.¹¹⁸ These results indicate that AhR signaling in T cells might be a novel therapeutic target for the treatment of high blood pressure.

Clinical Importance of PXR, CAR and AhR

Drug uptake transporters are now increasingly recognized as clinically relevant determinants of variable drug responsiveness and unexpected drug-drug interactions. Activation of these transporters during vascular therapy is likely to affect the effectiveness of many drugs and there is growing evidence for tissue-specific enhancement of the malignant phenotype.^{119,120} Exposure to some environmental factors (xenobiotics), such as pesticides and toxic compounds, has been shown to play an important role in the pathogenesis of vascular disease. The enzymes implicated in xenobiotic metabolism are regulated by an endogenous defense system comprising PXR, CAR and AhR. Both their levels and functional structures are determined by their coding genes or other molecules regulating their expressions. With the revelation of human genome sequences and frequency of sequence variations in the population, it is clear that the DNA sequences of these genes vary from subject to subject. Thus, genetic variation in these TFs may modify the regulation of relevant environmental factors and the associated risk of vascular disease. Genes in the CYP superfamily are highly polymorphic and mutations in CYP1A1, 1B1, CYP2A, 2B, 2C, 2E 2J, CYP3A, CYP4A, 4B, 4F, CYP5A1, CYP7A1, 7B1 and CYP8A1 are associated with vascular disease.^{121–123} Clopidogrel was selected for testing whether PXR regulation of vascular drug-metabolizing enzymes has a functional effect on the efficacy of cardiovascular drugs directly in the vessel wall.^{22,124,125} Clinical observations showed that PXR activation enhanced

responsiveness to clopidogrel. Polymorphic PXR expression correlates with clopidogrel non-responsiveness. The allelic variant CYP2C19*17 increases the bioactivation and patient responsiveness to clopidogrel. Hagedorn et al indicated that activation of PXR by progesterone metabolites, PXR-dependently increases vasorelaxation in pregnancy.¹²⁶

Some endogenous substances and other naturally occurring compounds also act as ligands for PXR and CAR.⁴ The initial discovery was that St. John's Wort and yin zhi huang are capable of activating PXR and CAR.^{127,128} *Salvia miltiorrhiza*, also known as danshen, has been reported to activate human PXR transcriptional activity to treat various vascular diseases, including hypertension, stroke, and hyperlipidemia.^{129,130} Garlic, guggul and *Ginkgo biloba*, commonly known as herbal medicines, are characterized as activators of CAR, which provides a molecular basis for the traditional therapeutic use of this herbal medicine in the treatment of many metabolic diseases.¹³¹ AhR alter the metabolism and pharmacokinetics of some drugs,¹³² which has implications for clinical practice. Flavonoids are present in fruits, vegetables, and beverages derived from plants, such as tea and red wine, which have been recognized as health-promoting and atherosclerotic vascular disease-preventing dietary supplements.¹³³ Flavonoids inhibit the activity of CYP1A1, 1A2, 2E1, and 3A4.¹³⁴ Sulforaphane (SFN) present in cruciferous vegetables shows a protective effect on inflammatory damage induced by LPS in human vascular ECs.¹³⁵ SFN revealed activation of AhR transformation and induced CYP1A1 mRNA expression.¹³⁶ Besides the exogenous compounds, various classes of endogenous compounds, such as eicosanoids, indirubin, bilirubin, biliverdin, tryptophan and cAMP, are able to activate AhR and thus play vital roles in vascular health and the immune system.^{49,137–140}

Conclusions

Recent findings from many laboratories have clearly suggested that the xenobiotic NRs, PXR and CAR, not only have their "conventional" functions, but also have interesting crosstalk in their participation in drug metabolism, inflammation, lipid and

glucose homeostasis and bile acid metabolism. These discoveries suggest PXR as a novel target for vascular diseases through protection from circulating toxins and oxidative stress, anti-inflammatory role and promoting bile acid and cholesterol metabolism and efflux. The health or vascular benefits of a number of natural products or drugs (eg, statins) may, in part, be via vascular PXR activation (Figure 4). Local upregulation of CYPs in the vasculature suggests that generally PXR may protect the vasculature from disruptions to vascular homeostasis and inflammation, and regulate tone. Zhou et al found that PXR activation in wild-type mice increased the levels of the atherogenic lipoproteins, very-low-density lipoprotein (VLDL) and LDL, whereas in ApoE^{-/-} mice, PXR increased atherosclerosis by diminishing the levels of the antiatherogenic ApoA-IV and increasing lipid accumulation in macrophages.¹⁴¹ The therapeutic potential of CAR in vascular disease, however, remains to be defined (Figure 5). CAR has been determined as a potential target in the prevention and treatment of atherosclerosis.¹⁴² However, there is limited information on the relative contribution of CAR in reduction of atherosclerosis systemically vs. locally in the vessel wall because the vasculature system expresses all AhR-regulated genes in a species- and tissue-specific manner. Nowadays, genetic, clinical and basic scientific studies all support the theory that AhR activation contributes to the development and progression of atherosclerosis and hypertension (Figure 6). AhR is also a link between the development of CVD and the "detoxification" system. Therefore, PXR, CAR and AhR have the potential to be both novel therapeutic targets for vascular disorders and be utilized by a number of current drugs and natural products to give vascular protection.

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Supplementary Files

Supplementary File 1

Non-standard abbreviations and acronyms

Please find supplementary file(s);
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