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CYP2B6: New Insights into a Historically Overlooked Cytochrome P450 Isozyme

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Abstract

Human CYP2B6 has been thought to account for a minor portion (< 1%) of total hepatic cytochrome P450 (CYP) content and to have a minor function in human drug metabolism. Recent studies, however, indicate that the average relative contribution of CYP2B6 to total hepatic CYP content ranges from 2% to 10%. An increased interest in CYP2B6 research has been stimulated by the identification of an ever-increasing substrate list for this enzyme, polymorphic and ethnic variations in expression levels, and evidence for cross-regulation with CYP3A4, UGT1A1 and several hepatic drug transporters by the nuclear receptors pregnane X receptor and constitutive androstane receptor. Moreover, 20- to 250-fold interindividual variation in CYP2B6 expression has been demonstrated, presumably due to transcriptional regulation and polymorphisms. These individual differences may result in variable systemic exposure to drugs metabolized by CYP2B6, including the antineoplastics cyclophosphamide and ifosfamide, the antiretrovirals nevirapine and efavirenz, the anesthetics propofol and ketamine, the synthetic opioid methadone, and the anti-Parkinsonian selegiline. The potential clinical significance of CYP2B6 further enforces the need for a comprehensive review of this xenobiotic metabolizing enzyme. This communication summarizes recent advances in our understanding of this traditionally neglected enzyme and provides an overall picture of CYP2B6 with respect to expression, localization, substrate-specificity, inhibition, regulation, polymorphisms and clinical significance. Emphasis is given to nuclear receptor mediated transcriptional regulation, genetic polymorphisms, and their clinical significance.

Keywords

CYP2B6; pregnane X receptor (PXR); constitutive androstane receptor (CAR); polymorphism; induction; inhibition; substrate; clinical significance

Introduction

Cytochrome P450s (CYPs) are recognized as the predominant phase I enzymes and probably the most important catalysts among all drug metabolizing enzymes. Although there are more than 270 *CYP* gene families present among all species, human *CYP* genes are arranged into 18 families and 42 subfamilies, consisting of 59 active genes [1-3]. In humans, approximately 15 CYP enzymes among families 1-4 are responsible for xenobiotic metabolism, including over 90% of the phase I-dependent metabolism of clinically used drugs [4,5]. In contrast to the predominant hepatic CYP isozyme CYP3A4, CYP2B6 has been thought traditionally to account for a minor portion of total hepatic CYP content and play a negligible role in human

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drug metabolism [6,7]. This paradigm was established because 1) early studies demonstrated the absence of CYP2B6 mRNA or protein expression in a significant portion of human livers [6,8,9], and 2) no selective substrate probes or chemical inhibitors of CYP2B6 were available to facilitate assessment of its relative contribution to drug metabolism [10,11]. However, recent studies using more sensitive and specific immunochemical detection methods have reported higher expression levels of CYP2B6 in all human liver samples tested, which exhibited 20- to 250-fold interindividual variability in its expression [10,12-18]. In parallel, CYP2B6 activities have been reported to vary among human liver microsomes from approximately 25-fold using S-mephenytoin as substrate [10] to 80-fold using bupropion as substrate [19]. Such interindividual differences in hepatic CYP2B6 expression and enzymatic activities may result in variable systemic exposure and therapeutic response to the growing list of drugs and chemicals now known to be metabolized by CYP2B6.

During the past several years, a number of investigations have focused on elucidating the predominant mechanisms underlying interindividual variability in CYP2B6 expression, including polymorphisms in the *CYP2B6* gene, transcriptional suppression by cytokines [20, 21], transcriptional activation by inducers [22,23], enzyme inhibition, and allosteric activation [11]. Although the relative contributions of each of these factors to CYP2B6 variation remain unclear, particularly *in vivo*, it is suspected that genetic polymorphisms and/or differences in gene regulation are the major contributors. Compared with the well established polymorphic CYPs such as CYP2D6 and CYP2C19, the genetic variations of CYP2B6 and its potential clinical significance have only been realized in recent years. To date, more than 50 DNA variations with determined haplotypes have been identified from the *CYP2B6* gene (www.imm.ki.se/CYPalleles/cyp2b6.htm). The consequence of these polymorphic alleles is displayed in a variety of alterations in CYP2B6 expression and enzymatic activity (reviewed in [24]).

In addition to genetic polymorphisms, another source of variation in hepatic CYP2B6 protein expression and catalytic activity involves transcriptional activation of the corresponding gene in response to xenobiotic exposure. In contrast to the well studied rodent CYP2Bs, there has been relatively limited information available regarding the potency and magnitude of human CYP2B6 induction, the spectrum of agents capable of such induction, and the underlying mechanisms. Initial studies reported an increase in CYP2B6 mRNA or protein expression by known CYP3A4 inducers such as dexamethasone (DEX), phenobarbital (PB), and rifampicin (RIF) [12,25,26]. Subsequently, *in vitro* CYP2B6 induction was observed after treatment with cyclophosphamide (CPA), calcium channel antagonists, 3-hydroxy-3-methyl-glutaryl-CoA (HMGCoA) reductase inhibitors, and thiazolidinediones [27-30]. Accumulating evidence revealed that induction profiles of the CYP2B6 enzyme were remarkably associated with the activation of several liver enriched transcription factors such as pregnane X receptor (PXR), constitutive androstane receptor (CAR), glucocorticoid receptor (GR), and vitamin D receptor (VDR), in a direct and/or indirect manner.

The first comprehensive review of CYP2B6 in human xenobiotic metabolism was published eight years ago [11]. Since then, a plethora of new basic and clinical research on CYP2B6 has been reported raising awareness and interest in this CYP enzyme. The scope of this communication does not allow detailed discussion of all aspects of CYP2B6 as an enzyme of consequence. We chose to highlight the recent progress in our understanding of the mechanisms underlying interindividual variability in CYP2B6 expression. Focus will be given to the two speculated major contributors: genetic polymorphisms and nuclear receptor mediated regulation of CYP2B6 expression. In addition, the clinical relevance of CYP2B6 genetic variation, transcriptional regulation, and associated toxicity and drug-drug interactions will be discussed.

General Characteristics of CYP2B6

Although PB-induced synthesis of the microsomal drug metabolizing enzyme systems was observed over 40 years ago, the first cDNA of the major PB-inducible CYP (CYP2B) was cloned and sequenced from rat liver almost twenty years later [31,32]. Subsequently, the human orthologue was isolated using the rat CYP2B cDNA as a probe in the late 1980s [33]. Located on the long arm of chromosome 19, CYP2B6 is mapped with closely related pseudogene CYP2B7 and several other members of the CYP2 gene family, [33,34]. The 48kDa CYP2B6 protein was originally detected in only a limited number of hepatic samples with extremely low expression (<1% of the total hepatic CYP content)-largely due to the lack of a selective and efficient antibody. At that time, it was estimated that CYP2B6 only played a negligible role in human drug metabolism. However, this conception has been challenged by recent investigations, using more specific antibodies which demonstrated that the average relative expression of CYP2B6 ranges from 2% to 10% of the total hepatic CYP content [Fig. (1A)] [10,12-14,18]. In addition, the importance of the CYP2B6 in xenobiotic biotransformation has also been rediscovered based on: 1) the identification of an increasing number of novel substrates of this isozyme, 2) the inducibility of human CYP2B6 by an array of structurally diverse chemicals, and 3) the relatively high numbers of CYP2B6 genetic polymorphisms that contribute to the large interindividual variation in activity across the human population.

