Review Article

Theme: Natural Products as Therapeutic Modulators
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Activation of Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR) by Herbal Medicines

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Abstract. Pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are transcription factors that control the expression of a broad array of genes involved not only in transcellular transport and biotransformation of many drugs, other xenochemicals, and endogenous substances, such as bile acid, bilirubin, and certain vitamins, but also in various physiological/pathophysiological processes such as lipid metabolism, glucose homeostasis, and inflammation. Ligands of PXR and CAR are chemicals of diverse structures, including naturally occurring compounds present in herbal medicines. The overall aim of this article is to provide an overview of our current understanding of the role of herbal medicines as modulators of PXR and CAR.

KEY WORDS: constitutive androstane receptor; herbal medicine; pregnane X receptor.

INTRODUCTION

Nature has provided us with a vast array of chemical substances of medicinal value. According to Koehn and Carter (1), “of the 877 small molecule new chemical entities introduced between 1981 and 2002, roughly half (49%) were natural products, semi-synthetic natural product analogues, or synthetic compounds based on natural product pharmacophores.” Paclitaxel, tacrolimus (FK-506), and topotecan are a few of the many examples of natural product-based small molecule drugs that have been developed and subsequently approved for use in clinical pharmacotherapy. While natural products continue to serve as a platform for various drug discovery and development programs in search of novel single molecular entities, they have a much longer and richer history of use as plant-based herbal remedies in various traditional medicine systems (e.g., Ayurvedic medicine and traditional Chinese medicine). In contrast to the abundance of scientific information on the biological activities and mechanisms of action of single molecular entities, considerably less is available for herbal medicines, at least in English language scientific journals. However, with the ever growing popularity of herbal medicines, especially among consumers in North America, there is increasing interest in the Western scientific community to unravel the mystique of herbal medicines by analyzing their chemical composition, elucidating their biochemical, cellular, and

molecular actions, and identifying the chemical constituents responsible for their biological effects. A potential outcome of these scientific efforts is the discovery of novel therapeutic opportunities.

Members of the superfamily of nuclear receptors are ligand-activated transcription factors. These include endocrine receptors (e.g., estrogen receptor and androgen receptor), adopted orphan receptors [e.g., constitutive androstane receptor and pregnane X receptor (PXR)], and orphan receptors [e.g., Nur-related protein 1 (NURR1)] (2). Nuclear receptors represent potential therapeutic targets because they play a vital role in various biological processes of fundamental importance. Thus, considerable efforts are spent in drug discovery programs to identify nuclear receptor agonists and antagonists that may possess the desired pharmacological activity. Among the members of the nuclear receptor superfamily, two of them are the focus of this review article: (1) PXR (gene designation *NR1I2*) (3,4), which is also known as steroid and xenobiotic receptor (5) and pregnane-activated receptor (6) and (2) constitutive androstane receptor (CAR; gene designation *NR1I3*), which was originally referred to as MB67 (7). PXR and CAR regulate the expression of an overlapping set of genes involved in the bioactivation, detoxification, and transport of various drugs, endogenous substances (e.g., bilirubin, bile acid, and various vitamins), and environmental toxicants (8,9). Recent studies have indicated that these receptors play a regulatory role in various physiological and pathophysiological processes, such as lipid metabolism, glucose homeostasis, and inflammatory response (10). Collectively, the available evidence suggests that PXR and CAR may be useful targets for pharmaco-

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logical intervention in various conditions, including hepatic steatosis, cholestatic liver disease, hyperbilirubinemia, osteoporosis, and inflammatory diseases (10,11).

Various chemicals have been identified as ligands for PXR and CAR. These include not only drugs and other xenochemicals, but also endogenous substances and other naturally occurring compounds (12). Since the initial discoveries that *Hypericum perforatum* (St. John's wort) and yin zhi huang (a traditional Chinese herbal decoction consists of extracts from *Artemisia capillaries*, *Gardenia jasminoides* Ellis, *Rheum officinale* Baill, and *Scutellaria baicalensis* Georgi) are capable of activating PXR (13,14) and CAR (15), respectively, subsequent studies by various investigators have identified other herbal medicines as modulators of these receptors. Therefore, the overall aim of this article is to provide an overview on the effect of specific herbal medicines on the activity of PXR and CAR.

PREGNANE X RECEPTOR

CYP3A1 (3) and *CYP3A4* (4–6) are prototypic target genes for rat PXR and human PXR, respectively, but it is now known that PXR regulates the expression of a broad array of genes involved in biotransformation and transport of endogenous substances, natural products, drugs, and other xenochemicals. Other examples of PXR target genes include the various cytochromes P450 (e.g., *CYP2A6*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP3A5*, *CYP3A7*, *CYP4F12*, *CYP24*, and *CYP27A1*), uridine diphosphate (UDP)-glucuronosyltransferases (e.g., *UGT1A1*, *UGT1A3*, *UGT1A4*, *UGT1A6*, and *UGT1A9*), sulfotransferases (e.g., *Sult2a1*), glutathione *S*-transferases (e.g., *Gsta2* and *GSTA4*), and carboxylesterases (8,9,16). Drug transporter genes regulated by PXR include *ABCB1* (P-glycoprotein), *Abcc2* (multidrug resistance-associated protein 2), *Abcc3* (multidrug resistance-associated protein 3), and *SLC21A6* (organic anion transporting polypeptide 2 or oatp2) (8,9,16).

Experimental evidence obtained in the past decade have provided us with an understanding of the general steps involved in the activation of PXR (17). In the basal state, PXR is localized in the cytoplasm in a complex with heat shock protein 90 (HSP90) and CAR cytoplasmic retention protein (CCRP), as shown in experiments with mouse liver. Ligand binding leads to dissociation of PXR from HSP90 and CCRP. The resultant ligand-bound PXR translocates to the nucleus where it forms a heterodimer with another nuclear receptor known as retinoic acid receptor α (RXR α ; gene designation *NR2B1*). The ligand-PXR-RXR α complex binds to DNA response elements of a PXR target gene, resulting in increased gene transcription. The extent of PXR-mediated gene transcription is increased by coactivators, such as the p160/SRC family of coactivators, including steroid co-activator 1 (SRC-1), and peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α), and decreased by corepressors, such as nuclear receptor corepressor protein (NCoR), sterol regulatory element binding protein 1 (SREBP-1), and silencing mediator of retinoid and thyroid hormone receptors (SMRT), particularly the SMRT α isoform (17,18). PXR transcriptional activity is also influenced by other nuclear receptors or transcription factors (19). As examples, hepatocyte nuclear factor-4 α (HNF-4 α ; gene designation *NR2A1*) and glucocorticoid receptor (gene designation

NR3C1) have been shown to increase PXR transcriptional activity. In contrast, small heterodimer partner (gene designation *NR0B2*) suppresses PXR activity. The reader is referred to recent reviews on the details of the molecular mechanism of PXR activation (17) and the interplay between PXR with other nuclear receptors (19).

