Metabolism-based Drug-Drug Interactions: What Determines Individual Variability in Cytochrome P450 Induction?

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Abbreviations: P450, cytochrome P450; AhR, aryl hydrocarbon receptor; Arnt, Ah receptor nuclear translocator; CAR, constitutive androstane receptor; PXR, pregnane X receptor; RXR, retinoid X receptor; HNF4α, hepatocyte nuclear factor 4 alpha; Pgp, P-glycoprotein; SNPs, single nucleotide polymorphisms.
ABSTRACT

Individual variability in cytochrome P450 (P450) induction comprises an important component contributing to the difficulties in assessing and predicting metabolism-based drug-drug interactions in humans. In this commentary, we outline the major factors responsible for the individual variability in P450 induction, including variable transporter activity and metabolism of inducers in vivo, genetic variations of P450 genes and their regulatory regions, genetic variations of receptors and regulatory proteins required for induction, and different physiological and environmental elements. With a better understanding of the major determinants in P450 induction and a profile of the phenotypes of these determinants in each individual, it is believed that the individual variability in induction-mediated drug-drug interactions can be adequately evaluated.
As one of the core components in the issue of metabolism-based drug-drug interactions, induction of drug metabolizing enzymes, especially P450s, has posed a challenge to scientists in the field of drug discovery and development. Unlike enzyme inhibition, which is an instantaneous response, enzyme induction is a relatively slow regulatory process involving transcriptional activation of the gene or stabilization of mRNA or protein. The net effect is to increase the amount of functional drug metabolizing enzymes, resulting in altered pharmacokinetics and/or pharmacodynamics (Kolars et al., 1991; Dilger et al., 2000), and even potential toxicity of therapeutic agents (Seeff et al., 1986). The impact that induction has on the therapeutic efficacy of a drug and the resulting potential economic loss for pharmaceutical companies (Lin and Lu, 2001) undoubtedly warrants the value of investment in early assessment of human enzyme induction potential.

The challenge arises from different responsiveness to inducers among individuals, both quantitatively and qualitatively, sometimes even within the same individual under different physiological or disease status. As a result, it is extremely difficult, if not impossible, to quantitatively predict the drug interactions derived from enzyme induction (Lin, 2000), not to mention predicting the degree of interaction in each individual. Until recently drug interaction derived from induction has not received as much attention as that due to inhibition, despite enzyme induction in rats by phenobarbital or aromatic hydrocarbons was observed more than 40 years ago (Conney, 2003). In addition to the factors responsible for the variability in enzyme inhibition (Lin and Lu, 2001), additional elements involved in the regulatory process of enzyme induction can potentially have an impact on individual variability in drug interactions, such as aryl hydrocarbon receptor (AhR) variation (both quantitative and qualitative) in CYP1A induction (Ma and Lu, 2003; Smart and Daly, 2000), pregnane X receptor (PXR) variation in CYP3A induction (Hustert
et al, 2001; Zhang et al, 2001), plus the variations of their respective regulatory proteins (Cao and Hegele, 2000). Nevertheless, in recent years, a variety of in vitro human test systems, including cell lines, cultured liver slices and hepatocytes, have been established to measure specific P450 protein levels, enzyme activity, or mRNA (Riley, 2001; Okino and Whitlock, 2000), and an appreciable number of drugs have been identified as P450 inducers in man (Handschin and Meyer, 2003). More importantly, the identification of PXR and constitutive androstane receptor (CAR), and the growing understanding of their role in induction of drug metabolism (Handschin and Meyer, 2003) have shed light upon the underlying mechanism for P450 induction. In this paper, we will outline the factors contributing to individual variability in P450 induction with a focus on evidence obtained from human in vivo and in vitro studies. Animal data will be used only when human studies are not available. Since the origins of individual variability in CYP1A induction have been detailed in a separate paper (Ma and Lu, 2003), the discourse in this paper will be centered mainly on CYP3A, CYP2C and CYP2B subfamilies. Individual variability in basal level expression of P450s in the absence of exogenous inducers is not the subject to be considered in this paper. As pointed out by others (Wojnowski, 2004; Burk and Wojnowski, 2004; Schuetz, 2004), factors contributing to different P450 expression levels among individuals are complex, requiring further investigations and understanding.

Factors Determining Individual Variability in P450 Induction

Different endpoints have been measured in tissues and body fluids to evaluate P450 induction in human studies (Table 1). Following treatment with an inducer, an increased amount of metabolites from P450 probe substrates in urine (Zhou et al, 1990) or a decreased AUC of probe substrates (Backman et al, 1996; Villikka et al, 1997) are indicative of P450 induction. In addition, induction in human can also be evaluated by measuring the increase of mRNA, protein or
enzyme activity of a specific P450 in human tissues following treatment with an inducer (Watkins et al, 1989; Kolars et al, 1992; Diaz et al, 1990). As described in the review by Lin and Lu (2001), large inter-individual variability in P450 induction has been recorded in the literature from human studies using various measurements. In addition to the polymorphisms of P450 genes, genetic variations of nuclear receptors and regulatory proteins that modulate the transcriptional processes of P450 expression, intracellular and tissue concentration of inducers, physiological factors (hormones, development, and disease) and environmental elements (diet and pollutants) can all impact induction of drug metabolizing enzymes. Thus, it is the interaction of genetic and epigenetic factors that determines an individual’s responsiveness to a given inducer. In contrast to differences resulting from genetic factors, however, differences resulting from epigenetic factors may be short-lived. Traditionally, the most unpredictable component of drug responses has been attributable to genetic variability. With the advent of facile tests for genotypes at a given gene locus, the variation caused by epigenetic factors is now considered more unpredictable, whether between individuals or within the same person from one day to the next (Morgan, 2001).