1. CYP2B6 distribution and localization

Different from the centrilobular distribution of several major CYPs such as CYP3A4 and CYP2E1, hepatic CYP2B6 is diffusely expressed across zone 1 and zone 3 in the parenchymal cells throughout the hepatic lobules [35]. Immunohistochemistry staining indicates that even after chemical induction, the patterns of zonal distribution for CYP3A4, CYP2E1, and CYP2B6 in liver are well maintained, suggesting these enzymes execute regionally-selective *in situ* metabolism in the liver. For instance, the analgesic acetaminophen is metabolized to the more toxic intermediate, N-acetyl-quinoneimine (NAPQI), by CYP2E1 and CYP3A4, and the primary lesion in acetaminophen toxicity is located in the centrilobular region where both CYP2E1 and CYP3A4 are highly expressed [36,37]. Therefore, it is reasonable to foresee that chemicals preferentially metabolized by CYP2B6 to more toxic metabolites may cause regionally-specific damage based on the location of CYP2B6 expression. Like most other CYP isoforms, CYP2B6 is primarily expressed in the liver and involved in the first-pass metabolism of ingested drugs, but has also been detected in several extrahepatic tissues including: brain, kidney, intestine, endometrium, bronchoalveolar macrophages, peripheral blood lymphocytes and skin [26,38,39]. The recently discovered CYP2B6 expression in human brain was primarily found in the neuron and astrocytes in a regional- and cell-specific manner [40]. In the same study, the authors observed a significantly higher level of CYP2B6 expression in the brains of smokers and alcoholics compared with non-smoking and non-alcoholic controls. Intriguingly, cells expressing higher CYP2B6, such as cerebellar Purkinje cells and hippocampal pyramidal neurons, are those known to be damaged in alcoholics; thus indicating higher levels of brain CYP2B6 expression may put certain individuals at higher risk of neuronal damage. Another recent study demonstrated that variable renal expression of CYP2B6 was responsible for tubular biotransformation of ifosfamide to the nephrotoxic metabolite, chloroacetaldehyde, limiting its therapeutic use [41]. Overall, the functional significance of CYP2B6 in certain extrahepatic tissues has not yet been fully elucidated but, it has become evident that tissue-specific expression of CYP2B6 may cause local accumulation of active metabolites and facilitate target-organ damage. Given that most pharmaceuticals and toxicants are limited by end-target toxicities, the extent and localization of CYP2B6 extrahepatic expression may influence the bioavailability of therapeutics and toxic burden in their target tissues.

2. Substrates of CYP2B6

Initially, CYP2B6 was known to metabolize only a limited number of xenobiotics, due, at least in part, to the inefficient detection of CYP2B6 expression and the lack of selective substrate probes or chemical inhibitors. During the past several years, the development of specific monoclonal antibodies and the application of recombinant DNA techniques have rapidly increased the number of drugs recognized as CYP2B6 substrates. These developments reflect both an increased research interest in this enzyme and an increase in phenotyping studies which include recombinant CYP2B6. In 1999, Ekins and Wrighton [11] published the first comprehensive review of CYP2B6 in xenobiotic metabolism and summarized over 50 chemicals known to be metabolized by CYP2B6. Since then, a large number of CYP2B6 substrates including clinically used therapeutics, recreational drugs, endogenous chemicals, pesticides and environmental chemicals have been identified (reviewed in [42,43]). Although CYP2B6 shares an overlapping substrate spectrum with other CYP enzymes including CYP3A4, a distinct affinity for specific drug substrates and unique enzymatic biotransformation reactions distinguishes its pharmacotoxicological significance. The use of human liver microsomes and recombinant CYP isoforms identified CYP2B6 as the sole enzyme responsible for the N-demethylation of the antiepileptic mephobarbital, while identifying CYP2Cs responsible for the 4-hydroxylation [44,45]. Extensive studies by Kupfer and colleagues have revealed that CYP2B6 is the only CYP enzyme to catalyze both O-demethylation and ortho-hydroxylation of the endocrine disrupter methoxychlor, while other CYPs are only capable of one metabolic reaction [46-48]. In addition, CYP2B6 has been demonstrated to be predominantly responsible for the biotransformation of clinically important pharmaceuticals, such as bupropion, artemisinin, propofol, methadone, and pethidine [14,19, 49-52]. An updated list of drugs metabolized, at least in part, by CYP2B6 has been summarized in Table 1.

As the role of CYP2B6 in xenobiotic metabolism increases, so does the likelihood of CYP2B6 mediated drug-drug interactions. These newly identified substrates for CYP2B6 have been evaluated for specificity and potency in order to identify an *in vivo* probe. Bupropion is generally accepted as the traditional CYP2B6 probe, however there are documented observations of non-CYP2B6 mediated metabolism of this substrate [53,54]. In fact, it is not unusual for CYP2B6 substrates to also be substrates for other CYP isoforms—most commonly CYP3A4, CYP2C9 and CYP2C19. A specific metabolite or difference in affinity is what separates the CYP2B6-mediated catalysis from that of other CYP isoforms. For example, bupropion hydroxylation is specific to CYP2B6 and occurs at low concentrations of substrate, while hydrometabolites are generated by other enzymes at much higher concentrations [14, 19]. Recently, the metabolism of bupropion by CYP2B6 has also been shown to be stereo-selective. Bupropion is used clinically as a racemic mixture of R- and S-enantiomers and CYP2B6 selectively catalyzes the hydroxylation of (S)-bupropion over that of (R)-bupropion. Given the documented limitations of bupropion as an *in vivo* probe for CYP2B6 activity, the stereo-specific catalysis allowed researchers to identify the (S,S)-hydroxybupropion metabolite as a more specific *in vivo* probe [55]. Nevertheless, stereo-selective catalysis by CYP2B6 is not unique to bupropion metabolism. Methadone is another clinically-important drug given as a racemic mixture and CYP2B6 metabolism is stereo-selective toward the S-enantiomer. (R)-methadone mediates the narcotic effect by activating the μ -opioid receptor, but (S)-methadone inhibits the cardiac potassium channel, contributing to the cardiac side effects of methadone treatment [56]. Investigating these stereo-specific behaviors, researchers discovered that decreased CYP2B6 activity was associated with high plasma concentrations of (S)- but not (R)-methadone and a greater risk of cardiac side effects and death [56]. The stereo-selective behavior of CYP2B6 has real probative or clinical consequences during treatment with bupropion or methadone, respectively, suggesting that other chiral substrates of CYP2B6 catalysis could exhibit similar stereo-specific outcomes.