PXR is expressed predominantly in liver, although it has also been detected in various extrahepatic tissues, including small intestines (3,4,6), colon (4,6), kidney (20,21), brain capillaries (21), and mammary tissue (22). In addition, studies with human specimens have shown localization of PXR in mammary (22) and endometrial tumors (23). Interestingly, a tissue-specific PXR activator has been identified. With the use of PXR-humanized mice, it has been shown that rifaximin is a gut-specific activator of human PXR (24). Chemical activation of PXR may also be species-dependent. Whereas rifampicin activates human PXR, it does not activate rodent PXR (25). By comparison, PCN activates rodent PXR, whereas it has little or no effect on human PXR activity (25). Other compounds have also been identified as agonists and antagonists of PXR (12). These include synthetic drugs of various therapeutic classes and diverse chemical structures, naturally occurring compounds, endogenous substances, including bile acids and vitamins, and environmental toxicants. In contrast to the volume of information on PXR activation by single chemical entities (12,16), considerably less is known about the effect of complex chemical mixtures, such as herbal medicines, on PXR activity (26). St. John's wort was the first herbal medicine shown to activate PXR (13,14). Since then, various other herbal medicines have also been identified as activators of PXR (Table I). The following is an overview of our current knowledge on the effect of specific herbal medicines on PXR activity.

ACTIVATION OF PXR BY HERBAL MEDICINES

Coleus forskohlii

Coleus forskohlii, which is also known as *Plectranthus barbatus*, is a plant used in traditional Ayurvedic medicine for the treatment of various conditions, including hypertension, congestive heart failure, respiratory disorders, and hypothyroidism (27). Among the approximately 20 individual chemical constituents that have been identified in *C. forskohlii* extract, the best characterized is forskolin, which is a diterpene present in the root of the plant. Forskolin activates adenylate cyclase, increases cAMP levels, and stimulates the protein kinase A signaling pathway (28). Various herbal preparations of *C. forskohlii* are available, including extracts standardized to 10% forskolin.

An alcoholic extract of *C. forskohlii* (as root extract in powder form) of undefined chemical composition has been reported to activate mouse PXR based on the experimental finding indicating that the extract increases *Cyp3a11* messenger RNA (mRNA) expression in primary hepatocytes isolated from wild-type mice, whereas it has little or no effect on *Cyp3a11* mRNA expression in hepatocytes isolated from PXR knockout mice (29) (Table II). As mentioned previously, *Cyp3a11* is a gene subject to regulation by PXR (3). It is not known which individual chemical constituent(s) is directly responsible for or contributes to the activation of

Table I. *In Vitro* Effect of Herbal Extracts on Human PXR Activity as Assessed in Cell-Based Reporter Gene Assays

Herbal Extract	Concentration (or volume)	Reporter activity (fold-increase over control)	Reporter activity (relative to 10 μ M rifampicin)	Reference
<i>Agauria salicifolia</i>	63 μ g/ml ^a	5	Not determined	(74)
<i>Clausena anisata</i>	125 μ g/ml ^a	4	Not determined	(74)
<i>Commiphora mukul</i> (Gugulipid®)	1:4,000 dilution ^b	4	0.8	(36)
<i>Cyphostemma hildebrandtii</i>	N/A ^c	2	Not determined	(74)
<i>Elaeodendron buehneri</i>	63 μ g/ml ^a	4	Not determined	(74)
<i>Ginkgo biloba</i>	100 μ g/ml ^d	3	0.1	(41)
	200 μ g/ml ^d	6	0.2	(41)
	400 μ g/ml ^d	14	0.5	(41)
	800 μ g/ml ^d	32	1	(41)
	100 μ g/ml ^d	5–6	0.8	(42)
<i>Glycyrrhiza uralensis</i> (gan cao)	1:500 dilution ^d	4–5	0.8	(70)
<i>Humulus lupulus</i> (hops)	10 μ l ^b	4	0.6	(46)
<i>Hypericum perforatum</i>	10 μ g/ml ^b	5–6	0.7 ^e	(14)
(St. John's wort)	7–75 μ g/ml ^b	6–8	1–1.2	(13)
<i>Hypoxis hemerocallidea</i>	N/A ^c	2	0.3 ^f	(75)
<i>Jatropha multifida</i>	500 μ g/ml ^a	6	Not determined	(74)
<i>Pteridium aquilinum</i>	250 μ g/ml ^a	4	Not determined	(74)
<i>Piper methysticum</i> (kava kava)	100 μ g/ml ^d	10	0.3	(59)
<i>Radix angelicae sinensis</i> (dang gui)	1:500 dilution ^d	4–5	0.8	(70)
<i>Radix astragali</i> (huang qi)	1:500 dilution ^d	2	0.3	(70)
<i>Rhei rhizoma</i> (da huang)	1:500 dilution ^d	3–4	0.6	(70)
<i>Salvia miltiorrhiza</i>	100 μ g/ml ^b	2–3	0.4	(65)
(danshen)	100 μ g/ml ^d	no effect	0	(65)
<i>Schisandra chinensis</i>	1:1,000 dilution ^b	6–8	1.2	(70)
(wu wei zi)	1:1000 dilution ^d	4–6	0.8	(70)
<i>Sclerocarya birrea</i> Sond	16 μ g/ml ^a	3	Not determined	(74)
<i>Sterculia africana</i>	125 μ g/ml ^a	2	Not determined	(74)
<i>Sutherlandia frutescens</i>	N/A ^c	2	0.4 ^f	(75)
Tian xian	16 μ g/ml ^b	10–20	0.2	(73)
	31 μ g/ml ^b	20–30	0.3	(73)
	63 μ g/ml ^b	40–50	0.7	(73)
	125 μ g/ml ^b	50–60	0.8	(73)
	250 μ g/ml ^b	65	1	(73)
<i>Turraea holstii</i>	63 μ g/ml ^a	4	Not determined	(74)

PXR pregnane X receptor

^a Methanolic extract^b Ethanolic extract^c Information not available^d Aqueous extract^e Twenty micromolar rifampicin^f Concentration of rifampicin not reported

mouse PXR by *C. forkohlii* extract. However, candidate compounds include forskolin and 1,9-dideoxyforskolin, which is another diterpene present in the roots of *C. forkohlii*. Each of these chemicals has been shown to act as an agonist of mouse PXR, as judged by their ability to bind to the ligand-binding domain of PXR, recruit coactivator (i.e., SRC-1) to PXR, and dissociate corepressor (i.e., NCoR1) from PXR (29). Both forskolin (29,30) and 1,9-dideoxyforskolin (29) also activate human PXR activity *in vitro*. Based on the reported *in vitro* EC₅₀ of 0.4–12 μ M in human PXR activation by forskolin (Table III) and plasma forskolin concentration of 4 μ M (31), this compound is predicted to be capable of activating PXR *in vivo* (31).