With the induction of CYP3A4 by rifampin as a simplified model depicted in Fig 1, we outline in Table 2 the major determinants responsible for the individual variability and possible consequences in P450 induction. They include (1) tissue and intracellular concentrations of inducers, which are modulated by P-glycoproteins (Pgp) and other transporters, and depend upon the metabolism of inducers by P450s and other enzymes; (2) genetic variations of P450 coding sequences and their regulatory elements; (3) genetic variations of nuclear receptors and regulatory proteins for transcriptional expression of P450 genes; and (4) physiological and environmental factors, such as hormonal homeostasis, disease status, age and diet.
Individual Variability in Tissue Concentration of Inducers: Modulation by Pgp and Other Transporters

The extent of enzyme induction is dose-dependent. Therefore, individual differences in the intracellular concentration of inducers could influence variation in drug-inducible expression of P450s and other drug-metabolizing enzymes. One of the important elements in the modulation of intracellular concentration of inducers is the efflux or influx mediated by a variety of transporters when the inducers also serve as substrates for the transporters, such as rifampin (Schuetz et al., 1996) and omeprazole (Pauli-Magnus et al., 2001). Although it is extremely difficult to document individual variability in tissue concentration of inducers (particularly in liver) among human subjects, the important role of Pgp, a well known drug-efflux transporter, in determining drug concentration in cells and tissues has been demonstrated in a number of studies utilizing either human derived cell cultures or animal models. Schuetz et al (1996) elegantly tested the hypothesis that Pgp has a role in modulating the extent of CYP3A induction through regulating intracellular concentration of an inducer. It is the first evidence demonstrating that in MDR1 overexpressed human colon carcinoma cells, rifampin induction of CYP3A mRNA and protein was reduced and required greater rifampin concentrations compared with parental cells. It was also found in the same study that lower doses of rifampin were required for induction of CYP3A proteins, and that the magnitude of CYP3A induction was greater at all doses of rifampin in Pgp knockout mice compared with wild type mice, a result consistent with enhanced accumulation of rifampin in Pgp knockout mice. The pharmacological relevance of these findings can be extended to the individual variability in human CYP3A induction. Since there exists large individual variation in the content of Pgp among human livers (Schuetz et al, 1995), it is conceivable that such variation in human
expression of Pgp and other transporters would contribute to individual variability in drug-metabolizing enzyme induction.

The degree of expression and the functionality of Pgp can vary due to genetic polymorphisms of Pgp genes (Woodahl and Ho, 2004) and the presence of a variety of agents down-regulating, inhibiting or up-regulating this transporter (Lin, 2003). Recently, Hoffmeyer et al (2000) identified polymorphisms in the human MDR1 gene and reported a significant correlation of a polymorphism in exon 26 (C3435T) of MDR1 with the expression level and function of Pgp. They found that individuals homozygous for this polymorphism had significantly reduced duodenal MDR1 expression and increased digoxin (a Pgp substrate) plasma levels. Yamauchi et al (2002) investigated the correlation of MDR1 gene polymorphisms with tacrolimus-induced neurotoxicity in patients after liver transplantation and concluded that mutation at position 2677 in exon 21 was one of the important factors responsible for the toxic event. Other studies dealing with either increased human plasma levels of phenytoin and fexofenadine (Kerb et al, 2001, Kim et al, 2001), Pgp expression in human placenta (Tanabe et al, 2001), or altered efflux of rhodamine 123 in human CD56+ natural killer cells (Hitzl et al, 2001) further substantiated the relation of MDR1 gene polymorphisms to Pgp expression and function. Given the facts that a number of single nucleotide polymorphisms (SNPs) in MDR1 gene have been observed (Tanabe et al., 2001), that different allelic frequencies in different ethnic populations have been reported (Kim et al., 2001), and that some SNPs are associated with altered Pgp expression and functionality (Brinkman, 2002), it can be concluded that genetic polymorphisms of Pgp genes can contribute to individual variability in intracellular or tissue concentration of an inducer.

The scenario of drug-drug interaction derived from Pgp inhibition or induction has been extensively reviewed (Lin, 2003; Ayrton and Morgan, 2001). Analogous to the effect of P450
inhibition and induction on clearance, Pgp inhibition results in an increase in the systemic exposure and tissue distribution of drugs that are subject to Pgp efflux, while the opposite would be seen in Pgp induction (Lin, 2003). Given that the genetic polymorphisms of Pgp genes may lead to abnormal Pgp expression and functionality, it can be speculated that allelic variants may display different responses to inhibitors or inducers, leading to various changes in intracellular and tissue levels of drugs. However, there is no report available in the literature investigating whether variants may respond to inducers differently in relation to the wild type.