Although the exact percentage of drugs metabolized by CYP2B6 is not known currently, it exceeds earlier estimates. As recently as 2001, CYP2B6 was listed as only contributing <1% of CYP-mediated drug metabolism [57], yet, more recent publications recognize that the rising numbers of clinically-relevant CYP2B6 substrates considerably exceed and contradict those previous estimations. Several publications cite an estimate of the CYP2B6 contribution to drug metabolism at ~8%, meanwhile Xie and Evans [58] suggested that CYP2B6 is capable of metabolizing 25-30% of known clinical drug substrates for CYP3A4 [55,59,60]. More substantially, a comprehensive summary published in 2002 organized metabolism data for over 1300 substances (including drugs and other compounds) on all relevant CYP enzymes, including CYP2B6; from which we calculated a CYP2B6 contribution to drug metabolism of 7.6% [61]. While these 1300 substrates may not contain all drugs of interest, it's a useful cross-section to generate documented estimates of CYP2B6, which till now have been lacking. Based on all recent data, we present an updated evaluation of major CYPs by their relative hepatic expression and relevant contribution to drug metabolism, with specific emphasis in CYP2B6 [Fig. (1)]. Indeed, mounting evidence thus far indicates that CYP2B6, an isozyme expressed at a relatively low, yet variable, level functionally plays a significant role in the biotransformation of clinically-relevant drugs. These characteristics of CYP2B6 suggest a similarity to CYP2D6, which is only modestly expressed in liver (<4%) but involved the metabolism of over 25% clinically used drugs [62].

3. Inhibition of CYP2B6

Inhibition of drug metabolizing enzymes is one of the major concerns in both clinical practice and drug development. Although CYP inhibitors have clinical significance in metabolism-mediated drug-drug interactions in general, identification of selective and potent chemical inhibitors are extremely important for delineating the specific roles of particular CYPs in metabolism/detoxification of therapeutics and environmental toxicants. Based on known species-specific CYP activation, several selective rodent CYP2B inhibitors identified previously, such as orphenadrine, were shown to exert nonspecific CYP inhibition when applied to human *in vitro* studies. This emphasizes the limitation of direct extrapolation of animal data to humans. While the previous search for chemical inhibitors of CYP2B6 may have been biased toward pharmaceutical drugs because of their potential therapeutic use, some environmental chemicals have been discovered as potent CYP2B6 inhibitors. For instance, chlorpyrifos is the most widely used organophosphate insecticide and a potent inhibitor of CYP2B6 enzymatic activity [63]. In exposed populations, this inhibition may potentiate the occurrence of harmful pesticide-drug interactions.

Recently, a comprehensive approach was taken to investigate 227 structurally diverse compounds for their ability to inhibit CYP2B6 enzymatic activity [60]. Thirty out of the 227 compounds displayed 50% or greater inhibition of CYP2B6 at the concentration of 30 μ M, while most lacked any inhibitory potential. Among these inhibitors, six drugs exhibited potent inhibition of recombinant human CYP2B6 activity with IC₅₀ values lower than 1 μ M. Specifically, the anti-clotting agents, clopidogrel and ticlopidine were identified among the most potent inhibitors of CYP2B6 activity [60]. In order for CYP2B6 inhibitors to be effective as research tools, candidate compounds need to be specific as well as potent. In a recent publication, the selectivity of four known inhibitors of CYP2B6 activity [2-phenyl-2-(1-piperidinyl)propane (PPP), thioTEPA, clopidogrel and ticlopidine] was analyzed to evaluate the utility of each as a phenotyping tool. ThioTEPA demonstrated a minimal selectivity that rendered it insufficient to be used for phenotyping, while PPP, clopidogrel and ticlopidine displayed significant selectivity and the anti-clotting agents possessed a greater potency. The authors concluded PPP served as a selective probe for CYP2B6 inhibition, but restricted its use to *in vitro* studies only, as PPP is not approved for human administration. The next selective inhibitors, clopidogrel and ticlopidine, already approved as clinical agents, could be used in

clinical studies under circumstances that promote selectivity [64]. Most of the CYP2B6 inhibitors known to date are identified utilizing *in vitro* enzyme incubations but, these data alone cannot dictate the potential magnitude of *in vivo* CYP2B6 related drug-drug interactions. Ultimately, these studies provide the most likely drug candidates for further clinical investigation.

CYP2B6 Induction and Transcriptional Regulation

Hepatic CYP2B genes represent the most inducible CYP isoforms by PB-type compounds in most mammalian species. Rodent CYP2B enzymes have been studied extensively as prototypical models for the well-known PB-mediated CYP induction phenomenon [65,66]. Comparatively, it is only in recent years that a growing numbers of reports regarding the potency and magnitude of human CYP2B6 induction became available. The regulation of CYP2B6 transcription has been recognized as one of the major contributors for the observed dramatic interindividual variations in this isozyme. Clinical consequences of elevated CYP2B6 expression both beneficial and detrimental have increased the pharmacologic importance of this CYP isozyme.

1. CYP2B6 inducers

Although PB has been realized as a potent CYP2B inducer in multiple species including rodents and humans, significant species-specific differences in the regulation and substrate specificity of the mammalian CYP enzymes have hindered the direct extrapolation of data obtained from animal to man. For instance, 1,4-bis[3,5-dichloropyridyloxy]benzene (TCPOBOP) and pregnenolone 16 alpha-carbonitrile (PCN) are potent CYP2B and CYP3A inducers in rodent but not in humans. Conversely, 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehydeO-(3,4-dichlorobenzyl)-oxime (CITCO) and RIF induce human CYP2B6 and CYP3A4, but not their rodent counterparts [67-70]. The lack of physiologically relevant CYP expression in most immortalized cell lines prevents their use in metabolic studies or in identifying human specific CYP inducers. However, the establishment of human primary hepatocyte cultures has provided an *in vitro* tool that mimics the expression and inducibility of *in vivo* conditions. Primary human hepatocytes have become the gold-standard model for the *in vitro* testing of drugs and their metabolic fate [71]. In human primary hepatocyte cultures, CYP2B6 is well maintained and robustly induced by known CYP2B inducers, such PB. In some individuals, the elevated CYP2B6 protein content even challenges that of CYP3A4, known to be the most abundantly expressed of CYP isoforms. Using this *in vitro* model, several typical CYP3A4 inducers such as RIF and dexamethasone (DEX), have shown the capability to induce CYP2B6 expression [21,23]. Recently, we have evaluated a total of thirty commercially available compounds including weak, moderate and potent inducers of CYP3A4 for their ability to induce CYP2B6 expression in two separate studies [72,73]. Interestingly, the majority of the CYP3A4 inducers displayed efficacious dose-dependent induction of CYP2B6, indicating these two enzymes may share overlapping regulatory mechanisms.

