Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance

Ulrich M. Zanger,1,2* and Kathrin Klein1,2

1 Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany
2 The University of Saarbrücken, Saarbrücken, Germany

INTRODUCTION

The cytochrome P450 (CYP) enzyme CYP2B6 is one of about a dozen human CYPs that are primarily involved in the biotransformation of drugs and other xenobiotics. The CYP2B6 gene and its closely related pseudogene, CYP2B7, are located in a tandem head-to-tail arrangement within a large CYP gene cluster on the long arm of chromosome 19 (Hoffman et al., 2001; Figure 1). The orthologous genes in dog, mouse, and rat are termed CYP2B11, Cyp2b10, and CYP2B1, respectively, but in contrast to other mammalian species, CYP2B6 is the only functional isozyme of its subfamily in humans (Nelson et al., 2004). Owing to the existence of extensive genetic polymorphism as well as strong inhibitors and inducers, its activity is highly variable in the population. For some clinically used drugs including the antiretroviral agents efavirenz, nevirapine, cyclophosphamide, efavirenz, ketamine, and methadone. CYP2B6 is one of the most polymorphic CYP genes in humans and variants have been shown to affect transcriptional regulation, splicing, mRNA and protein expression, and catalytic activity. Some variants appear to affect several functional levels simultaneously, thus, combined in haplotypes, leading to complex interactions between substrate-dependent and -independent mechanisms. The most common functionally deficient allele is CYP2B6*6 (I172H, K262R), which occurs at frequencies of 15 to over 60% in different populations. The allele leads to lower expression in liver due to erroneous splicing. Recent investigations suggest that the amino acid changes contribute complex substrate-dependent effects at the activity level, although data from recombinant systems used by different researchers are not well in agreement with each other. Another important variant, CYP2B6*18 (I328T), occurs predominantly in Africans (4–12%) and does not express functional protein. A large number of uncharacterized variants are currently emerging from different ethnicities in the course of the 1000 Genomes Project. The CYP2B6 polymorphism is clinically relevant for HIV-infected patients treated with the reverse transcriptase inhibitor efavirenz, but it is increasingly being recognized for other drug substrates. This review summarizes recent advances on the functional and clinical significance of CYP2B6 and its genetic polymorphism, with particular emphasis on the comparison of kinetic data obtained with different substrates for variants expressed in different recombinant expression systems.

Keywords: bupropion, cyclophosphamide, cytochrome P450, drug metabolism, drug–drug interaction, efavirenz, pharmacogenetics, pharmacogenomics