In addition to efflux via Pgp, uptake through other transporters can also be an important determinant of intracellular and tissue concentrations of an inducer. Tirona et al recently (2003a) demonstrated that human liver-specific organic anion transporting polypeptide-C (OATP-C) mediates the hepatocellular uptake of rifampin. Since several naturally occurring OAPT-C variants (Tirona et al, 2001) have shown markedly reduced rifampin transport activity, the expression of these OATP-C variants can potentially weaken rifampin-mediated PXR activation. These findings suggest that uptake transporters such as OATP-C may affect the degree to which hepatic drug-metabolizing enzymes and transporters are induced in vivo by rifampin and perhaps other drugs. Considering the frequencies of functionally relevant polymorphisms in OATP-C, variability in this transporter activity may be a major determinant of the extent of observed inter-subject variability in induction of P450 enzymes and transporters (Tirona et al, 2003a).

**Individual Variability in Tissue Concentration of Inducers: Modulation by P450 and other Drug-metabolizing Enzymes**

Another important element in the modulation of intracellular concentration of inducers is metabolism by P450s and other drug-metabolizing enzymes. Individual variation in these enzymes has been well established (Shimada et al, 1994; Lin and Lu, 1998; Lin and Lu, 2001, Meyer and
Zanger, 1997), both qualitatively (genetic polymorphism) and quantitatively (enzyme expression level). Thus, since many inducers are subject to metabolism by P450s and other drug-metabolizing enzymes, two scenarios can be expected to be responsible for different intracellular inducer levels: (1) subjects with different metabolic capacity for an inducer may exhibit different degrees of induction and (2) two inducers with similar induction potency but different metabolic susceptibility may result in different degrees of induction.

The first situation is exemplified by CYP1A2 induction in humans by omeprazole. The major metabolic pathway of omeprazole is the 5-hydroxylation by CYP2C19, a polymorphic P450 (Andersson, 1996). This pathway in human liver microsomes and the in vivo clearance of omeprazole exhibit large individual variations, which correlate with CYP2C19 phenotypes (Chiba et al, 1993; Sohn et al, 1992). The difference in the rate of 5-hydroxylation between poor metabolizers (PM) and extensive metabolizers (EM) leads to different tissue levels of omeprazole available for CYP1A induction. For instance, at a therapeutic dose (40 mg), omeprazole failed to show CYP1A2 induction in subjects with a CYP2C19 EM phenotype (Rost et al, 1992), but the inductive effect was revealed at a higher dose (120 mg) in the same subjects (Rost et al, 1994). In contrast, CYP1A2-mediated caffeine metabolism, as measured by the caffeine breath test, was induced at the 40 mg omeprazole dose in subjects with a CYP2C19 PM phenotype. Clearly, individual variations in the metabolic rate of omeprazole affect the intracellular concentration of the inducer, contributing to variability of CYP1A2 induction.

Good examples are difficult to find for the second scenario. Rifampin and rifabutin are two compounds with very similar chemical structures, and their in vitro induction potencies in a PXR reporter gene system are comparable. However, rifampin is a much more potent CYP2C and CYP3A inducer than rifabutin in humans. It is known that both compounds are deacetylated at the
25-position, a major metabolic pathway, but rifabutin is metabolized much more rapidly than rifampin by the deacetylating enzyme in human liver slices and human liver microsomes (Jamis-Dow et al, 1997). Both compounds are also hydroxylated by P450. In agreement with the in vitro data, rifabutin displayed a clearance 7-fold greater than that for rifampin in a clinical study (Brogden et al, 1994). These results suggest that its slower metabolism and elimination from human could make rifampin a more potent P450 inducer than rifabutin. It was also found that individual variability exists in liver B-esterases, enzymes responsible for the deacetylation of both rifampin and rifabutin (Jamis-Dow et al, 1997), and this difference could contribute to individual variability in P450 induction by these rifamycins.