Because CYP2B6 shares mechanisms of transcriptional regulation with several other important drug metabolizing genes, a large number of CYP2B6 inducers have also been shown to induce CYP3A4, UGT1A1, and several drug transporters, as well. For example, known CYP2B6 inducers, PB, RIF, clotrimazole (CLZ), phenytoin (PHN), and carbamazepine (CMZ) induce a pleiotropic set of drug metabolizing gene products including CYP3A4, CYP2C9, UGT1A1, and efflux drug transporter MDR1 [74-77]. Among CYP2B6 inducers, some are also substrates for CYP2B6 and therefore accelerate their own metabolism affecting clearance or toxicity. Examples of auto-induction include the widely used anticancer therapeutics CPA and ifosfamide, the non-nucleoside reverse transcriptase inhibitor efavirenz (EFV), and the antimalarial artemisinin [73,78-80]. Alternatively, other drugs such as RIF increase the

biotransformation of co-administered substrates without significantly affecting their own metabolic fate.

Recently, several studies have focused on the CYP2B6 expression in extrahepatic tissues, such as brain. CYP2B6 expression in the brain of the African Green monkey, which shares 91% amino acid identity with human CYP2B6, was induced by the antidepressant/smoking cessation agent, bupropion. However, CYP2B6 expression in the liver was unaffected, suggesting that CYP2B6 expression might be regulated by tissue-specific mechanisms [81]. In addition, CYP2B6 expression is inducible under stressed conditions such as fasting or energy restriction [82-85]. The use of nutritional restriction as a selective pressure gave rise to a novel HepG2-derived cell line. Functional analysis of this new cell line revealed that CYP2B6 was efficiently induced by PB treatment and by activation of the energy-sensing AMPK pathway, although similar inductions are absent in the parent HepG2 cells [84]. Therefore, it is reasonable to speculate that changes in CYP2B6 expression under these stress conditions may suggest an involvement of CYP2B6 in pathways of energy metabolism, homeostasis, and the metabolism of endobiotics; although direct evidence for such involvement has yet to be identified.

2. Nuclear receptor-mediated regulation of CYP2B6

Xenobiotic induction of CYP2B genes in many species has been realized for a long time. However, substantial progress in our understanding of the underlying mechanisms of this induction has only been achieved during the past ten years. Transfecting the 5'-flanking promoter region of CYP2B1/2 into intact rat liver or hepatocytes demonstrated PB-mediated induction of CYP2B expression and promoter deletion experiments delineated this induction to a 163 bp DNA sequence located from -2155 to -2318 bp relative to the CYP2B1/2 transcriptional start site [86,87]. This sequence, termed the PB-responsive element (PBRE) or PB-responsive unit (PBRU), also conferred PB induction of reporter gene expression, regardless of its orientation to the reporter promoter, identifying the PBRE/PBRU as a functional enhancer element. Similar studies conducted with primary mouse hepatocytes delineated PB responsiveness to a minimum sequence of 51 bp at -2339 through -2289 of the *Cyp2b10* promoter, termed the phenobarbital-responsive enhancer module (PBREM) [88]. Subsequently, the PBREM sequence was discovered in the rat *CYP2B1*, *CYP2B2*, and human *CYP2B6* genes, demonstrating a highly conserved sequence [88-90]. Sequence analysis of the PBREM revealed that this element was composed of a nuclear factor 1 (NF1) binding site and two nuclear receptor-binding sites (NR1 and NR2), with each conforming to imperfect direct repeat (DR4) motifs of the AGGTCA hexameric sequence [88]. Sequence comparison of the PBREM across species revealed that the NR1 site is the most conserved of the three elements. These findings suggested that the NR1 site is, quantitatively, the most important motif within the PBREM for mediating CYP2B induction by PB-type inducers [23,88,89]. Using *in vitro* cell-based reporter assays, the nuclear receptors RXR, CAR, LXR, thyroid receptor (TR), HNF4, and COUP-TF were screened for their ability to transactivate mouse PBREM in HepG2 cells. These results lead to the identification of CAR as the first nuclear receptor able to activate PBREM-driven reporter gene expression [88]. Subsequent work demonstrated that CAR bound to the NR1 and NR2 motifs as a heterodimer with RXR and NR1-affinity chromatography demonstrated that CAR-RXR binding to NR1 increased after PB treatment. In mouse hepatocytes and HepG2 cells, heterologous reporter constructs containing the *Cyp2b10* or CYP2B6 PBREM were activated by a large number of compounds reported to induce CYP2B genes in rodents, including PB, TCPOBOP, CLZ, chlorpromazine, and metyrapone [89,91]. These compounds also induced endogenous expression of *Cyp2b10* in mouse hepatocytes and CYP2B6 in CAR-transfected HepG2 cells. Transfection assays using reporter plasmids containing the NR1 site revealed that binding of CAR-RXR to the NR1 site alone was sufficient to activate PBREM. This was substantiated by the finding that CAR-RXR bound to the NR1 motif with greater affinity than the NR2 motif [23]. The definitive association of

CAR and CYP2B induction in response to PB-type compounds was established by demonstrating loss of Cyp2b10 induction by PB and TCPOBOP in CAR-null mice [92].

In addition to CAR, PXR has been implicated in CYP2B regulation. Several studies have shown that both mouse and human PXR have the ability to bind to the putative PBREM in the CYP2B promoter and activate the human PBREM and mouse Cyp2b10 promoter [23,93]. Similar to CAR, the human PXR-RXR heterodimer bound the NR1 site of human PBREM with greater affinity than the NR2 site [23]. In animal studies, PXR ligands, DEX and PCN significantly enhance the expression of Cyp2b10 in mice, and PXR-null mice lose that PCN-mediated Cyp2b10 induction [94,95]. Overall, these data suggest PXR is capable of regulating CYP2B expression, but the outcome of CYP2B induction is likely co-regulated by both PXR and CAR.

2.1. Human CAR and PXR mediated CYP2B6 expression—Substantial progress on the molecular mechanisms underlying CAR regulation of *CYP2B* gene expression has been achieved in rodents, particularly in mice and rats. However, information pertaining to hCAR regulation of CYP2B6 is relatively limited. At present, the majority of our understanding of hCAR regulation of CYP2B6 has been extrapolated from data obtained with rodent CAR. Although human CAR exhibits some common characteristics with its rodent counterparts, such as nuclear translocation after PB treatment, binding to the PBREM, and activation by either direct ligand binding or indirect mechanisms, there are distinct biochemical and biological differences between rodent CAR and hCAR. For example, TCPOBOP can bind and activate mCAR but not hCAR; CLZ can deactivate hCAR but has no effect on mCAR; and the androstane metabolites, androstanol and androstenol repress the constitutive activity of mCAR, but not hCAR [96-98]. In addition, the prototypical CYP2B inducer PB, which selectively activates mCAR but not mouse PXR, is a potent activator of both hCAR and hPXR [97,99]. Because of documented species-specific differences, it is difficult to extrapolate data describing rodent CAR regulatory mechanisms to human CYP2B6 induction.