VARIABILITY OF EXPRESSION AND TRANSCRIPTIONAL REGULATION

Cytochrome P450 2B6 is primarily expressed in the liver where its contribution to the total microsomal P450 pool has been estimated several levels of gene expression from the initial mRNA transcript to splice variants (pre-mRNA splicing and mRNA expression) to altered proteins, and affect function in various ways including substrate-dependent and substrate-independent effects. Several previous reviews are available that cover the biochemical pharmacology, molecular genetics, and pharmacogenetics of this enzyme at various degrees of detail (Elkins and Wrighton, 1999; Turpeinen et al., 2006; Hodgson and Rose, 2007; Zanger et al., 2007; Wang and Tompkins, 2008; Mo et al., 2009; Turpeinen and Zanger, 2012). The purpose of this review is to summarize recent advances in areas that have an impact on variable expression of CYP2B6 and the mechanisms and impact of CYP2B6 polymorphism, as observed by various in vitro approaches as well as in in vivo studies, and to discuss their functional and clinical implications.
to be within a range of about 1–10%, with a large inter-individual variability at protein level of roughly 100-fold (see Zanger et al., 2007 for review and references therein). Although some earlier studies reported expression in only a fraction of human livers, newer studies with better antibodies found CYP2B6 to be present in all investigated human adult liver samples (Hofmann et al., 2008) while up to one-third of pediatric samples contained no detectable protein (Croom et al., 2009). In the latter study, ontogenic differences were studied in liver microsomes from 217 pediatric liver donors. Hepatic median CYP2B6 protein levels were about twofold higher in the period between birth and 30 days postnatal compared to fetal samples, and protein levels varied already over 25-fold in both of these age groups (Croom et al., 2009). Maturation effects may further depend on genotype, as suggested in a study on HIV-infected children treated with efavirenz (Sueyoshi et al., 1999; Wang et al., 2003; Fauchette et al., 2004, 2007). One of the most important factors contributing to intra- and inter-individual variability is enzyme induction, i.e., de novo protein synthesis following exposure to certain chemicals. Regulation of CYP2B gene expression represents the archetypal example of enzyme induction (Remmer et al., 1973). Human CYP2B6 is strongly inducible by several drugs including "classical" inducers such as rifampicin, phenytoin, and phenobarbital involving a so-called phenobarbital-responsive enhancer module (PRRE) at −1.7 kb of the CYP2B6 gene promoter, and a distal xenobiotics-responsive enhancer module (XEREM, −8.5 kb), to which pregnane X receptor (PXR, NR1I2) and/or constitutive androstane receptor (CAR, NR1I3) bind to mediate increased transcription (Sueyoshi et al., 1999; Wang et al., 2003; Fauchette et al., 2004, 2007). Since other CYPs are regulated by overlapping sets of nuclear receptors, CYP2B6 is often co-induced with CYP2C enzymes and CYP3A4. CYP2B6 inducers identified to date include cyclophosphamide (Corrot et al., 1999), hyperforin (Goodwin et al., 2001), artemisinin, and carba-mazepine (Oscarson et al., 2006; Desta et al., 2007), metamizole (Saussele et al., 2007; Qin et al., 2012), ritonavir (Kharasch et al., 2008), the insect repellent N,N-diethyl-m-toluamide (DEET, Das et al., 2008), statins (Redlich et al., 2010), efavirenz (Ngaimisi et al., 2010; Habtewold et al., 2011). Interestingly, in the latter study, gender influenced the inducibility of efavirenz-8-hydroxylation, which was higher in women than in the men (Ngaimisi et al., 2010). In addition to therapeutic drugs, pesticides were found to be powerful inducers of CYP2B6 and other CYPs through interaction with both PXR and CAR (Das et al., 2008). Induction of CYP2B6 and other cytochromes P450 and its clinical consequences has been reviewed by others (Pellonen et al., 2008; Mo et al., 2009).

Sex differences in liver expression have been observed in a number of studies. Females liver donors had higher amounts of CYP2B6 mRNA (3.9-fold), protein (1.7-fold), and enzyme activity (1.6-fold); compared to male subjects in a study of 80 ethnically mixed samples (Lamba et al., 2003). In a study with 235 Caucasian liver donors, female samples had 1.6-fold higher expression level of CYP2B6 mRNA; however, this difference did not translate into higher protein and activity levels and no sex difference was found when only liver donors without presurgical drug exposure were considered (Hofmann et al., 2008). Discrepancy effects of sex on pharmacokinetics of CYP2B6 substrates, which may be due to other confounders such as age or smoking status, were also found in vivo. Higher bupropion hydroxylation rates were found in adolescent females compared to males (Stewart et al., 2001) but not in adults (Hess et al., 1997). For efavirenz, several studies reported elevated plasma concentrations in female compared to male patients, which is in contrast to the above-mentioned in vitro findings and may be explained by other factors such as differences in body fat content and distribution (Burger et al., 2006; Nyakutira et al., 2008; Mukonzo et al., 2009). The influence of age on CYP2B6 expression may also depend on sex, as only males showed a significant increase of liver CYP2B6 at higher age (Yang et al., 2010).
Besides liver, CYP2B6 is also consistently expressed in different parts of the respiratory and gastrointestinal tracts, including lung and nasal mucosa, and also in skin and the kidneys (Choudhary et al., 2003; Dietrich et al., 2008; Thelen and Dressman, 2009; Le decor et al., 2010). The significance of CYP2B6 in these extrapulmonary tissues is currently unknown, but it should be remembered that the enzyme is probably the most important one for many environmental toxins such as pesticides, and its presence in tissues with barrier function may thus contribute substantially to protection against these chemicals. In addition, the presence of CYP2B6 in brain has been demonstrated in human and primate brain tissue samples and smoking, alcohol consumption, and genetic polymorphism have been suggested to contribute to its variability in this organ (Mikys et al., 2003). In general, CYP levels in extrapulmonary tissues are far below those of liver, but the localization to specific regions in the brain may contribute to the activation or inactivation of centrally acting drugs and to neurological side effects of certain medications or abused drugs, e.g., “ecstasy” [1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), see below]. This may also explain why efficacy for some centrally acting drugs is not well correlated to their plasma levels. The potential role of brain-expressed CYPs including CYP2B6 in the biotransformation of centrally acting drugs has been reviewed by others (Meyer et al., 2007; Ferguson and Tyndale, 2011).