**Genetic Polymorphism of P450s and their Regulatory Regions**

Recent advances in human gene analysis have revealed numerous genetic mutations in both coding and non-coding regions for human P450s involved in drug metabolism (Nagata and Yamazoe, 2002). Some mutations can cause enzyme products with abolished, reduced, altered or increased enzyme activity (Ingelman-Sundberg, 2001). Thus, some variants may fail to express the enzymes (2A6*4, 2D6*5) or express enzymes with no activity (2A6*2, 2C19*2, 2C19*3, 2D6*4), altered substrate specificity (2C9*3), reduced affinity for substrate (2D6*17, 3A4*2), or decreased stability (2D6*10). On the other hand, a number of variants retain the same phenotypic activity as their wild type alleles, and some even show increased activity (2D6*2xn). In addition to significantly contributing to highly variable basal activities of respective P450 isozymes (Özdemir et al., 2000; Lamba et al., 2002; Bertilsson, 1995), these variants are likely to possess different responsiveness to an inducer, leading to distinct inducibility. This possibility has been implicated in a number of human studies intended to investigate genotype-phenotype correlations.
A straightforward example is that CYP2C19 genotype determines the activity and inducibility of S-mephenytoin hydroxylase (Zhou, 2001; Zhou et al., 1990; Feng et al., 1998; de Morais et al., 1995). The 4'-hydroxylation of S-mephenytoin is primarily mediated by CYP2C19, and this reaction has been used as an in vivo probe for CYP2C19. Genetic deficiency of CYP2C19 is attributed to a number of SNPs, including the alleles CYP2C19*2 (a splice site mutation), CYP2C19*3 (a premature stop codon), CYP2C19*4 (an A-G transition in the initiation codon), and CYP2C19*5 (an amino acid mutation). These genetic polymorphisms separate people into two phenotypes: EMs and PMs of S-mephenytoin. It has been shown that almost 100% Asian and 85% Caucasian PMs possess two mutations (CYP2C19*2 and *3), while two additional defective alleles (CYP2C19*4 and *5) contribute to the PM phenotype in Caucasian population (Zhou 2001). Using mephenytoin as a probe, Zhou et al (1990) found that induction of CYP2C19 activity by rifampin was only observed with EMs, but not with PMs, suggesting a possibility of either no induction at all or only induction of the enzyme with impaired activity in PMs. Furthermore, a gene-dosage effect was also reflected in the extent of inducibility. The amount of 4'-hydroxy mephenytoin excreted in the 0-24 h urine in subjects homozygous for CYP2C19*1 was increased by 203.9 ± 42.5%, whereas that of subjects heterozygous for either one CYP2C19*2 or CYP2C19*3 was only increased by 69.6 ± 4.1%, indicating that the functional allele is an important determinant for the inducibility of CYP2C19 (de Morais et al., 1995). However, depending on the probe substrate, inducer, and target enzyme, different phenotypes may give rise to different clinical outcomes. For example, propafenone is metabolized in humans by CYP2D6-mediated hydroxylation (a major metabolic pathway) and by CYP3A4- and CYP1A2-dependent N-dealkylation (a minor pathway). When propafenone was used as a P450 probe substrate, rifampin treatment caused a two-fold increase in systemic clearance of propafenone in CYP2D6
PMs, but had little effect on the clearance in EMs (Dilger et al., 1999), because rifampin induced CYP3A4 in PMs where N-dealkylation by 3A4 and 1A2 became dominant due to the lack of CYP2D6 activity. Furthermore, many inducers are substrates of the induced enzymes. Therefore, variation in the activity of a P450 enzyme due to a genetic polymorphism can affect the degree of auto-induction. For instance, variants with a defective CYP1A1 gene were found to express higher levels of CYP1A1 mRNA than wild-type cells in the absence of exogenous inducers (Hankinson, 1995). A simple explanation is that an endogenous agonist(s) of AhR, which is normally metabolized by CYP1A1 in wild-type cells, is accumulated in CYP1A1 deficient variant cells, leading to an increased expression of CYP1A1 mRNA. By the same token, variation in the activity of a P450 enzyme due to a genetic polymorphism can affect the extent of induction of another P450 enzyme since different tissue concentrations of the inducer can be achieved due to the different activities in EMs and PMs of the enzyme that metabolizes the inducer. An example supporting this notion is the clinical study of CYP1A2 induction by omeprazole in EMs and PMs of S-mephenytoin hydroxylase, which has been discussed above.

The impact of genotype on inducibility varies depending on the site of mutation, inducer, and gene coding region of the enzyme. For instance, an insertion mutation at the regulatory region of human CYP2E1 (from positions -2270 to -1672) not only enhanced the metabolic activity of the enzyme, but also potentiated its inducibility associated with obesity and ethanol intake (McCarver et al., 1998). Obesity and ethanol intake had no impact on the activity of chlorzoxazone 6-hydroxylation (an in vivo probe for CYP2E1) in wild type subjects, but significantly increased chlorzoxazone metabolism in subjects with this mutation, possibly due to an interruption of CYP2E1-negative regulatory element or alteration in transcription factor cooperativity (McCarve et al., 1998). Similarly, Sachse et al. (1999) found that a SNP in intron 1 of CYP1A2 gene at
position 734 (C→A, CYP1A2*1F) downstream of the first transcribed nucleotide was associated
with high inducibility of CYP1A2 in Caucasian smokers but not in nonsmokers. The A/A
genotype may be either a direct cause of increased CYP1A2 activity or genetically linked to a
polymorphism conferring high inducibility. However, a polymorphism identified at position -2964
in the 5’-flanking region of human CYP1A2 (CYP1A2*1C) significantly decreased the activity in
smokers, but not in nonsmokers. This suggests that this point mutation does not affect the
constitutive expression of the gene but primarily influences the in vivo induction of CYP1A2 by
smoking, presumably attributed to differential binding of a protein factor(s) to the wild-type and
variant allele sequences (Nakajima et al., 1999). Kinetic analysis revealed that F186L, a CYP1A2
variant which was newly identified along with five other novel nonsynonymous nucleotide
alterations in human CYP1A2 gene and displayed the most profound reduction in CYP1A2
activity, showed a \( V_{max} \) only 5% of that of the CYP1A2 wild type, as measured for the
ethoxyresorufin \( O \)-deethylation (Murayama et al., 2004). However, whether this variant is
associated with altered inducibility of CYP1A2 remains unclear.