***Commiphora mukul* (Guggul)**

Commiphora mukul, which is also known as *Commiphora wightii* or guggul tree, is indigenous to India, Pakistan,

and Bangladesh. It has medicinal value in traditional Ayurvedic medicine (32). Extracts of guggul (available commercially as Gugulipid®), which is the gum resin from the bark of the *C. mukul* tree, is available as an over-the-counter dietary supplement in various Western countries, including the USA. It is used by consumers as a naturally occurring cholesterol-lowering agent (33). Chemical analysis indicates that guggul consists of a mixture of diterpenes, sterols, steroids, esters, and higher alcohols. (*E*)-Guggulsterone [*cis*-4,17(20)-pregnadiene-3,16-dione] and (*Z*)-guggulsterone [*trans*-4,17(20)-pregnadiene-3,16-dione] are the active compounds with cholesterol-lowering action. Mechanistic studies have proposed that these two pregnane derivatives act by antagonizing the farnesoid X receptor (gene designation *NRIH4*) (34) and up-regulating the expression of the bile acid export pump (35).

Gugulipid® extract is capable of activating human and mouse PXR, as assessed in an *in vitro* cell-based luciferase

Table II. *In Vitro* Effect of Herbal Extracts on PXR Target Gene Expression

Herbal extract	Concentration (or volume)	Cell culture model	Target gene expression	Reference
<i>Agauria salicifolia</i>	63 µg/ml ^a	HepG2 cells ^b	CYP3A4 mRNA: 2-fold ↑	(74)
<i>Coleus forskohlii</i>	2,000–16,000 ^c dilution	Mouse hepatocytes (wild-type)	<i>Cyp3a11</i> mRNA: ↑	(29)
		Mouse hepatocytes (PXR knockout)	<i>Cyp3a11</i> mRNA: no effect	(29)
<i>Commiphora mukul</i> (Gugulipid®)	1:4,000 dilution ^c	Human hepatocytes	CYP3A4 mRNA: 4-fold ↑	(36)
<i>Cyphostemma hildebrandtii</i>	Not available	HepG2 cells	CYP3A4 mRNA: no effect	(74)
<i>Elaeodendron buchananii</i>	63 µg/ml ^a	HepG2 cells	CYP3A4 mRNA: no effect	(74)
<i>Ginkgo biloba</i>	100 µg/ml ^d	Human hepatocytes	CYP3A4 mRNA: 5–18 fold ↑	(42)
	200 µg/ml ^d	LS180 cells ^e	CYP3A4 mRNA: 2-fold ↑	(41)
		LS180 cells	CYP3A5 mRNA: 2-fold ↑	(41)
		LS180 cells	ABCB1 mRNA: 2-fold ↑	(41)
	400 µg/ml ^d	LS180 cells	CYP3A4 mRNA: 3-fold ↑	(41)
		LS180 cells	CYP3A5 mRNA: 2-fold ↑	(41)
		LS180 cells	ABCB1 mRNA: 3-fold ↑	(41)
	800 µg/ml ^d	LS180 cells	CYP3A4 mRNA: 3-fold ↑	(41)
		LS180 cells	CYP3A5 mRNA: 4-fold ↑	(41)
		LS180 cells	ABCB1 mRNA: 6-fold ↑	(41)
<i>Humulus lupulus</i> (hops)	0.8 µl ^c	Human hepatocytes	CYP3A4 mRNA: no effect	(46)
		Human hepatocytes	ABCB1 mRNA: no effect	(46)
	4 µl ^c	Human hepatocytes	CYP3A4 mRNA: no effect	(46)
		Human hepatocytes	ABCB1 mRNA: no effect	(46)
	20 µl ^c	Human hepatocytes	CYP3A4 mRNA: 5-fold ↑	(46)
		Human hepatocytes	ABCB1 mRNA: no effect	(46)
	100 µl ^c	Human hepatocytes	CYP3A4 mRNA: 9-fold ↑	(46)
		Human hepatocytes	ABCB1 mRNA: 2-fold ↑	(46)
<i>Hypericum perforatum</i> (St. John's wort)	7–75 µg/ml ^c	Human hepatocytes	CYP3A4 mRNA: ↑	(13)
	0.8 µl ^c	Human hepatocytes	CYP3A4 mRNA: no effect	(46)
		Human hepatocytes	ABCB1 mRNA: no effect	(46)
	4 µl ^c	Human hepatocytes	CYP3A4 mRNA: 3-fold ↑	(46)
		Human hepatocytes	ABCB1 mRNA: no effect	(46)
	20 µl ^c	Human hepatocytes	CYP3A4 mRNA: 8-fold ↑	(46)
		Human hepatocytes	ABCB1 mRNA: < 2-fold ↑	(46)
	100 µl ^c	Human hepatocytes	CYP3A4 mRNA: 8-fold ↑	(46)
		Human hepatocytes	ABCB1 mRNA: < 2-fold ↑	(46)
<i>Piper methysticum</i> (kava kava)	100 µg/ml ^d	Human hepatocytes	CYP3A4 mRNA: 5-fold ↑	(59)
<i>Schisandra chinensis</i> (wu wei zi)	1:1,000 dilution ^c	Human hepatocytes	CYP2C9 mRNA: ↑	(70)
		Human hepatocytes	CYP3A4 mRNA: ↑	(70)
<i>Sclerocarya birrea</i> Sond	16 µg/ml ^a	HepG2 cells	CYP3A4 mRNA: no effect	(74)
<i>Sterculia africana</i>	125 µg/ml ^a	HepG2 cells	CYP3A4 mRNA: no effect	(74)
Tian xian	4 µg/ml ^c	Mouse hepatocytes ^f (expressing human PXR)	CYP3A4 mRNA: 3-fold ↑	(73)
		Mouse hepatocytes (wild type)	<i>Cyp3a11</i> mRNA: 10-fold ↑	(73)
		Mouse hepatocytes (<i>Cyp3a11</i> knockout)	<i>Cyp3a11</i> mRNA: no effect	(73)
	31 µg/ml ^c	Mouse hepatocytes ^f (expressing human PXR)	CYP3A4 mRNA: 7-fold ↑	(73)
		mouse hepatocytes (wild type)	<i>Cyp3a11</i> mRNA: 30-fold ↑	(73)
		Mouse hepatocytes (<i>Cyp3a11</i> knockout)	<i>Cyp3a11</i> mRNA: no effect	(73)
	250 µg/ml ^c	Mouse hepatocytes ^f (expressing human PXR)	CYP3A4 mRNA: 18-fold ↑	(73)
		Mouse hepatocytes (wild type)	<i>Cyp3a11</i> mRNA: 65-fold ↑	(73)
		Mouse hepatocytes (<i>Cyp3a11</i> knockout)	<i>Cyp3a11</i> mRNA: no effect	(73)
<i>Turraea holstii</i>	63 µg/ml ^a	HepG2 cells	CYP3A4 mRNA: no effect	(74)
	125 µg/ml ^a	HepG2 cells	CYP3A4 mRNA: 4-fold ↑	(74)