**THE CHEMICAL INTERACTION PROFILE OF CYP2B6**

Recent studies have revealed crystal structures of the CYP2B6 wild-type and K262R variant in complex with various inhibitors at providing first views into its active site and its plasticity to adopt different conformations when binding different ligands (Gay et al., 2010; Shah et al., 2011). Wilderspin and Hulbert, 2012). Substrates of CYP2B6 are usually fairly lipophilic, neutral or weakly basic non-planar molecules with one or two hydrogen bond acceptors (Lewis et al., 1999, 2004). The CYP2B6 substrate selectivity comprises many diverse chemicals, including not only clinically used drugs but also many environmental chemicals such as pesticides (Turpeinen et al., 2006; Hodgson and Rose, 2007; Turpeinen and Zanger, 2012). Therapeutically important drugs metabolized primarily by CYP2B6 include the prodrug cyclophosphamide, which is converted to the direct precursor of the cytotoxic metabolites, phosphoramide mustard and acrolein, by 4-hydroxylation (Huang et al., 2000; Roy et al., 2005), the non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz, which is 8-hydroxylated to become pharmacologically inactive (Ward et al., 2003; Desta et al., 2007), and between ticlopidine and ketamine (Pellet et al., 2011). Furthermore, certain non-pharmaceutical substrates are the insecticide and endocrine disruptor methoxychlor, the extensively used insect repellent N,N-diethyl-m-toluamide (Des et al., 2008), profenofos and other pesticides (Abbas and Pekkonen, 2012), as well as the tobacco-specific nitrosoamine, 4-(methylheterosaminos)-1-(3-pyridyl)-1-butanone (NNK, Smith et al., 2003), aflatoxin B1 (Cade et al., 1997), and others (Hodgson and Rose, 2007; Abbas and Pekkonen, 2012).

Several structurally unrelated drugs have been shown to inhibit CYP2B6 and many of them do that in a mechanism-based, irreversible manner (Turpeinen et al., 2006; Turpeinen and Zanger, 2012). The thienopyridine derivatives clopidogrel and ticlopidine are prodrugs that selectively inhibit platelet aggregation and have been in clinical use for the prevention of atherothrombotic events for several years. Both of them are potent mechanism-based inhibitors of CYP2B6 (Richter et al., 2004; Zhang et al., 2011a). The established anticancer agent, thiTEPA (N,N′,N′′-triethylenethiophosphoramide) was also found to be a highly selective and mechanism-based CYP2B6 inhibitor (Rae et al., 2002; Hazleton et al., 2004; Richter et al., 2005). A comparison of several selective inhibitors revealed that 2-phenyl-2-(1-piperidinyl)propene is probably the most selective CYP2B6 inhibitor in vitro (Walsky and Obach, 2007). Recent in vitro observations identified the progesterone receptor antagonist, mifepristone (RU486, Lin et al., 2009), the anti-Parkinsonian agent selegiline (the R-enantiomer of deprenyl) (Sridar et al., 2012), methadone (Anumagama et al., 2012), and tamoxifen (Sridar et al., 2012) as potent mechanism-based inhibitors. In vivo drug-drug interactions have been reported, for example, between thiTEPA and cyclophosphamide (Hustema et al., 2000), clopidogrel and bupropion (Turpeinen et al., 2005a), voriconazole and efavirenz (Le et al., 2008; Jeong et al., 2009), clopidogrel and efavirenz (Jiang et al., 2012), and between ticlopidine and ketamine (Poltorani et al., 2011). Furthermore, certain non-pharmaceutical compounds like particular benzopyridine derivatives have been characterized as very potent inhibitors of CYP2B6 (Korhonen et al., 2009).
et al., 2007) and have been utilized for structural modeling experiments (Gay et al., 2010).