While the CYP3A subfamily comprises the most important P450 enzymes involved in drug
metabolism, reports on the association of genetic polymorphisms in CYP3A genes and their
inducibility are sparse, and even the origin of the enormous interindividual variability in their basal
eexpression/activity (e.g. CYP3A4 expression by more than 50-fold, and in vivo CYP3A4 function
by at least 20-fold (Eichelbaum and Burk, 2001)) is not fully understood. Although more than 30
SNPs have been identified in CYP3A4 genes in coding and noncoding regions (Sata et al., 2000;
Dai et al., 2001, Westlind et al, 1999) and altered activities have been identified in some in vitro
studies (Lamba et al., 2002), these coding variants may contribute to but are unlikely to be the
major cause of interindividual differences in CYP3A-dependent clearance, given their low allele
frequencies and limited alterations in enzyme expression or catalytic function. Large interind individual variability in the basal enzyme expression/activity coupled with an unclear molecular mechanism makes the identification of polymorphism related inducibility of CYP3A extremely difficult. The only study in this regard was conducted by Wilkinson’s group to investigate genotype–phenotype associations for common CYP3A4 and CYP3A5 variants in the basal and induced metabolism of midazolam in European- and African-American men and women (Floyd et al., 2003). It was found that the oral clearance of midazolam and the fold-increase in oral clearance after rifampin was significantly lower in subjects with homozygous CYP3A4*1B (the most common variant with an A-392G transition in the 5’-flanking region from the transcriptional initiation site) compared with wild-type and heterozygous subjects, while these two measures were significantly higher in subjects with homozygous CYP3A5*3. Trends consistent with a gene–dose effect were also apparent when measured as fold-increase in midazolam oral clearance after rifampin. However, no such associations were observed with systemic clearance of midazolam, presumably indicating more impact of rifampin treatment on intestinal than hepatic CYP3A. Except for this study, no comprehensive investigations have been reported in studying the inducibility of CYP3A variants, even with in vitro systems.

Genetic Variations of Receptors and other Regulatory Proteins

AhR, PXR and CAR play a central role in modulating P450 induction via transcriptional activation. It has been well established that AhR and CAR are present in the cytoplasm prior to being translocated into the nucleus upon activation. PXR is generally thought to be primarily retained in the nucleus, but recent findings (Squires et al, 2004; Kawana et al, 2003) indicate that PXR may also be located in the cytoplasm and similarly translocated into the nucleus upon activation. The activated receptors inside the nucleus bind to the target response elements as
heterodimers with another factor (Arnt for AhR, and RXR for PXR and CAR), leading to transcription of respective P450 genes. The mechanism of induction of CYP1A1/2 by polycyclic aromatic hydrocarbons has been well established (Whitlock, 1999), and the factors contributing to the individual variability in CYP1A induction comprehensively reviewed (Ma and Lu, 2003). On the other hand, the pivotal role played by PXR and CAR in the regulation of CYP3A, CYP2C, and CYP2B has only recently been elucidated (Waxman, 1999; Kliewer et al., 2002; Handschin and Meyer, 2003), and information on the factors contributing to the individual variability in P450s regulated through these two orphan nuclear receptors is limited.

PXR activates CYP3A genes in response to diverse chemicals, including certain natural and synthetic steroids (Waxman, 1999; Goodwin et al., 2002; Kliewer et al, 2002). CAR mediates widely studied induction of CYP2B and CYP3A genes by phenobarbital (PB) and other “PB-like” lipophilic chemicals (Waxman, 1999; Sueyoshi and Negishi, 2001). As depicted by Handschin and Meyer (2003) and Riley (2001), both CAR and PXR form heterodimers with RXR and these complexes bind to and transactivate several DNA response elements (direct repeats of AGGTCA motif separated by three or four nucleotides (DR3, DR4) and everted repeats separated by six nucleotides (ER6)), located in both the proximal and distal CYP3A4 regulatory region. It is believed that CAR, when bound to endogenous steroids related to androstanol and androstenol, is an inactive form and present in the cytoplasm. The presence of PB or PB-like inducers, however, triggers nuclear translocation of CAR, possibly via receptor dephosphorylation. Nuclear translocation may also be involved in PXR activation (Squires et al, 2004; Kawana et al, 2003), although this nuclear receptor generally has been thought to be present in the nucleus as an inactive form (Sueyoshi and Negishi, 2001; Goodwin et al., 2002). Once bound to its ligands, the activated PXR dimerizes with RXR. A direct correlation between ligand binding and receptor
activation has been demonstrated for PXR (Jones et al, 2000). Serving as a common heterodimerization partner for many orphan nuclear receptors, RXR can be further modulated by other factors, such as dexamethasone and glucocorticoid receptor.