In addition to hCAR, mounting evidence also suggests that hPXR may be a key receptor in the regulation of human *CYP2B6* gene expression [22,23,78,100]. hPXR ligands such as RIF, troglitazone, CLZ, hyperforin, and SR12813 have been shown to induce CYP2B6 transcription along with CYP3A4 [23,75,101]. CYP2B6 induction is highly correlated with the activation of hPXR for most compounds [73]. Among the efficacious CYP2B6 inducers evaluated to date, most activate hPXR but not hCAR, such as RIF and CLZ (CLZ was even reported as an hCAR deactivator by Moore et al [102]), whereas others can activate both hPXR and hCAR, such as PB. Recently, several relatively specific hCAR activators have been identified such as CITCO, PHN, EFV, nevirapine, carbamazepine, and bilirubin [70,73,99,103]. Together, this diverse group of compounds provides further evidence of the varied chemical properties of CYP2B6 inducers.

As the result of evaluating a total of 30 known CYP3A4 and/or CYP2B6 inducers in two separate studies, we noticed that hCAR is less flexible in its interactions with xenobiotics, when compared with the highly-promiscuous hPXR [73,101]. This observation is supported by the recently characterized crystal structure for the CAR ligand binding domain, which revealed the volume of the hCAR ligand binding pocket ($675 \text{ \AA}^3$) to be remarkably smaller than that of hPXR ($1290\text{-}1540 \text{ \AA}^3$) [104-107]. Despite these observations, there is still considerable substrate overlap between hCAR and hPXR and an apparent substrate-specific preference for relative induction of CYP2B and CYP3A. Activators of hPXR are known to induce both CYP2B6 and CYP3A4 with little bias; however we have observed that hCAR-selective activators exhibit preferred induction of CYP2B6 over CYP3A4. Studies utilizing *in vitro* EMSA and cell-based reporter assay demonstrated that while hPXR bound both DR4 from CYP2B6 and ER6 of CYP3A4 similarly, hCAR bound DR4 with much higher affinity than ER6 [101]. This binding preference of hCAR was further confirmed by the more

physiologically-relevant chromatin co-immunoprecipitation (ChIP) assay [Fig. (2)]. Together, these data support that the observed differential induction of CYP3A4 versus CYP2B6 by hCAR is governed by the preferential binding of hCAR to DR4 motifs in the CYP2B6 promoter compared with ER6 elements in the CYP3A4 promoter.

2.2. *In vitro* activation of human CAR—The limited numbers of recognized hCAR activators can be attributed to the lack of an efficient *in vitro* cellular system as a model for the hCAR activation. Furthermore, the activation mechanisms of CAR, which include both, direct ligand binding and indirect activation, decrease the utility of an *in vitro* binding assay. To date, accumulated evidence reveals two different mechanisms in the CAR-mediated induction of *CYP2B* genes by PB-type chemicals, including nuclear translocation and receptor activation in the nucleus [108,109]. In immortalized cell lines, CAR exhibits a high constitutive activity in the absence of ligand where it spontaneously accumulates in the nucleus [110]. However, in primary hepatocytes or intact liver, CAR is localized predominantly in the cytoplasm in the absence of inducers and translocates to the nucleus only upon exposure to xenobiotics [110]. Thus, retention of CAR in the cytoplasm provides a mechanism to prevent activation of *CYP2B* genes in the absence of inducers, but nuclear translocation of CAR does not always result in CAR activation and subsequent CYP2B induction. We observed that CLZ, a known hCAR deactivator and antagonist, efficiently translocates YFP-tagged hCAR from the cytoplasm into the nucleus in mCAR-null mice [Fig. (3)]. In addition, the PPAR activator, WY-146 also causes accumulation of mCAR in the nucleus without activation of this receptor [111]. Collectively, these data further illustrate the complexity of the mechanisms underlying CAR activation. Potential mechanisms involved in the cellular localization and nuclear trafficking of CAR has been proposed and intensively investigated. Several important discoveries in deciphering the molecular mechanisms of CAR activation have been documented in recent review articles [112-115].

Recently, a number of mRNA splice variants of the human CAR gene have been identified by several laboratories [116-118]. One of these variants, hCAR3 contains a 5 amino acid insertion (APYLT) between exon 7 and exon 8 within the ligand binding domain and was found to be prominently expressed in all tested human livers [119]. Interestingly, functional analysis revealed hCAR3 lacks the high basal activation in immortalized cell lines, but responds robustly to known hCAR agonist, CITCO [120]. The activation of CAR3 was further investigated in HepG2 cells by utilizing known hCAR activators, as well as 16 known CYP3A4 and/or CYP2B6 inducers [73]. Notably, all the known hCAR activators including CITCO, PB, PHN, EFV, nevirapine (NVP), and CMZ efficiently activated hCAR3, while hPXR activator, RIF showed no activation, indicating that the hCAR3 cell-based reporter assay may represent a novel avenue for predicting *in vitro* hCAR activation in a high throughput manner.

2.3. Responsive elements in CYP2B6 promoter—Although the PBREM is identified as the key promoter element mediating transcriptional activation of CYP2B genes in multiple species, a noticeable discrepancy observed between the robust CYP2B6 induction in human primary hepatocyte cultures and the relatively weak activation of PBREM by PXR and CAR in cell-based reporter assays in response to the same compounds suggests the importance of other regulatory elements. Additionally, hCAR target genes are not all induced to the same extent following hCAR activation; as CYP2B6 exhibits a greater induction compared to several other target genes such as CYP2C9 and UGT1A1 [77,121]. Together, these observations indicate that responsive elements other than PBREM may exist in the promoter region of the CYP2B6 gene and together with the PBREM are responsible for the maximum induction of CYP2B6 expression.

A computer-based search of the -20 kb upstream promoter region of CYP2B6 gene was performed and led to the identification of a XREM-like remote module located approximately

-8.5kb from the transcriptional start site of CYP2B6 [78]. This distal module contains a cluster of response elements including perfect and imperfect half-sites of the hexameric sequence AGGTCA organized as direct repeats (DR), everted repeats (ER), and inverted repeats (IR). Among the identified elements, the NR3 element (an imperfect DR4) exhibited only one base pair difference compared to the known NR1 and NR2 elements within the PBREM, and was unique to the entire -20kb region. PXR and CAR demonstrated an ability to activate the CYP2B6 distal XREM promoter in a ligand-dependent and independent manner, respectively and electrophoretic mobility shift assay (EMSA) revealed only the NR3 motif in the distal XREM specifically bound to PXR/RXR and CAR/RXR heterodimers. Site-directed mutagenesis of the distal XREM indicated the NR3 motif, alone, appears to be sufficient to confer CAR- and PXR-mediated reporter activation. Evaluation of a new promoter construct containing both the PBREM and the distal XREM displayed additive reporter activation in cell-based reporter assays, but exhibited synergistic reporter activation in PB-treated mice [78].