PXR pregnane X receptor

^a Methanolic extract^b Cultured HepG2 human hepatoma cells^c Ethanolic extract^d Aqueous extract^e Cultured LS180 human colon adenocarcinoma cells^f Cultured hepatocytes from transgenic mice expressing human PXR

Table III. PXR Activation by Individual Chemicals Present in Herbal Extracts: Experimentally Derived EC₅₀ and E_{max} Values

Chemical	EC ₅₀ (μM)		E _{max} (fold-increase over vehicle control)		Reference
	Human PXR	Mouse PXR	Human PXR	Mouse PXR	
Hyperforin	0.023	N/A ^a	6–7	N/A	(13)
	0.032 ^b	N/A	N/A	N/A	(52)
	0.04 ^c	N/A	N/A	N/A	(31)
	0.2 ^d	N/A	12 ^e	N/A	(93)
Forskolin	0.4	0.9	3	7	(29)
	1.5 ^c	N/A	N/A	N/A	(31)
	12.4 ^f	N/A	N/A	N/A	(30)
Cryptotanshinone	0.4	N/A	7	N/A	(65)
<i>E</i> -Guggulsterone	1.2 ^g	N/A	4 ^h	N/A	(36)
Tanshinone IIA	1.4	N/A	6	N/A	(65)
<i>Z</i> -Guggulsterone	1.4	2.4	8	11	(36)
Schisandrin A	2 ⁱ	1.25 ^j	13	7 ^k	(70)
Schisandrin B	2 ⁱ	1.25 ^j	13	5 ^k	(70)
Schisandrol B	2 ⁱ	N/A	10	N/A	(70)
1,9-Dideoxyforskolin	2.3 ^c	N/A	N/A	N/A	(31)

PXR pregnane X receptor

^a Data not available

^b EC₅₀ of 0.46 μM for rifampicin in the same study

^c EC₅₀ of 1.2 μM for rifampicin in the same study

^d EC₅₀ of 0.9 μM for rifampicin in the same study

^e E_{max} of 19-fold for rifampicin in the same study

^f EC₅₀ of 4.1 μM for rifampicin in the same study

^g EC₅₀ of 0.8 μM for rifampicin in the same study

^h E_{max} of 9-fold for rifampicin in the same study

ⁱ EC₅₀ of 2 μM for rifampicin in the same study

^j EC₅₀ of 0.5 μM for pregnenolone 16α-carbonitrile (PCN) in the same study

^k E_{max} of 13-fold for PCN in the same study

reporter gene assay (36). At the highest concentration (1:4,000 dilution) investigated, the extent of human PXR activation by Gugulipid® is approximately 80% of that by rifampicin (10 μM), which is a prototypic agonist of human PXR (Table I). By comparison, the extent of mouse PXR activation by the same concentration of Gugulipid® is similar to that by PCN (10 μM), a prototypic agonist of mouse PXR. The mechanism by which Gugulipid® activates PXR remains to be elucidated.

The effect of (*Z*)-guggulsterone and (*E*)-guggulsterone on PXR activity has also been studied. Both of these compounds activate PXR in *in vitro* cell-based reporter gene assays (34,36). Detailed dose–response experiments show that (*Z*)-guggulsterone activates human and mouse PXR with reported EC₅₀ values of 2.4 and 1.4 μM, respectively, and E_{max} values of 8- and 11-fold increase in reporter activity, respectively (36) (Table III). By comparison, (*E*)-guggulsterone activates human PXR activity with an EC₅₀ of 1.2 μM, which is comparable to the EC₅₀ (0.8 μM) obtained for rifampicin in the same study (36) (Table III). Consistent with the action of an agonist, both (*Z*)-guggulsterone and (*E*)-guggulsterone stimulate the interaction between PXR and a coactivator (i.e., SRC-1). Treatment of primary cultures of human hepatocytes with *E*-guggulsterone (10 μM) or Gugulipid® (16,000-fold dilution in ethanol) increases CYP3A4 mRNA expression (4-fold, Table II) to an extent similar to that obtained with 10 μM rifampicin (5-fold) (36). *E*-Guggulsterone and Gugulipid® also increases the expression of *Cyp3a11* mRNA in cultured mouse hepatocytes (36). Although Gugulipid® and guggulsterones activate PXR, this

effect does not appear to be linked to their hypolipidemic action. As reported previously in human studies, the administration of a known PXR agonist (rifampicin) does not decrease plasma levels of cholesterol (37).

Ginkgo biloba

Ginkgo biloba, which is a member of the *Ginkgoaceae* family, is the oldest living tree species (38). The roots and leaves of this tree contain bioactive constituents, such as terpene trilactones (e.g., ginkgolide A, ginkgolide B, ginkgolide C, and ginkgolide J, which are diterpenes, and bilobalide, which is a sesquiterpene) and flavonols (e.g., the aglycones and various glycosides of quercetin, kaempferol, and isorhamnetin). Cell culture and rodent studies have shown that *Ginkgo biloba* has a variety of biological actions, including antioxidant, anti-amyloidogenic, and anti-apoptotic activities (39). *G. biloba* is used for the self-medication of a variety of conditions, most commonly in the management of memory impairment, including those associated with dementia in neurodegenerative diseases, such as Alzheimer's disease (40). In certain jurisdictions (e.g., Germany), *G. biloba* is approved for the therapeutic treatment of dementia.