The mechanism of P450 expression via receptor-modulated transcriptional regulation underscores the possible contribution of altered expression or functions of these receptors and their regulatory proteins to the variable expression and inducibility of respective P450 enzymes. A more extensively studied area is AhR and its regulatory proteins (Ma and Lu, 2003). For instance, variable expression of AhR and Arnt mRNA has been described in human liver and lung tissues, which correlated with the CYP1A1 mRNA in peripheral blood cells from 20 healthy Japanese subjects (Hayashi et al., 1994). Recently, the variable expression of AhR and Arnt has been linked to individual variation of CYP1A1 inducibility based on the finding that, after controlling for gender and cigarette smoking, AhR levels positively correlated with CYP1A1 inducibility (Lin et al., 2003). Altered functions of AhR have been identified in human placenta samples that displayed more than 20-fold differences in AhR affinity for ligand binding between the “high” and the “low” CYP1A1 inducibility phenotypes (Okey et al., 1995). On the other hand, information on variable expression of CAR and PXR is just beginning to unveil. A recent report described individual variability of CAR and PXR gene expression and its correlation with CYP2B6 mRNA levels (Chang et al., 2003). These investigators measured CYP2B6, CAR and PXR mRNA expression and compared the levels in a panel of 12 individual human liver samples. There was a striking interindividual variability (240-fold) in hepatic CAR mRNA levels, which correlated well with the similar variability (278-fold) in CYP2B6 mRNA. Although less variable (27-fold), PXR mRNA also correlated well with CAR and CYP2B6 mRNA. Therefore, substantial interindividual differences exist in hepatic CAR and, to a lesser extent, PXR gene expression. It is speculated that
the variability in abundance of these transcription factors may contribute to the large interindivudual differences in CYP2B6 gene expression in human liver (Chang et al, 2003). However, it is not clear if such variation in CAR and PXR expression is associated with genetic polymorphisms and further linked to variable inducibility of the corresponding P450 enzymes. Such information would be very useful in understanding the contribution of transcriptional regulation to individual variability in P450 inducibility, and therefore, deserves more attention and efforts.

It has been established in rodent models that genetic variation in the AhR has powerful phenotypic effects on responses to dioxins and polycyclic aromatic hydrocarbons (Harper et al., 2002). For example, a single nucleotide difference at codon 375 leads to an AhR affinity to TCDD that is approximately 10-fold higher in C57BL/6J mice than in DBA/2 mice (Okey et al., 1989). As a consequence of this lowered affinity, DBA/2 mice are less sensitive to toxic effects of heterocyclic aromatic amine/amides and polycyclic aromatic hydrocarbons than are C57BL/6 mice (Nebert, 1989). In humans, however, the scenario is more complicated. While a number of AhR variants have been identified, and some have shown altered functions, they have not been clearly associated with phenotypic effects (Harper et al., 2002). On the other hand, some variant phenotypes have been reported, such as a difference of at least 10-fold in AhR binding potency in populations (Manchester et al., 1987) and induced CYP1A1 activity spanning a range of greater than 6-fold in human periphery blood lymphocytes (Micka et al., 1997), but genetic analyses have not revealed polymorphic variants as a clear explanation. This is a subject that needs to be further explored.

Since CYP3A gene polymorphisms do not appear to play a major role in highly variable basal and induced activity of the enzymes, genetic polymorphisms in PXR and CAR along with their
regulatory factors have been suggested to be important contributors. However, knowledge of PXR and CAR polymorphisms and their impact on variable induction among individuals is only in its infancy. The first report (Zhang et al., 2001) described approximately 40 single nuclear polymorphisms identified in the human PXR gene, including six SNPs in the coding region. An hPXR variant, R122Q, which alters an amino acid in the DNA binding domain (DBD) of PXR, reduces DNA binding activity and causes a slight attenuation of the rifampin-induced CYP3A4 promoter activity. Hustert et al (2001) later on reported three other hPXR variants, V140M, D163G, and A370T, all of which alter residues in the ligand binding domain (LBD) of PXR. D163G had reduced basal and rifampicin-induced activity relative to wild-type PXR when tested on a reporter gene construct containing the proximal promoter and distal enhancer of CYP3A4. In contrast, V140M and A370T exhibited modest increases in basal activity in this same assay. Interestingly, three of the silent PXR polymorphisms that do not result in amino acid substitutions correlate with alterations in the expression of CYP3A4 (Zhang et al., 2001). Thus, these natural PXR protein variants may play a role in the observed interindividual variability of CYP3A4 expression and may be involved in rare, atypical responses to drugs or altered sensitivities to carcinogens (Hustert et al., 2001). Four additional naturally occurring hPXR variants (R98C, R148Q, R381W, and I403V) have been identified and their functions characterized recently by Koyano et al (2004). An important finding was that the variant R98C, which alters an amino acid immediately after the fourth cysteine residue of the second zinc finger of the DBD, caused dramatic loss of both DNA binding activity and the transactivation of the CYP3A4 promoter/enhancer region. Such a change in both measures by R98C is more drastic than that by R122Q, described by Zhang et al (2001), although both variants are located in the DBD. Taken together, these studies suggest that hPXR variants with altered functions may influence the
expression and inducibility of P450 enzymes and transporters, which are transactivated by PXR. Nevertheless, further studies will be required to determine whether these and/or other PXR polymorphisms represent reliable predictors of CYP3A4 activity in vivo (Kliewer et al., 2002).