More recently, HepG2 cells stably-transfected with mouse CAR (Ym17) showed an unexpectedly robust synergistic induction of CYP2B6 gene by the co-treatment of TCPOBOP and okadaic acid (OA) [122]. Further experiments delineated this CAR-dependent synergistic effect to a 24-base pair sequence (-256/-233) named okadaic acid responsive element (OARE) in the CYP2B6 promoter. Although CAR did not directly bind to the OARE, chromatin immunoprecipitation and DNA affinity chromatography assays revealed that TCPOBOP-activated mCAR was indirectly associated with OARE; suggesting that in addition to direct binding of PBREM and XREM, CAR may also activate transcription via interactions with other factors [122,123]. Taken together, these newly identified distal and proximal elements enhance our appreciation of the intricate and multi-faceted induction of CYP2B6 expression in human. Deciphering the interaction between these distal and proximal modules *in vivo* will help our understanding of the dramatic interindividual variation of CYP2B6 expression.

2.4. GR in CYP2B6 induction—The role of the GR on the regulation of CYP2Bs has been a controversial and long-debated issue. Putative glucocorticoid responsive elements (GREs) were identified in mouse Cyp2b10 and rat CYP2B1/2 promoters, and the rat CYP2B2 element was activated by the synthetic glucocorticoid, DEX [67,90,124]. The consensus GRE in the rat *CYP2B2* gene was identified as part of the PBRU in the 5'-flanking region [90]. There are also indications that GR is required for maximal TCPOBOP induction of Cyp2b10 in mice, as submicromolar concentrations of DEX enhanced Cyp2b10 induction by TCPOBOP [94]. In addition, the GR antagonist RU486 blocked *in vivo* induction of Cyp2b10 by DEX in male mice and induction of rat CYP2B1/2 reporter constructs by PB in hepatoma cells [125,126]. Furthermore, GR-deficient mice showed no induction of Cyp2b10 in response to DEX and PCN and showed an attenuated induction in response to PB [98]. However, it is not clear whether CYP2B6 induction in humans is dependent upon GR. In contrast to the rat CYP2B2 promoter, the CYP2B6 promoter does not contain a functional GRE. CYP2B6 reporter activity was not induced in the presence of known inducers when GR alone was transfected with a PBREM reporter construct, suggesting GR does not play a direct role in CYP2B6 induction [75]. Nevertheless, an indirect role for human GR in CYP2B6 induction has been suggested by the ability of DEX-activated GR to increase expression of PXR and CAR in human hepatocytes [21,127]. Although submicromolar or micromolar concentrations of DEX are unable to induce CYP2B6 expression in primary human hepatocytes, similar concentrations of DEX can potentiate endogenous CYP2B6 induction and promoter activation by PXR activators such as PB in human hepatocytes and Huh7 cells, respectively [21,22,75]. In addition to increasing PXR and CAR expression, potential mechanisms underlying this interaction might include GR-mediated recruitment of specific coactivator proteins or facilitation of PXR and CAR binding to the CYP2B6 PBREM and XREM via protein-protein interactions [128].

CYP2B6 Gene Polymorphisms

Compared to the well established polymorphic CYPs such as CYP2D6 and CYP2C19, the genetic variations of CYP2B6 and its potential clinical significance have only been realized in recent years. Nevertheless, along with awareness of the remarkable interindividual variations in CYP2B6 expression and activity, the number of pharmacogenetic studies on this isozyme has been growing rapidly. To date, 28 characterized alleles, over 50 determined haplotypes and more than 100 described SNPs of the *CYP2B6* gene have been documented and are listed on the CYP allele nomenclature website (<http://www.cypalleles.ki.se>). Functional characterization has revealed a variety of phenotypic outcomes from these polymorphic alleles including significantly decreased catalytic activity or complete loss of CYP2B6 expression [16,129]. Genetic variations of CYP2B6 have been identified throughout the gene sequence including the 5' promoter, introns, and the coding region. Although the majority of identified CYP2B6 polymorphic SNPs do not encode functional changes and are relatively rare, several important SNPs have been identified that exhibit higher frequencies, up to 50% in certain populations, and exert significant impact on the pharmacokinetics of several important CYP2B6 substrate drugs such as the HIV reverse transcriptase inhibitor EFV and anticancer prodrug CPA [130-132].

1. SNPs in coding regions

The first systematic analysis of genetic polymorphism in CYP2B6 focused on the nine exons of the coding region [16]. This study led to the identification of nine single base mutations, of which five are nonsynonymous amino acid changes and four are silent mutations. Alone or in combination, these SNPs resulted in six different CYP2B6 alleles designated as CYP2B6*2 (64C>T), CYP2B6*3 (777C>A), CYP2B6*4 (785A>G), CYP2B6*5 (1459C>T), CYP2B6*6 (516G>T and 785A>G), and CYP2B6*7 (516G>T, 785A>G and 1459C>T). Among these alleles, CYP2B6*6, characterized with the combination of 516G>T in exon 4 and 785A>G in exon 5, was detected in about 15 - 40% of Asians and over 50% of African-Americans [16, 133,134]. Analysis of human liver mRNA discovered that aberrant splicing of the CYP2B6 gene resulted in the 2B6*6 allele which lacks exon 4, 5 and 6 and thus displays a reduced function of mRNA and protein [135]. Both heterozygous and homozygous carriers of CYP2B6*6 allele displays remarkably lower catalytic activity and a significant decrease in protein expression, compared with wild-type CYP2B6*1 allele carriers. CYP2B6*6 has been associated with aberrant EFV metabolism resulting in significantly elevated plasma concentrations and therapeutic toxicities. Another polymorphic allele, CYP2B6*5 detected in 14-25% of Caucasians, results in a significant decrease in protein expression and catalytic activity but only in female livers [15,16].

Another 19 alleles in the coding region have been defined through the dedicated works of several independent research groups [15,17,136]. Despite all alleles resulting in amino acid changes, the functional consequences of each on CYP2B6 expression/function varied extensively. A recent observation indicated that inclusion of the less common loss-of-function alleles greatly enhances the predictive value of CYP2B6 polymorphisms, specifically for elevated EFV plasma levels [137]. Novel alleles have been characterized such as CYP2B6*16 (785A>G and 983T>C), *18 (983T>C), *27 (593T>C), and *28 (1132C>T), as well as four phenotypic null alleles *8 (415A>G), *11 (136A>G), *12 (296G>A), and *15 (1172T>A) (<http://www.cypalleles.ki.se/cyp2b6.htm>). The signature changes of these nonsynonymous mutations are located in the coding region, but CYP2B6 expression and activity are not equally affected in these alleles. For instance, 2B6*6 was associated with greater reduction of S-mephenytoin N-demethylase activity compared with a modest decrease of protein expression, while 2B6*5 displayed significantly lower protein expression compared to only a slight reduction in catalytic activity [16,132]. Even though the underlying mechanisms for these

polymorphic phenotypes are largely unknown, it may be important to consider: 1) unknown co-existing SNPs in the promoter region or introns may affect gene expression; 2) some nonsynonymous mutations may modify the enzyme active center therefore resulting in substrate-specific changes; and 3) disease condition and preliminary drug treatment may be a potential confounding factor. Although the collective frequency of these alleles was relatively low, the clinical impact, whether individual or collective, could be significant in certain populations.