An extract of *G. biloba* containing known concentrations of terpene trilactones and flavonols has been shown to activate human PXR and mouse PXR, as assessed in an *in vitro* cell-based luciferase reporter gene assay (41). Detailed dose–response data indicate that the extract is effective in activating human PXR transcriptional activity at concentrations of 100–800 μg/ml (Table I). Activation of human PXR

by *G. biloba* extract was confirmed in a subsequent study (42) (Table I). Consistent with these findings, *G. biloba* extract is capable of inducing PXR target genes (Table II), including CYP3A4, as shown in PXR-expressing LS180 cells in culture (41) and in primary cultures of human hepatocytes (42). Although ginkgolide A and B have been reported to activate human PXR (42,43), the concentrations used in those cell culture experiments far exceed the levels present in an extract of *G. biloba*. Thus, it remains to be determined which chemical constituent(s) is responsible for the *in vitro* activation of PXR by *G. biloba* extract.

Humulus lupulus (Hop Extract)

Humulus lupulus is a plant that is cultivated in various regions of the world, including North America, South America, South Africa, and Australia (44). Hops, which are the flower cones of the plant, are used as a preservative in beer, and they give beer the characteristic bitterness, aroma, and flavor. Hop extract is used as herbal medicine for the treatment of a variety of conditions, including anxiety, insomnia, and restlessness. It also has estrogenic activity. As a result, hop extract has been investigated as a potential therapy for the management of postmenopausal symptoms (45). Chemicals present in hops include terpenes, bitter acids, chalcones, flavonol glycosides (i.e., those of quercetin, quercitrin, rutin, and kaempferol), and catechins (44). The bitter acids comprise of α -acids (e.g., humulone, cohumulone, and adhumulone) and β -acids (e.g., colupulone, lupulone, and adlupulone).

It has been shown that an ethanolic extract of hops of unknown chemical composition increases PXR-mediated transcriptional activity (46), as assessed in an *in vitro* cell-based luciferase reporter gene assay (Table I). Comparative analysis indicates that the extent of PXR activation by the ethanolic extract of hops is similar to that obtained with St. John's wort and Gugulipid®. Consistent with the finding that hop extract increases PXR activity, treatment of primary cultures of human hepatocytes with the extract increases CYP3A4 mRNA expression (Table II). Experiments with colupulone show that this compound (at concentrations of 3 and 10 nM) increases PXR activity. However, it remains to be demonstrated conclusively that colupulone is responsible for the human PXR-activating effect of hop extract. It is likely that colupulone is also an activator of rodent PXR because of previous findings showing that this β -acid is an inducer of hepatic CYP3A gene expression in mice (47) and rats (48).

Hypericum perforatum (St. John's wort)

H. perforatum is commonly known as St. John's wort. This plant has a long history of use as herbal medicine in Europe and is well known as an anti-depressant. The anti-depressant action of St. John's wort has been linked to its inhibition of synaptosomal reuptake of serotonin, norepinephrine, and dopamine (49). The chemical constituents in St. John's wort include naphthodianthrone such as hypericin and pseudohypericin (0.1–0.3%), phlorolucins such as hyperforin (up to 3%), flavonoids such as hyperoside, quercetin, and rutin (0.5–1%), carboxylic acids, xanthones, proanthocyanidins, anthraquinones, carotenoids, coumarins, and volatile oils

(e.g., α -pinene and cineole) (50). Hyperforin has been shown to have inhibitory effect on neurotransmitter reuptake (51).

As mentioned above, St. John's wort was the first herbal medicine reported to activate PXR (13,14) (Table I). The mechanism of human PXR activation by St. John's wort involves direct ligand binding to the receptor (14,52). Consistent with the finding that St. John's wort activates PXR, this herbal medicine is known to induce PXR-regulated genes, such as CYP3A4, in primary cultures of human hepatocytes (13) (Table II). Many of the clinical herb–drug interactions with St. John's wort can now be explained on the basis of PXR activation by this herbal medicine (53).

Chemical analysis identified hyperforin as a constituent in St. John's wort that activates human PXR (13,14). This compound activates human PXR transcriptional activity with an EC₅₀ value in low nanomolar concentrations (Table III), and it is one of the most potent activators of human PXR identified to date (31). Hyperforin is an agonist of human PXR as shown by the findings that it competes with ³H-SR12813 for binding to human PXR (13) and stimulates the interaction between human PXR and the coactivator SRC-1 (14). By comparison, other chemical constituents in St. John's wort, including hypericin, pseudohypericin, kaempferol, luteolin, myricetin, quercetin, quercitrin, isoquercitrin, amentoflavone, hyperoside, scopoletin, and β -sitosterol, have little or no effect on human PXR transcriptional activity when analyzed at a concentration of 10 μ M (13).

Piper methysticum (Kava Kava)

Piper methysticum, which is commonly known as kava or kava kava, is a Polynesian plant with medicinal value. Roots of *P. methysticum* have been used as herbal medicine and consumed as a beverage by natives in the South Pacific. Therapeutic uses of kava extracts include the management of anxiety and insomnia (54). The mechanism by which kava extract exerts its therapeutic effects is not known. Its biological activities include binding to the gamma-aminobutyric acid receptor (55) and inhibition of noradrenaline uptake (56). The chemical constituents in kava extract are arylethylene- α -pyrones, chalcones and other flavanones, and conjugated diene ketones (57). The kavalactones, which are the substituted 4-methoxy-5,6-dihydro- α -pyrones, are associated with pharmacological activity. The major kavalactones are dehydrokavain (desmethoxyyangonin), dihydrokavain, yangonin, kavain, dihydromethysticin, and methysticin. The use of kava extract in the Western world has been linked to the development of hepatotoxicity in some individuals, although it has been proposed that this may relate to the use of stems and leaves in commercial herbal preparations of kava, rather than the use of roots in traditional preparations of kava (58).