**PHARMACOGENETICS OF CYP2B6**

The CYPalleles website currently lists 37 distinct star-alleles, i.e., gene haplotypes with a distinct variant amino acid sequence or with demonstrated functional effect (last accessed: February 21st, 2015). More than 30 amino acid-changing single-nucleotide polymorphisms (SNPs) occur in different combinations and together with additional non-coding variants and many more SNPs not yet assigned to particular haplotypes. The worldwide variations in SNP frequencies have been reviewed recently (Li et al., 2012). This finding has been reproduced manifold in different ethnicities throughout the world (summarized by Telenti and Zanger, 2008; Rakhmanina and van den Anker, 2010). Three CYP2B6 polymorphisms, 15631G>C, 21011T>C, and an intron 3 SNP rs4803419, were also shown to be associated with efavirenz pharmacokinetics at genome wide significance (Holzinger et al., 2012).

The potent first-generation NNRTI of HIV-1 is recommended as initial therapy with two NRTIs in highly active antiretroviral therapy (HAART) regimes, but patients with subtherapeutic plasma concentrations can develop resistance and treatment failure, whereas those with too high plasma levels are at increased risk of central nervous system (CNS) side effects, which can lead to treatment discontinuation in a fraction of patients (King and Aberg, 2008). Q172H variant was furthermore associated with demonstrated functional effect (last accessed: February 21st, 2015). More than 30 amino acid-changing single-nucleotide polymorphisms (SNPs) occur in different combinations and together with additional non-coding variants and many more SNPs not yet assigned to particular haplotypes. The worldwide variations in SNP frequencies have been reviewed recently (Li et al., 2012).

Table 1 lists the most important variants in terms of frequency and functional impact and summarizes updated structural, functional, and frequency information for different ethnicities. Table 1 lists the most important variants in terms of frequency and functional impact and summarizes updated structural, functional, and frequency information for different ethnicities. In addition to the CYPalleles website, further valuable information about CYP2B6 SNPs and pharmacogenetics are available on the websites of The Pharmacogenomics Knowledgebase, the NCBI portal for short genetic variations, dbSNP, the 1000 Genomes Catalog of Human Genetic Variation, as well as the NHLBI exome sequencing project.

Table 1 | Summary data on selected genetic polymorphisms of CYP2B6

| CYP allele designation | Key mutation(s) | Location, protein effect | Allele frequencies | Functional effect |
|------------------------|-----------------|--------------------------|-------------------|------------------|
| CYP2B6*4               | g.18053(C:516)A>G | K262R (isolated)         | 0.00 AA, Af        | ↑ Expression, moderate substrate-dependent effects |
|                        | rs2279343       |                          | 0.04 Ca           |                  |
|                        |                 |                          | 0.05-0.12 As       |                  |
| CYP2B6*5               | g.25506(C:1458)C>T | R487C                    | 0.01-0.04AA, Af    | ↓ Expression, in part compensated by ↑ specific activity |
|                        | rs3211371       |                          | 0.09-0.12 Ca       |                  |
|                        |                 |                          | 0.05-0.12 Hs       |                  |
|                        |                 |                          | 0.01-0.04 As       |                  |
| CYP2B6*6               | g.15631(G:18053)G>T | Q172H,K262R              | 0.33-0.5 AA, Af    | ↓ Expression; activity with efavirenz in vivo; some other substrates show ↑ activity |
|                        | rs2365274       |                          | 0.10-0.21 As       |                  |
|                        |                 |                          | 0.14-0.27 Ca       |                  |
|                        |                 |                          | 0.62 PNG           |                  |
| CYP2B6*18              | g.21011(C:9837)T>C | I328T                    | 0.04-0.08 AA       | ↓ Expression and activity |
|                        | rs2839949       |                          | 0.05-0.12, Af      |                  |
|                        |                 |                          | 0.01 HS            |                  |
| CYP2B6*22              | g.402T>C         | promoter (TATA-box)      | 0.00 As, Ca, PNG   | ↑ Inducibility in vitro |
|                        | rs34223104      |                          | 0.00-0.025 AA, Af, As |                  |
|                        |                 |                          | 0.024 Ca, Hs       |                  |

1 According to CYPalleles nomenclature homepage http://www.cypalleles.ki.se.
2 Genetic (g.) and cDNA (c.) positions are given in bp.
3 Selected frequencies of individual ethnicities (Af, African American; Af-African; As, Asian; Ca, Caucasian; Hs, Hispanic; PNG, Papua New Guineans) compiled from dbSNP http://www.ncbi.nlm.nih.gov/SNP and from the literature cited in the text.