Alternative pre-mRNA splicing has been identified as a mechanism for regulating gene expression and for generating alternative forms of a wild-type protein gene product (Smith et al., 1989). The recent discovery of several splicing variants of PXR (Fukuen et al., 2002) and CAR (Savkur et al., 2003) in human liver may indicate a potential role for mechanism in the individual variability in P450 expression and induction. Nine splicing variants (SV) of human PXR were identified in tissues from a single human liver with seven of these variants being novel (Fukuen et al., 2002). The wild-type of human PXR has 434 amino acids containing both DBD and LBD. However, splicing events lead to truncated hPXR proteins that lack a large part of the LBD, or may have incomplete LBD. Some splicing variants lack the common CTG translation initiation codon and thus produce no protein. In the same study, the expression for all those splicing variants was determined in 15 different human livers and the pattern of expression levels of these transcripts was found to vary among liver samples (Fukuen et al., 2002). The result suggests that the hPXR is expressed as several different transcripts in liver tissues due to alternative as well as defective gene splicing, which may serve as a possible mechanism of interindividual differences in hPXR transcript profiles. As a result, this alternative splicing for hPXR may largely contribute to the interindividual variability in CYP3A4 and Pgp induction. In another study, Savkur et al (2003) identified two novel hCAR splice variants: hCAR2 encodes a receptor in which alternative splice acceptor sites are utilized resulting in a 4 amino acid insert between exons 6 and 7, and a 5 amino acid insert between exons 7 and 8, and hCAR3 encodes a receptor with exon 7 completely deleted resulting in a 39 amino acid deletion. Both hCAR2 and hCAR3 mRNAs are expressed in a pattern
similar to the initially described variant MB67 (hCAR1) with some key distinctions. Although the levels of expression vary depending on the tissue examined, hCAR2 and hCAR3 contribute 6-8% of total hCAR mRNA in liver. Analysis of the activity of these variants indicates that both hCAR2 and hCAR3 lose the ability to heterodimerize with RXR and lack transactivation activity. Although the role of hCAR2 and hCAR3 is currently unclear, these additional splice variants may provide increased diversity in terms of responsiveness to xenobiotics. However, the clinical relevance of these findings remains to be assessed with quantitative analysis of a large set of samples.

By analogy, genetic polymorphisms of regulatory proteins can be a potential contributor to the variable expression and induction of P450 enzymes. The only information available to date in this respect is on CYP1A induction, largely from in vitro and in vivo animal models (Ma and Lu, 2003). For example, the variant mouse hepatoma cell lines contain a defective Arnt gene derived from a point mutation, which not only reduces the DNA binding activity of Arnt, but also increases its susceptibility to proteolytic digestion, therefore significantly reducing its biological half-life (Numayama-Tsuruta et al., 1997). Although direct link of the Arnt defect to altered CYP1A induction has not been firmly established, a recent finding (Tomita et al., 2003) supports the critical role of Arnt in AhR-mediated induction of CYP1A. It was found that mice with the Arnt gene disrupted specifically in T cells were resistant to TCDD-induced thymic involution, a response observed in normal mice exposed to the inducer (Tomita et al., 2003). While several polymorphisms of human Arnt gene have been cited in the literature, their functional impact on human CYP1A induction has not been established (Scheel et al., 2002; Anttila et al., 2000; Cao and Hegele, 2000; Watanabe et al., 2001). As for the regulatory proteins for CYP3A induction, there are only a few reports on the polymorphisms of RXR (Hegele and Cao, 2001), but their
influence on CYP3A and CYP2B induction is unclear. The orphan nuclear receptor HNF4α is also an important regulator for PXR-mediated induction of CYP3A4 (Tirona et al, 2003b). Studies showed that adult mice with conditional hepatic deletion of Hnf4α had reduced basal and inducible expression of CYP3A. Therefore, genetic polymorphisms of HNF4α resulting in altered function would be expected to affect the induction of CYP3A4.

The expression of PXR and CAR is also subject to modulation by a variety of endogenous and exogenous substances (Handschin and Meyer, 2003). For instance, it has been found that glucocorticoids play a dual role in CYP3A4 expression, first by controlling the expression of PXR and CAR under physiological conditions (submicromolar concentrations) through the classical glucocorticoid receptor pathway, and second by activating the PXR under bolus or stress conditions (supramicromolar concentrations) (Pascussi et al., 2001). In contrast, the addition of interleukin-6 results in down-regulation of both CAR and PXR expression (Pascussi et al, 2000). Therefore, altered nuclear receptor levels in the presence of endogenous and exogenous substances can modify the expression of P450 genes, contributing to the individual variability in P450 activity and inducibility.