Kava extract activates human PXR transcriptional activity, as determined in cell-based reporter gene assays (59) (Table I). Dose–response data indicate that PXR activation is evident over the range of 5–1,000 μ g/ml (60). The chemical constituent(s) responsible for PXR activation by kava extract has yet to be identified, although it has been shown that kavain, yangonin, desmethoxyyangonin, methysticin, dihydrokavain, and dihydromethysticin at a concentration of 50 μ M do not activate either human PXR or rat PXR (61).

***Salvia miltiorrhiza* (Danshen)**

Salvia miltiorrhiza is a perennial flowering plant native to Japan and China (62). The roots of *S. miltiorrhiza*, known as danshen, are used in traditional Chinese medicine. It is used to treat various conditions, including coronary artery diseases such as angina and myocardial infarction, hyperlipidemia, hypertension, arrhythmia, stroke, and peripheral vascular disease (63). The chemical constituents of danshen include water-soluble phenolic acids, such as salvianolic acid and lithospermic acid B, and the more lipophilic abietane type diterpene quinones, such as tanshinone I, tanshinone IIA, tanshinone IIB, and cryptotanshinone (64). These chemicals all contribute to the anticoagulant, antithrombotic, antioxidant, and other biological activities of danshen.

An ethanolic extract of danshen has been reported to activate human PXR transcriptional activity in a cell-based reporter gene assay (65). At a concentration of 100 µg/ml, the magnitude of PXR activation by the extract is approximately one half of that by a known PXR agonist, rifampicin (10 µM) (Table I). Interestingly, water extracts of danshen do not result in PXR activation. The chemical constituent(s) contributing to the effect of PXR activation by danshen is not known. However, tanshinone IIA and cryptotanshinone, but not tanshinone I, are capable of increasing human PXR transcriptional activity when analyzed at a concentration of 2 µM (65) (Table III). Danshen may also be an activator of mouse PXR, as suggested by the finding that an ethyl acetate extract of danshen increases hepatic microsomal CYP3A protein levels in mice (66). It remains to be determined whether danshen has any PXR-activating effects in humans, given that it is usually ingested as extracted powder or as one of the several herbs as part of a traditional Chinese medicine regimen.

***Schisandra chinensis* (Wu Wei Zi)**

Schisandra chinensis is a deciduous woody vine found in the northwestern China, far eastern Russia, and Korea (67). As one of the commonly used herbs in traditional Chinese medicine, the berries of *S. chinensis* is known as “wu wei zi,” which means five flavor berry because it is salty, sweet, sour, astringent, and bitter. Wu wei zi is used in traditional Chinese medicine as a tonic to treat a variety of conditions, including stress. In recent years, it has been investigated as a hepatoprotectant (68). Dibenzocyclooctene lignans are the biologically active chemical constituents in the berries of *S. chinensis* (69). These include schisandrol A, schisandrol B, schisandrin A (also known as deoxyshisandrin), and schisandrin B (also known as γ-schisandrin).

Both aqueous and ethanolic extracts of wu wei zi at a concentration of 1:1,000 have been shown to activate human PXR transcriptional activity in a cell-based reporter assay (70). The degree of PXR activation by the extracts is similar to that by rifampicin (10 µM) in the same experiment (Table I). Consistent with the finding that wu wei zi extract activates human PXR, it is also capable of increasing CYP2C9 and CYP3A4 gene expression in primary cultures of human hepatocytes (Table II). Experiments with individual dibenzocyclooctene lignans indicate that schisandrol B, schisandrin A, and schisandrin B

activate human PXR with a similar efficacy and potency as rifampicin (Table III). Relative to these compounds, schisandrol A is also efficacious, but it is less potent. Wu wei zi extract and the four dibenzocyclooctene lignans are also able to activate mouse and rat PXR (70).

Tian Xian

Tian xian (or tien-hsien) is a Chinese herbal remedy that consists of multiple herbs, including *Hedyotis diffusa*, *Radix ginseng*, *Radix astragali*, *Polyporus umbellatus*, *Radix clematidis*, *Radix trichosanthis*, *Semen impatientis*, *Solanum nigrum*, *Calculus bovis*, and *Venenum bufonis* (71). It is marketed as anticancer herbal therapy and is available commercially in several dosage forms, such as capsule, tablet, liquid, suppository, ointment, and plaster. The very limited amount of scientific information on tian xian suggests that it has immunomodulating effect (71) and is capable of inhibiting proliferation of tumor cells by inducing apoptosis (72). An ethanolic extract of tian xian at concentrations of 16–250 µg/ml has been shown to activate human PXR transcriptional activity in a cell-based reporter gene assay (73). The fold induction in the reporter activity by the 250 µg/ml concentration of the extract is comparable to that by rifampicin (10 µM) (Table I). As shown in the mammalian two-hybrid assay, tian xian extract stimulates recruitment of a coactivator (i.e., SRC-1) to human PXR and dissociation of a corepressor (i.e., NCoR) from the receptor, suggesting that the extract acts as an agonist of human PXR. Tian xian extract (4–250 µg/ml) also increases the expression of a PXR target gene (CYP3A4) in cultured hepatocytes from transgenic mice expressing human CYP3A4 (Table II). The PXR-activating effect of tian xian is not species specific because it also appears to be an activator of mouse PXR, as suggested by the finding that it induces hepatic *Cyp3a11* gene expression in wild-type mice but not in PXR knockout mice.

Other Herbal Medicines

As shown in Table I, various other herbal medicines have also been identified as activators of human PXR, as assessed by cell-based reporter assays. These include (1) aqueous extracts of various herbs in traditional Chinese medicines, such as *Glycyrrhiza uralensis* Fisch (gan cao), *Rhei rhizoma* (da huang), *Radix angelicae Sinensis* (dang gui), and *R. astragali* (huang qi) (70); (2) Tanzanian plants, such as *Jatropha multifida*, *Agauria salicifolia*, *Elaeodendron buchanaanii*, *Turraea holstii*, *Clausena anisata*, *Sclerocarya birrea* Sond, *Cyphostemma hildebrandtii*, and *Sterculia africana* (74); and (3) *Hypoxis hemerocallidea* and *Sutherlandia frutescens*, which are used in Africa in the management of HIV infection and AIDS (75).