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|                        |                 |                          | 0.05-0.12 Hs       |                  |
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|                        |                 |                          | 0.14-0.27 Ca       |                  |
|                        |                 |                          | 0.62 PNG           |                  |
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|                        |                 |                          | 0.024 Ca, Hs       |                  |
with increased neurotoxicity and other CNS side effects (Haas et al., 2004; King and Aberg, 2008; Lubomirov et al., 2010; Ribaudo et al., 2010; Maimbo et al., 2011) with HAART-induced liver injury (Yimer et al., 2011), and with efavirenz treatment discontinuation and the associated risk of developing drug resistance (Ribaudo et al., 2006; Lubomirov et al., 2011; Wyen et al., 2011). Importantly, compound heterozygotes of 516T and another low activity allele (e.g., *11, *18, *27, *28) also predict high efavirenz plasma levels (Rotger et al., 2007; Ribaudo et al., 2010). In prospective, genotype-based dose adjustment studies the therapeutic dose of efavirenz could be successfully reduced and CNS-related side effects decreased (Gatanaga et al., 2007; Gatanaga and Oka, 2009). Using pharmacokinetic modeling and simulation it was suggested that a priori dose reduction in homozygous CYP2B6*6 patients would maintain drug exposure within the therapeutic range in this group of patients (Nyakutira et al., 2008).

The in vitro data that have accumulated over the years on the CYP2B6*6 allele draw a more complex picture with functional consequences on various levels including pre-mRNA splicing, protein expression, as well as substrate-dependent changes in enzyme activity and different sensitivity toward irreversible inhibition. While early studies using recombinantly expressed enzyme variants found higher 7-ethoxycoumarin O-deethylase activity for the Q172H variant (Ariyoshi et al., 2001; Inno et al., 2003), in genotyped human livers (ex vivo), the *6 allele has been associated with approximately 50–75% decreased protein levels (Lang et al., 2001; Desta et al., 2007; Hofmann et al., 2008). An explanation for decreased protein expression was provided based on the observation that the c.516G>T SNP coding for Q172H in exon 4 (rs3745274, Table 1) was correlated to increased amounts of a hepatic splice variant that lacked exons 4–6, and concurrently decreased amounts of the normal functional transcript. Recombinant expression of minigene constructs in mammalian cells proved that the c.516G>T variant was causally involved in erroneous splicing and lower expression of functional mRNA and protein (Hofmann et al., 2008). It has been hypothesized that binding of splice factors to an exonic splicing enhancer(s) located in exon 4 could be affected by the variant (Zanger and Hofmann, 2008; Sadee et al., 2011). Although reduced expression in liver appears to satisfactorily explain increased efavirenz plasma concentrations in individuals with *6/*6 genotype (Desta et al., 2007), recent in vitro data of expressed variants seem to indicate that the amino acid substitutions contribute to changes in catalytic activity of the enzyme. Structurally this is not easy to comprehend, because the Q172H and Lys262Arg amino acid changes occur in regions of the protein that are not directly located at the active site or that have been identified as substrate recognition sites (Figure 2).

Concerning efavirenz and also other substrates, the available in vitro data are however, not well in agreement with each other. Table 2 summarizes kinetic parameters for bupropion and efavirenz for CYP2B6 enzyme variants obtained from different recombinant expression systems. Using E. coli expression system, Zhang et al. (2011b) purified six N-terminally truncated expressed variants to homogeneity and reconstituted them with NADPH:cytochrome 450 reductase (POR) at a molar ratio of 1:2

![FIGURE 2](image-url)
Table 2: Kinetic properties of recombinantly expressed CYP2B6 protein variants with bupropion and efavirenz.