2. SNPs in non-coding regions

In addition to the coding regions, SNPs in DNA regulatory sequences and introns also have an impact on gene expression. Given the fact that CYP2B6 is a highly inducible isozyme which was regulated primarily at the transcriptional level, identification of SNPs in the 5'-flanking promoter has been investigated by several research groups [15,138,139]. Initially, Lamba et al., sequenced a -3.8 kb of the 5'-flanking promoter region that included the PBREM, and a distal promoter region which contains the XREM from a total of 80 human liver samples [15]. This study led to the discovery of nine SNPs occurring at more than 1% in the studied ethnic populations. Among these SNPs, -750T>C substitution occurred at the highest frequencies of 49% in Caucasians, 83% in African-Americans and 79%, in Hispanics. Although the -750T>C mutation is located in a putative HNF1 site, functional characterization revealed that alleles associated with this mutation only resulted in moderate or negligible changes in protein and mRNA expression [15]. Recently, a novel haplotype 2B6*22 which combined 4 promoter mutations (-1848C>A, -801G>T, -750T>C, and -82T>C), has been identified from 98 Caucasian DNA samples and demonstrated an enhanced transcriptional activation up to 9-fold compared with the reference CYP2B6 promoter [138]. The 2B6*22 allele illustrates that promoter polymorphisms could be a novel mechanism by which mutations can enhance transcription. Further functional examination revealed that the -82T>C mutation converted a putative TATA box into a functional CCAAT/enhancer-binding protein binding site, which alone is responsible for the 2B6*22 enhanced activation. The clinical relevance of this gain-of-function mutation in the promoter remains to be seen, but has the potential to contribute to an "ultrarapid metabolizer" phenotype of CYP2B6. With respect to CYP2B6 expression, the search for SNPs has yet to identify a polymorphism in the PBREM or XREM, which are responsible for the majority of xenobiotic induced CYP2B6 expression.

Clinical Relevance of CYP2B6

It has recently been discovered that CYP2B6 plays an critical role in the biotransformation of several clinically important drugs, including CPA, EFV, ifosfamide, tamoxifen, nevirapine, and artemisinin [49,140-142]. The clinical importance of enzyme inhibition, altered expression and polymorphic alleles in regards to CYP2B6 can be exemplified effectively with two clinically-important compounds, EFV and CPA. Both pharmaceuticals have narrow therapeutic indices, dose-limiting toxicities and large interindividual variations in plasma drug concentrations.

The antiretroviral EFV is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and part of the combination therapy for HIV patients. Pharmacokinetic analysis revealed that EFV has a relatively narrow therapeutic index and its plasma concentration displays dramatic interindividual variations [143,144]. Using human liver microsomes and recombinant CYPs, Ward et al. [145], were the first to document that CYP2B6 was the enzyme primarily responsible for the metabolism of EFV and identified 8-hydroxy-EFV and 8,14-dihydroxy-EFV as primary metabolites. Thereafter, pharmacogenetic studies have become focused on deciphering the relevance between polymorphic CYP2B6 alleles and the known variations in EFV plasma concentrations. Elevated plasma concentrations of EFV are responsible for CNS-related side effects such as difficulty sleeping, dizziness and depression and contribute to the

high discontinuation rate and eventual therapeutic failure [146]. Among the identified polymorphic alleles, recent evidence confirms that CYP2B6*6, predominantly the 516G>T mutation, is strongly associated with elevated EFV plasma concentration and the appearance of CNS-related side-effects [147-149].

The considerable frequency of the 2B6*6 allelic expression in a variety of distinct ethnic populations allows for CYP2B6 genotyping to be used as a tool for EFV dose determination. For example, 2B6*6 occurs at relatively high frequencies in populations of West Africans and Papua New Guineans where HIV infection has become a significant health concern, and identification of the “slower metabolizer” phenotype would greatly benefit EFV dose adjustment and alleviate drug toxicity in clinical practice. Genotype-specific dosing of EFV, in a Japanese cohort study, led to a decrease in EFV dose by 33% or 66% and a decrease in plasma concentrations proportionate to the reduction in dose; all while maintaining therapeutic effectiveness and improving CNS-related symptoms [150].

Although the role of CYP2B6 polymorphisms in EFV metabolism has been firmly established, evidence regarding drug-drug interactions between EFV and concomitant therapeutics is limited. By design and practice, this is a drug used in combination therapy, frequently in addition to herbal or nutritional supplements and in conjunction with other drugs for treatment of opportunistic infections and complications of HIV-infected patients [145]. Because hepatic CYP2B6 is highly inducible by a broad array of clinical drugs including EFV itself, the likelihood of induction-related interactions and EFV therapeutic failure is of concern.

CPA is another important drug whose therapeutic outcome is significantly affected by CYP2B6. CPA is an alkylating agent widely used in the treatment of breast cancer, sarcomas, and leukemia/lymphomas [151,152]. CPA is a prodrug which requires activation to the 4-hydroxy metabolite, predominantly via CYP2B6, which ultimately produces the DNA-alkylating, phosphoramidate mustard causing therapeutic cytotoxicity [153]. Alternatively, CPA is subject to inactivation exclusively by CYP3A4-mediated N-dechloroethylation yielding the neurotoxic metabolite, chloroacetaldehyde [154]. It is the production of the chloroacetaldehyde metabolite that limits the use of CPA and narrows its therapeutic index. As such, selective activation of CYP2B6 or inhibition of CYP3A4 could greatly enhance the formation of the therapeutically beneficial 4-hydroxy-CPA metabolite without simultaneously increasing the rate of the toxic N-dechloroethylation.

The relationship between CYP2B6 expression and the therapeutic potential of CPA was demonstrated recently by Waxman and colleagues [155], who reported that delivery of an adenovirus encoding a CYP2B6 expression cassette into a number of human cancer cell lines resulted in increased intracellular CPA 4-hydroxylation and increased cytotoxicity. Recently, we have demonstrated that several selective hCAR activators preferentially induce CYP2B6 rather than CYP3A4 in human primary hepatocyte cultures [73,101]. Accordingly, if hCAR selectivity for CYP2B6 is also true *in vivo*, co-administration of CPA with a selective hCAR activator should facilitate enhanced production of its 4-hydroxy metabolite without simultaneously increasing formation of the chloroacetaldehyde metabolite. This observation provides the opportunity to develop therapeutic regimens that optimize beneficial versus detrimental toxic effects of CPA in cancer treatment.

Significant research progress has been made to enhance the therapeutic potential of CPA by forced CYP2B6 expression [155] or altered amino acid composition [156], but only recently have researchers investigated the effect of CYP2B6 polymorphisms on CPA biotransformation. Following CPA treatment, patients display significant differences in metabolism causing up to a 10-fold difference in exposure to CPA metabolites across patients [157]. The CYP2B6*6 allele, more specifically the 516G>T mutations, has been correlated to

an increase in enzymatic activity, higher substrate clearance and a decreased half-life of CPA [158,159]. In addition, a number of other characterized SNPs in the promoter and introns have been found to be significantly related to decreased CPA 4-hydroxylation and the appearance of adverse side-effects, such as leukocytopenia [158]. While few are known, the effects of CYP2B6 polymorphisms on CPA metabolism and efficacy appear to be in contrast to the influence of CYP2B6 polymorphic alleles on EFV metabolism, suggesting that functional changes resulting from specific allele expression could be substrate specific.