Summary: Herbal Medicines as Activators of PXR

Various herbal extracts are capable of activating PXR, as shown in *in vitro* cell-based reporter gene assays (Table I). In some cases, such as *H. perforatum* (St. John's wort), *G. biloba*, *S. chinensis*, and tian xian, the fold increase in reporter activity is similar to that obtained for rifampicin, which is a known agonist of human PXR (4). Among the individual chemical constituents

investigated for their ability to activate PXR in *in vitro* reporter gene assays, hyperforin is the most potent (EC_{50} in low nanomolar concentrations), whereas the EC_{50} values for the others are considerably greater but are comparable to that reported for rifampicin (approximately 1 μ M) (Table III). As shown in this review article, for many of the herbal extracts investigated for their effect on PXR, the conclusion was drawn based on results obtained solely from *in vitro* cell-based reporter gene assays. In other cases, reporter activity data were corroborated by results showing coactivator recruitment (14,29,30,73), ligand binding to the receptor (13), and induction of PXR target gene expression not only in cultured human and mouse hepatocytes but also hepatocytes isolated from PXR knockout mice and transgenic mice expressing human PXR (Table II). Whether any of the herbal extracts are capable of activating PXR *in vivo* in humans is still largely not known, except for *H. perforatum* (St. John's wort), which has been shown to increase the clearance of drugs that are metabolized by CYP3A4 (16, 53).

CONSTITUTIVE ANDROSTANE RECEPTOR

CAR is expressed predominantly in liver (7) and also in small intestines (76). Similar to PXR, CAR regulates the expression of a wide array of genes involved in biotransformation and transport of endogenous substances, naturally occurring compounds, drugs, and other xenochemicals (16). There is overlap between CAR and PXR target genes (8,9,76). For example, PXR regulates the expression of both *CYP2B6* and *CYP3A4*, whereas CAR preferentially regulates *CYP2B6* as a consequence of its weaker binding to the PXR response element in the *CYP3A4* promoter (77). Mouse *Cyp2b10*, human *CYP2B6*, and rat *CYP2B1* were the first genes shown to be under the regulatory control of CAR (16,17). Other examples of CAR-regulated genes include *CYP2C8*, *CYP2C9*, and *CYP2C19*, phase II conjugation enzymes, such as UDP-glucuronosyltransferase *UGT1A1*, sulfotransferase *Sult2a1*, and glutathione *S*-transferases *Gsta1*, and transporters, including P-glycoprotein (*ABCB1*), certain organic anion transporting polypeptides, such as OATP2 (*Slc21a6*), and multidrug resistance-associated proteins, including Mrp1 (*Abcc1*), Mrp2 (*Abcc2*), and Mrp4 (*Abcc4*) (8,9,16). In addition, CAR has also been shown to regulate the repression of enzymes involved in gluconeogenesis, such as phosphoenolpyruvate carboxykinase 1 (PEPCK1), and beta-oxidation enzymes, such as carnitine palmitoyltransferase 1 (78). Overall, CAR regulates a broad array of genes of fundamental importance, such as bioactivation, detoxification, and transport of drugs, other xenochemicals, and endogenous substance. Therefore, alteration in CAR function may impact not only pharmacokinetics, efficacy, and toxicity of drugs but also endocrine homeostasis, energy metabolism, and cell proliferation/tumorigenesis (78).

In contrast to PXR, CAR is constitutively active (17). In the basal state, CAR is localized in the cytoplasm in a complex with HSP90 and CCRP. Upon binding to an agonist, CAR is dissociated from HSP90 and CCRP, and the ligand-bound CAR translocates to the nucleus, where it forms a heterodimer with RXR α and recruits coactivators and dissociates corepressors. The CAR–RXR α –coac-

tivator complex binds to DNA response elements in CAR target genes, resulting in increased gene transcription. SRC-1, transcription factor Sp1, and signal cointegrator-2 are examples of coactivators of CAR (17), whereas NCoR is an example of a corepressor of CAR (79). Interestingly, CAR activation may also occur without direct binding of the ligand to CAR, and this is exemplified by the activation of CAR by phenobarbital and various other compounds (12). The reader is referred to recent reviews on the mechanistic details of direct and indirect activation of CAR (17,80) and the interplay between CAR and other nuclear receptors (19).

Species-dependent chemical modulation of CAR activity has been reported (12,16). For example, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, which is an environmental chemical, is an agonist of mouse CAR. 6-(4-Chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-[3,4-dichlorobenzyl]oxime (CITCO), which is an imidazole derivative, is an agonist of human CAR. Another example is meclizine. This drug is an agonist of mouse CAR, but it is not an agonist of human CAR. In fact, meclizine is an inverse agonist of human CAR. Androstanol and androsteneol are efficacious inverse agonists of mouse CAR but not human CAR. Various synthetic drugs and other single chemical entities have also been identified as agonists (12), indirect activators (12), inverse agonists (12), and antagonists of CAR (81). Investigations in recent years have identified several herbal medicines as modulators of CAR. The following is an overview of our current knowledge on the effect of specific herbal medicines on CAR activity.

MODULATION OF CAR ACTIVITY BY HERBAL MEDICINES

Allium sativum (Garlic)

Commonly known as garlic, the root bulb of the *Allium sativum* plant has been used for medicinal purposes in certain cultures for thousands of years. Various biological activities have been shown for garlic, including antithrombotic activity and lipid-lowering activity (82). Although various chemicals are present in garlic oil, volatile sulfur-containing compounds account for the majority (83). These sulfur-containing compounds include diallyl sulfide, diallyl disulfide, and diallyl trisulfide. Garlic oil has been suggested to be an activator of rat CAR (84) based on the finding that it increases hepatic CYP2B mRNA expression to a greater extent in male Wistar-Kyoto rats than in female Wistar-Kyoto rats (Table IV). The reasoning is that CAR protein is expressed to a much greater level in male Wistar-Kyoto rats than in female Wistar-Kyoto rats (85). However, no other experimental approaches have been used to support the conclusion that garlic oil is an activator of rat CAR. Among the diallyl sulfides investigated, only diallyl disulfide shows preferential induction of hepatic CYP2B in male Wistar-Kyoto rats (84). Garlic oil and diallyl disulfide do not appear to activate human CAR, as suggested by the finding that they do not increase *in vivo* CYP2B6 transcriptional activity in mice transiently transfected with a CYP2B6-

Table IV. *Ex Vivo* Effect of Herbal Extracts on CAR Target Gene Expression

Herbal extract	Dosage	Animal model	Target gene expression	Reference
<i>Allium sativum</i> (garlic oil)	300 mg/kg single oral dose	Wistar-Kyoto rats	Hepatic CYP2B mRNA: ↑ (male>female by 9-fold)	(84)
Yin zhi huang	10 ml/kg/day orally for 3 days	Wild-type mice	Hepatic <i>Cyp2b10</i> mRNA: ↑	(15)
			Hepatic <i>Ugt1a1</i> mRNA: ↑	(15)
		CAR knockout mice	Hepatic <i>Cyp2b10</i> mRNA: no effect	(15)
			Hepatic <i>Ugt1a1</i> mRNA: no effect	(15)
		Transgenic mice expressing human CAR	Hepatic <i>Cyp2b10</i> mRNA: ↑	(15)
			Hepatic <i>Ugt1a1</i> mRNA: ↑	(15)

CAR constitutive androstane receptor

luciferase reporter construct containing NR1, which is a CAR-specific binding element.

hepatic expression of CYP2B (89), which are under the regulatory control of CAR (17).