| Variant      | System | Bupropion hydroxylation | Efavirenz 8-hydroxylation | Reference                  |
|--------------|--------|-------------------------|---------------------------|----------------------------|
|              |        | $K_{m}$ (μM) | $V_{max}$ (%) | CL int (%) | $K_{m}$ (μM) | $V_{max}$ (%) | CL int (%) |             |
| 2B6.1        | COS-1  | 87                   | 100                      | 100          | 2.07          | 100                      | 100          | Radloff et al. (2013) |
| E. coli      | 95     | 100                   | 100                      | 100          | 73            | 100                      | 100          | Zhang et al. (2011b)   |
| Sf9          | 64     | 100                   | 100                      | 100          | 77            | 100                      | 100          | Arionyosh (2011)       |
| 2B6.6        | COS-1  | 72                   | 81                       | 98           | 1.21          | 107                      | 183          | Radloff et al. (2013)  |
| E. coli      | 380    | 175                   | 43                       | 100          | 198           | 563                      | 20           | Zhang et al. (2011b)   |
| Sf9          | 63     | 139                   | 143                      | 100          | 12.4          | 81                       | 50           | Arionyosh (2011)       |
| 2B6.4        | E. coli| 162                   | 60                       | 35           | 8.8           | 133                      | 49           | Xu et al. (2012)       |
| Sf9          | 63     | 139                   | 143                      | 100          | 5.5           | 73                       | 96           | Zhang et al. (2011b)   |
| 2B6.5        | COS-1  | 65                   | 44                       | 59           | 9.16          | 169                      | 142          | Arionyosh (2011)       |
| E. coli      | 134    | 66                    | 47                       | 100          | 1.15          | 46                       | 83           | Radloff et al. (2013)  |
| 2B6.6        | COS-1  | 63                   | 81                       | 43           | 8.8           | 133                      | 49           | Xu et al. (2012)       |
| E. coli      | 162    | 60                    | 35                       | 100          | 5.5           | 73                       | 96           | Zhang et al. (2011b)   |
| Sf9          | 63     | 139                   | 143                      | 100          | 9.16          | 169                      | 142          | Arionyosh (2011)       |

Only studies which determined kinetic parameters ($K_m$, $V_{max}$, or $CL_{int}$) were included.

and measured efavirenz and bupropion kinetics. Using Sf9 insect cell cotransfection, CYP2B6.1, 2B6.4 and 2B6.6 were expressed in the presence of 10-fold excess of POR, i.e., under saturating conditions, to measure efavirenz kinetics (Ariyoshi et al., 2011). Another study determined both bupropion and efavirenz kinetics in protein preparations also derived from insect cells in the presence or absence of cytochrome b5 (CYB5) but at somewhat more variable ratios in regard to POR (Xu et al., 2012). Radloff et al. (2013) used the COS-1 expression system, where P450 monooxygenase activity is supported by endogenously expressed POR, to determine bupropion and efavirenz kinetics for several novel CYP2B6 variants in comparison to the known variants 2B6.1, 2B6.5, and 2B6.6.

The compilation of data in Table 2 shows that differences between the variants were masked by differences between the expression systems. For example, efavirenz $K_m$ was moderately decreased (58%) for COS-1 cell-expressed 2B6.6 compared to 2B6.4 but moderately larger for both insect cell-expressed proteins. The E. coli-expressed variant showed, however, 27-fold increased $K_m$. While the COS-1 proteins had almost identical $V_{max}$, one of the insect cell proteins had decreased $V_{max}$ (81%), while the other had increased activity (133%). Again, the E. coli-expressed variant showed the biggest difference of almost sixfold higher activity for the variant. Similar discrepancies, albeit less dramatic, were found with bupropion as substrate (Table 2).

This data-comparison illustrates the problems that still exist for erroneous splicing caused by the c.516G>T SNP (Desta et al., 2009). Enzyme variants may interact differently with the electron donors and catalytic differences could thus depend on reconstitution conditions. In addition, N-terminal modifications required to achieve high expression in E. coli may interact with the DNA-polymorphisms to be analyzed. In the COS-cell system, the POR:P450 ratio can neither be controlled nor quantified because expression of P450 is too low for spectral quantitation.

Taken together, the data from expression systems indicated that catalytic differences may exist between CYP2B6.6 and CYP2B6.1. However, except for the E. coli study, the differences were rather modest and at present it cannot be concluded with certainty whether the CYP2B6.6 variant is catalytically more or less active compared to the wild-type, at least for bupropion and efavirenz.

Taken all evidence together, the decrease in hepatic expression due to lower susceptibility to inhibition of the K262R variant and nor quantified because expression of P450 is too low for spectral quantitation.