Conclusions and Future Perspectives

The importance of CYP2B6 in drug metabolism/detoxification has been firmly established through a plethora of studies within the past 10 years. Although it was regarded historically as a minor drug-metabolizing enzyme with insignificant roles in pharmacology and toxicology, increasing evidence has demonstrated that CYP2B6 is expressed in all human livers with variable amounts and at much higher levels than previously estimated. CYP2B6 is a highly inducible CYP isozyme and the chemical-mediated induction of CYP2B6 is regulated at the transcriptional level through complex interactions of nuclear receptors such as CAR, PXR, and to a lesser extent GR and VDR. Moreover, CYP2B6 is a polymorphic enzyme that exerts significant gene-drug interactions for several clinically-important drugs such as EFV and CPA.

Although our understanding of the function of CYP2B6 in drug metabolism has increased tremendously, there are several unsolved issues yet to be answered. To date, both hPXR and hCAR have been recognized as primary transcription factors mediating chemically-induced CYP2B6 expression. However, the exact contribution of these receptors to the observed CYP2B6 induction is not clear either quantitatively or qualitatively because hPXR and hCAR have, to an extremely large extent, overlapping profiles of activators and target genes. It is expected that identification of a highly selective activator/deactivator for hPXR and hCAR would provide novel tools for deciphering the distinct role each receptor plays in the regulation of CYP2B6 expression.

The homeostatic functions of CYP2B6, including the metabolism of endogenous chemicals and the regulation of energy homeostasis have been eluded to in recent observations, but not yet fully investigated. Recently, several studies revealed that conditions of fasting, or calorie-restriction, markedly enhance the hepatic expression of Cyp2b10 in the mouse model. In addition, several anti-diabetic drugs, as well as sterol synthesis inhibitors have been shown to induce CYP2B expression, *in vitro*. Together, these data indicate that CYP2B6 may play a novel role in energy metabolism and response to stress.

Exciting new discoveries of CYP2B6 gene-drug interaction with real clinical consequences have been made from the clinical studies on CYP2B6 polymorphisms, especially for HIV antiretrovirals, such as EFV, but unfortunately, the studies on CYP2B6-mediated drug-drug interactions have been largely overlooked at these clinical stages. Given the increasing numbers of clinically used drugs identified as high affinity substrates for CYP2B6 and the highly inducible nature of CYP2B6 expression, the likelihood of undesired CYP2B6-mediated drug-drug interactions is high. A better understanding of CYP2B6 with respect to enzymatic function, gene expression, and genetic polymorphism will eventually enable more accurate prediction of metabolic outcomes to gain the highest therapeutic benefit and lowest toxic cost from drug treatment.

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Abbreviation

CYP	cytochrome P450
DEX	dexamethasone
PB	phenobarbital
RIF	rifampicin
CPA	cyclophosphamide
PXR	pregnane x receptor
CAR	

	constitutive androstane receptor
GR	glucocorticoid receptor
VDR	vitamin D receptor
NAPQI	N-acetyl-quinoneimine
PPP	2-Phenyl-2-(1-piperidiny)propane
TCPOBOP	1,4-bis(3,5-dichloropyridyloxy)benzene
PCN	pregnenolone 16 alpha-carbonitrile
CITCO	6-(4-chlorophenyl)imidazo(2,1-b)(1,3)thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime
UGT1A1	UDP-glucuronosyltransferase 1A1
CLZ	clotrimazole
PHN	phenytoin
CMZ	carbamazepine
MDR1	multi-drug resistance transporter 1
PBRE	phenobarbital-responsive element
PBRU	phenobarbital-responsive unit
PBREM	phenobarbital-responsive enhancer module
NF1	nuclear factor 1
NR1	nuclear receptor binding site 1
RXR	retinoid X receptor
LXR	liver x receptor

HNF1/HNF4

hepatocyte nuclear factor

COUP-TF

chicken ovalbumin upstream promoter-transcription factor

YFP

yellow fluorescent protein

EFV

efavirenz

NVP

nevirapine

XREM

xenobiotic-responsive enhancer module

EMSA

electrophoretic mobility shift assay

OA

okadaic acid

OARE

okadaic acid responsive element

SNP

single nucleotide polymorphism

HIV

human immunodeficiency virus

NNRTI

non-nucleoside reverse transcriptase inhibitor

CNS

central nervous system

Fig. 1. Pie charts for hepatic CYP expression and their contribution to metabolism of clinically-used drugs. (A) Relative hepatic expression of CYP content. (B) Contribution to drug metabolism. Due to substrate overlap among CYP isozymes, the total of contributions is moderately >100%.

Fig. 2.

Human CAR preferentially binds DR4 containing CYP2B6-PBREM over ER6 containing CYP3A4-PXRE. ChIP assay was performed in a V5-hCAR stable HepG2 cell line (Yh-18) [160]. (A) PCR amplification efficiency was tested by using in put DNA. (B) DNA extracted from V5-antibody or anti-mouse IgG precipitated chromatin was analyzed by PCR. DEC2 was used as a non hCAR target gene control as described previously [161].

Fig. 3.

Human CAR translocates in CAR $-/-$ mouse liver in response to CLZ. A fluorescent-hCAR (EYFP-hCAR) was introduced into CAR $-/-$ mice through tail vein injection, and mice was then treated with CLZ (90 mg/kg body weight), or DMSO, intraperitoneally. Animals were sacrificed 7 h later, and frozen liver sections were prepared for analysis by confocal microscopy as described previously [99].

Table 1

Clinically-relevant substrates of CYP2B6 enzyme

Drug Classification	CYP2B6 Substrate	Reference
Chemotherapeutics	Cyclophosphamide	[28,130,154,162]
	Ifosfamide	[153,154,162]
	Tamoxifen	[141,163]
Anti retrovirals	Efavirenz	[131,132,145]
	Nevirapine	[140,164]
Anti depressant	Bupropion	[14,19,53,54,165]
Anti epileptic	Mephobarbital	[44]
	Valproic Acid	[166]
Anti arrhythmic	Mexiletine	[167]
	Procainamide	[168]
Anti malarial	Artemisinin	[49,79,80]
Anti inflammatory	Tazofelone	[169]
	Aminopyrine	[170,171]
	Antipyrine	[172]
Anesthetics	Propofol	[50,173]
	Ketamine	[174,175]
	Pentobarbital	[176]
	Ropivacaine	[177]
	Lidocain	[178]
	Sevoflurane	[179]
Opioids	Methadone	[51,180]
	Pethidine	[52]
MAO Inhibitor (Anti Parkinsonian)	Selegiline	[4,181]
Psychotropic	Diazepam	[10,182,183]
	Temazepam	[10,182]
	Clotizazepam	[184]
	Midazolam	[10,182]
Steroids	Estrone	[185,186]
	Testosterone	[178]