***Commiphora mukul* (Guggul)**

As mentioned above, guggul extract (Gugulipid®) is capable of activating PXR (36). Whether the extract modulates CAR activity is not known, although it is possible that it may be an inverse agonist of CAR. The reason is that the *cis*- and *trans*-stereoisomers of guggulsterone, which are constituents in guggul extract, decreases the basal transcriptional activity of mouse CAR (86), suggesting that these compounds are inverse agonists of mouse CAR. Consistent with this possibility, *cis*- and *trans*-guggulsterone have been shown to dissociate a coactivator (i.e., SRC-1) from mouse CAR, as determined in a mammalian two-hybrid assay. However, whether the guggulsterones act as a mouse CAR inverse agonist depends on the relative cellular abundance of CAR and PXR. In cases where CAR expression is high and PXR expression is low or negligible, these compounds act as inverse agonist of mouse CAR in that they repress transcription of a target gene (e.g., *Cyp2b10*). In contrast, when CAR expression is low or negligible and PXR expression is high, the guggulsterones increases *Cyp2b10* mRNA expression. Given the pronounced interindividual differences in CAR and PXR expression in human liver (87), these findings illustrate another level of complexity in predicting the action of a given drug on the functional activity of these receptors in an individual.

Ginkgo biloba

In a recent study, an extract of *G. biloba* known as EGb 761 (100 µg/ml) weakly increased (~2-fold relative to vehicle-treated control group) CAR transcriptional activity in cultured HepG2 cells, as shown in an *in vitro* cell-based reporter assay (42). The result is somewhat difficult to interpret because, in the same study, treatment with CITCO, which is a known agonist of human CAR (88), did not increase CAR activity when compared to the vehicle-treated control group (42). In another experiment that used a splice variant of human CAR (designated as hCAR3), EGb 761 extract (100 µg/ml) increased hCAR3 activity by approximately 2-fold, whereas CITCO (1 µM) increased it by 7-fold. It is possible that *G. biloba* activates rat CAR because the *in vivo* administration of an extract of *G. biloba* to rats increases

Yin Zhi Huang

Yin zhi huang is a traditional Chinese herbal decoction consists of extracts from *A. capillaries*, *G. jasminoides* Ellis, *R. officinale* Baill, and *S. baicalensis* Georgi (90). This herbal remedy has a long history of use in Asia in the treatment of neonatal jaundice because it decreases serum levels of bilirubin (91). The notion that yin zhi huang activates CAR comes from experiments performed in mice (15). Administration of this herbal remedy (10 ml kg⁻¹ day⁻¹ for 3 days) decreases serum levels of bilirubin in wild-type mice but not in CAR knockout mice (Table IV). The alteration in serum bilirubin levels are accompanied by an increase in mRNA expression of CAR-regulated genes *Cyp2b10* and *Ugt1a1*. These effects of yin zhi huang can also be demonstrated in transgenic mice expressing human CAR (Table IV).

It remains to be determined which chemical constituent is responsible for the CAR-activating effect of yin zhi huang. A candidate compound is 6,7-dimethylesculetin (scoparone), which is a coumarin derivative present in yin zhi huang. The administration of 6,7-dimethylesculetin (100 mg/kg twice daily for 3 days) decreases serum bilirubin levels and increases hepatic *Cyp2b10* and *Ugt1a1* mRNA expression in wild-type mice but not in CAR knockout mice. Consistent with these findings, 6,7-dimethylesculetin stimulates nuclear translocation of CAR and increases hepatic *Cyp2b10* mRNA expression in cultured hepatocytes isolated from mice expressing human CAR.

Summary: Herbal Medicines as Modulators of CAR

Among the few herbal extracts studied to date, yin zhi huang is the best characterized herbal activator of CAR, as determined by experiments conducted in cell culture and various animal models (15). The finding that yin zhi huang activates CAR provides a molecular basis for the traditional therapeutic use of this herbal medicine in the treatment of neonatal jaundice (91).

CONCLUDING REMARKS

In recent years, various herbal medicines and some of their chemical constituents have been identified as activators of PXR and CAR. As mentioned above, many of the studies

were performed by conducting *in vitro* cell-based reporter assays, usually in a cell line (e.g., HepG2 cells). It has been shown that data from reporter assays (e.g., EC₅₀ values in PXR-dependent reporter assays) correlate with data (e.g., IC₅₀ values) obtained from direct ligand-binding assays ($r^2=0.65$) (92) and target gene expression analysis (e.g., CYP3A4 mRNA) in human hepatocytes ($r^2=0.85$) (93). However, interpretation of reporter assay data is not always straightforward. As shown in Tables I and II, an increase in PXR reporter activity is not necessarily accompanied by an increase in PXR target gene expression. In the case of CAR, the use of *in vitro* cell-based reporter assays is complicated by the high CAR activity in the basal state and the spontaneous nuclear translocation that occurs in cell lines (94). Some of the limitations of the *in vitro* approach to studying PXR and CAR activities may be overcome by: (1) conducting *in vivo* and/or *ex vivo* experiments in PXR knockout mice (95), CAR knockout mice (96), or transgenic mice that express human PXR and/or human CAR (15,97,98) or (2) performing *in vivo* gene transcription assays in rodents (99,100). Ultimately, to overcome any species differences in the pharmacokinetics of a given herbal extract, *in vivo* investigations are needed to determine whether it is capable of modulating PXR or CAR functional activity in humans. Future efforts in detailed chemical analysis will also be needed to identify the specific chemical constituent(s) responsible for the PXR/CAR-activating effects of the whole extract. Overall, with the appreciation that PXR and CAR may serve as potential therapeutic targets (10,11), the discovery of specific herbal medicines and some of their chemical constituents as *in vitro* modulators of PXR and CAR will provide a basis for targeted pharmacodynamic studies in the future.

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