CYP2B6*6 SNP-related functional differences were also observed with inhibitors. In contrast to the wild-type enzyme the recombinantly expressed K262R variant was not inactivated by efavirenz, but both enzymes were reversibly inhibited by 8-hydroxyefavirenz (Bumpus et al., 2006; Bumpus and Hollenberg, 2008). Lower susceptibility to inhibition of the K262R variant and the CYP2B6.6 double variant compared to CYP2B6.1 was also found with respect to sertraline and clopidogrel, as well as several other potent drug inhibitors of CYP2B6 (Talakad et al., 2009).

These data indicate a role of genetic polymorphisms in drug–drug interaction sensitivity of CYP2B6, a finding that warrants further investigation in vivo.
OTHER CYP2B6 VARIANTS AND OTHER SUBSTRATES — IN VITRO STUDIES

The two amino acid changes that together constitute the *6 allele also occur in isolation. At least 12 additional null or low-activity alleles have been described and analyzed with various substrates (Lang et al., 2004; Klein et al., 2005; Rotger et al., 2007; Watanabe et al., 2010; Honda et al., 2011). Although they are rather rare in all investigated populations they may have profound effects on drug metabolism if present in compound heterozygous genotypes, e.g., in combination with *6 or *18 (Rotger et al., 2007). The CYP2B6*22 allele is a gain-of-function variant associated with increased transcription in vitro (Zukunft et al., 2005) and with increased activity in vivo (Rotger et al., 2007). It was shown that a -82T>C exchange alters the TATA-box into a functional CCAAT enhancer binding protein binding site that causes increased transcription from an alternative downstream initiation site (Zukunft et al., 2005). Interestingly, the -82T>C polymorphism also confers synergistically enhanced CYP2B6 inducibility by the PXR ligand rifampicin in human primary hepatocytes (Li et al., 2010).

New variants are discovered preferentially in previously uncharacterized ethnic groups. Restrepo et al. (2011) described two novel combinations of known amino acid variants in a Colombian population. Structural variants including a novel CYP2B6/2B7P1 duplicated fusion allele (CYP2B6*30) were found when individuals from various ethnicities were screened for copy number variations (Maris et al., 2012). Furthermore, three novel and five previously uncharacterized amino acid variants in different combinations (CYP2B6*33 to *37) were identified by resequencing the CYP2B6 gene in a Rwandese cohort of HIV-1-infected patients (Radloff et al., 2013). The variants were then functionally studied by COS-1 cell expression and by in silico prediction tools. At least four of the variants were shown to result in complete or almost complete loss of function with bupropion and efavirenz as substrates. The detailed comparison of in vitro functionality of the variants with in silico prediction tools including a thorough substrate docking simulation analysis points at the challenge to deal with the hundreds of new variants that exist in all populations as currently uncovered by next generation sequencing approaches and large scale population projects (see links above).

Table 3 | Properties of recombinantly expressed CYP2B6 protein variants with other clinical substrates.

| Variant | K_{m} (μM) | V_{max}(%) | K_{m} (μM) | V_{max}(%) | K_{m} (μM) | V_{max}(%) | K_{m} (μM) | V_{max}(%) |
|---------|------------|------------|------------|------------|------------|------------|------------|------------|
| 28B:1   | 3.1        | 100        | 48.2       | 100        | 1.84       | 100        | 2.68       | 100        |
| 28B:6   | 6.72       | 416        | 56.6       | 169        | 1.97       | 254        | 1.62       | 99         |
| 28B:4   | 2.73       | 196        | 45.8       | 147        | 1.09       | 1094       | 2.75       | 74         |
| 28B:5   | 6.87       | 56         | 70.1       | 85         | 0.80       | 441        | 5.1        | 72.4       |

1) O-Demethylation
2) N-Demethylatation
3) Desulfation
4) 4-Hydroxylation
References:
(Honda et al., 2011)
(Crane et al., 2012)
(Watanabe et al., 2010; Honda et al.)
CLINICAL STUDIES WITH DIFFERENT DRUGS

The widely used anticancer and immunosuppressant prodrug cyclophosphamide depends on bioactivation to 4-hydroxycyclophosphamide for cytotoxic activity. Bioactivation is highly variable in cancer patients and has been attributed mainly to CYP2B6 in vitro and in vivo with contributions from CYP2C19 and CYP1A2 (Chang et al., 1993; Raccor et al., 2012). The case of cyclophosphamide 4-hydroxylation deserves particular attention, as it exemplifies substrate-dependent effects of CYP2B6 pharmacogenetics. Cyclophosphamide 4-hydroxylation was initially reported to be enhanced in liver genotype CYP2B6*6/*6 (Xie et al., 2003), which was confirmed in several later in vivo studies (Xie et al., 2006; Nakajima et al., 2007; Torimoto and Kuhgo, 2008). However, other in vivo studies analyzing pharmacokinetics or clinical outcome also presented contradictory or negative results (Singh et al., 2007; Ekhart et al., 2008; Melanson et al., 2010; Yoo et al., 2010; Raccor et al., 2012). In vitro, insect cell-expressed recombinant CYP2B6.4 [K262R] had lower activity for cyclophosphamide 4-hydroxylation (Ariyoshi et al., 2011; Raccor et al., 2012). The CYP2B6*4 and CYP2B6*6 variants thus display mirror-inverted catalytic activities toward efavirenz and cyclophosphamide, in that the former variant is the catalytically more active one with efavirenz, whereas the opposite is true for the latter variant (Table 3). A direct comparison of catalytic properties of the two variants with the reference enzyme expressed in insect cells supports this inverse behavior of the two variants toward these proteins. Clinical studies have then contributed to identify the variants that are important in vivo, and in vitro studies are again needed to identify and mechanistically explain causal variants. Nevertheless, CYP2B6 pharmacogenetics has yet to be fully explored, especially with respect to combined effects of the involved variants on both expression and catalytic properties, the latter of which additionally depend on the substrate. While the relevance for HIV-1 therapy with efavirenz is well established and translational approaches have already been clinically tested, an increasing number of studies suggest clinical relevance for additional drug substrates.

CONCLUSION

The polymorphism of the CYP2B6 gene has initially been studied by reverse genetics approach, i.e., starting from the identification of genetic variants in DNA and liver samples, followed by in vitro characterization of genotyped livers and expressed variant proteins. Clinical studies have then contributed to identify the variants that are important in vivo, and in vitro studies are again needed to identify and mechanistically explain causal variants. Nevertheless, CYP2B6 pharmacogenetics has yet to be fully explored, especially with respect to combined effects of the involved variants on both expression and catalytic properties, the latter of which additionally depend on the substrate. While the relevance for HIV-1 therapy with efavirenz is well established and translational approaches have already been clinically tested, an increasing number of studies suggest clinical relevance for additional drug substrates.

ACKNOWLEDGMENTS

Work in the authors’ laboratory was supported by the German Federal Ministry of Education and Research (Virtual Liver Network grant 0315755), the 7FP EU Initial Training Network program, FightingDrugFailure’ (GA-2009–238132), and by the Robert-Bosch Foundation, Stuttgart, Germany.

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CYP2B6 allele variants were also investigated in the context of the synthetic α-opioid receptor agonist, methadone, which is metabolized by CYPs 3A4/5, 2B6, and 2D6, and used as a maintenance treatment for opioid addiction. In “6/*6 carriers, (S)-methadone plasma levels were increased leading to potentially higher risk of severe cardiac arrhythmias and methadone associated deaths (Crettol et al., 2005; Eap et al., 2007; Buntten et al., 2011). Methadone dose requirement for effective treatment of opioid addiction was shown to be significantly reduced in carriers of this genotype (Levrn et al., 2011).

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In addition to efavirenz, CYP2B6 genotype also affects plasma levels of the antineuronal drug nevirapine (Prenak et al., 2007; Mahungu et al., 2009). The impact of the CYP2B6 516G>T polymorphism on nevirapine exposure was found and quantified in a pharmacometric analysis of nevirapine plasma concentrations from 271 patients genotyped for 198 SNPs in 45 ADME (absorption, distribution, metabolism, and excretion) genes and covariates (Lehr et al., 2011). Moreover, nevirapine-related cutaneous adverse events, which are most likely major histocompatibility complex (MHC) class I-mediated, were significantly influenced by CYP2B6 polymorphism while hepatic side effects, most likely MHC class II-mediated, were unaffected by CYP2B6 (Yuan et al., 2011).

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