**Individual Variability due to Physiological and Environmental Factors**

The presence of endogenous ligands of AhR, PXR and CAR (Handschin and Meyer, 2003; Ma and Lu, 2003) suggests that the expression of P450 enzymes can vary under different physiological conditions where levels of these endogenous ligands fluctuate accordingly. Indeed, in humans and animals, infections or inflammatory stimuli cause changes in the activities and expression levels of various forms of P450 in the liver, as well as in extrahepatic tissues such as kidney and brain (Morgan, 1997). In most cases, P450s and their activities are suppressed, but some are unaffected or induced under these conditions. Numerous experimental studies have shown that suppressed
activities are related to a decreased level of P450 enzymes in response to cytokines (Moochhala, 1991; Morgan, 1993; Morgan, 1997). For instance, interleukin-6 strongly represses the inducibility of CYP3A4 mRNA and protein in primary human hepatocytes (Muntane-Relat et al., 1995). A recent investigation of the underlying mechanism demonstrated that interleukin-6 rapidly and markedly decreases the expression of PXR and CAR mRNAs. In parallel, interleukin-6 decreases both rifampicin- and phenobarbital-mediated induction of CYP2B6, CYP2C8, CYP2C9, and CYP3A4. As the transcriptional activity of PXR and CAR is not affected by interleukin-6 in cell-based reporter assays, the data suggest that the loss of CYP2 and CYP3 inducibility results from the negative regulation of PXR and CAR gene expression by this cytokine (Pascussi et al, 2000). Similarly, Goodwin et al (2003) found that, in mice, inhibition of bile acid biosynthesis after dietary manipulation (low dose bile acid feeding) or gene disruption (Cyp7a1-/-) resulted in the repression of Cyp3a11 expression, while high-dose bile acid feeding resulted in the up-regulation of Cyp3a11, likely through the action of PXR by regulating the concentration of bile acid and its secondary metabolites. Liver diseases, such as hepatitis with severe liver failure or cirrhosis, impair P450 activities by 20-80%, with CYP1A2 being the most sensitive enzyme (Farrell, 1999). However, CYP1A2 is induced in diabetic conditions under certain circumstances in humans (Cheng and Morgan, 2001). Interestingly, sometimes changes in P450 expression levels do not parallel inducibility in certain disease status. In searching for evidence in humans for the effects of renal failure on CYP3A4 activity and inducibility, Dowling et al (2003) recently found that end-stage renal disease patients had 28% lower baseline erythromycin breath test values than controls (P <.05); however, enzyme induction capacity after rifampin administration was similar between groups (P > 0.70).
Gender can be an important physiological factor affecting the extent of induction of P450 enzymes (Postlind et al., 1993). Levels of induced CYP1A1 activity in lymphocytes as measured by ethoxyresorufin O-deethylation showed a gender difference with women having a significantly lower induced activity compared with men (Smart and Daly, 2000). Differential induction of CYP3A4 by rifampin between men and women has been demonstrated in a study (Gorski et al., 2003) in which CYP3A4 activity was measured based on the systemic and oral clearance of midazolam (a CYP3A4 probe substrate). A significant (P = .0023) effect of gender was noted in the extent of induction of the oral clearance of midazolam, resulting in greater induction in men than in women. In contrast, the extent of midazolam systemic clearance induction was greater in women than in men (P = .0107). The result indicates that gender-related differences exist in the extent of intestinal and hepatic CYP3A induction by rifampin. The extent of induction at hepatic and intestinal sites was inversely dependent and reflected the independent regulation of CYP3A expression at these sites (Gorski et al., 2003). Overall, it appears that gender-related difference in response to an inducer may vary with the P450 isoform, substrate, and site.

Aging is accompanied by marked changes in the physiology of many organs, as well as in their constituent cells. Since the liver plays a major role in drug clearance, it is not surprising that aging has been reported to diminish hepatic capacity, particularly the clearance of drugs that undergo mandatory oxidation by P450 enzymes. A number of studies have documented significant age-related declines in the amounts, specific activities, and rates of induction of liver P450s in inbred male rats. However, on the basis of a variety of clinical tests, most liver functions in humans appear to be well preserved. Most in vitro studies using nonhuman primate or human liver tissue did not detect age-related deficiencies in P450 activities (Schmucker, 2001). On the other hand, age-related induction of P450 enzymes remains controversial. Although age-related reduced
induction of drug metabolism has long been reported in some studies (Salem et al., 1978; Twum-Barima et al., 1984), other investigators have found similar extent of induction in old and young individuals (Fromm et al., 1998; Crowley et al., 1988; Dilger et al, 2000). The reason for these discrepancies remains to be further exploited. According to the conflicting data from different pharmacokinetic studies, it is very difficult to generalize the effect of aging on the induction response (Lin and Lu, 2001).

The pioneering studies by Conney and co-workers in the 1970s showed that in human subjects fed charcoal-broiled beef, Brussels sprouts, or cabbage, the metabolism of phenacetin (a CYP1A2 probe substrate) was enhanced, indicating the induction of CYP1A2 by dietary components (Conney et al, 1976; Pantuck et al, 1979). In addition, there was considerable individual variability in the inductive effect of the diet on phenacetin metabolism. The central role of AhR, PXR and CAR in modulating the expression and induction of respective P450 enzymes has provided a mechanistic insight into many previous findings that consumption of a particular food modulates the activity of drug-metabolizing enzyme system. Many dietary components have been identified as ligands for certain nuclear receptors. For example, the induction of CYP1A by cigarette smoking is attributable to the aryl hydrocarbons from the tar fraction of tobacco. Further studies revealed that the aryl hydrocarbons are agonists of AhR (Postlind et al, 1993). Similarly, some dietary flavonoids can bind to AhR as antagonists or agonists, depending on their concentrations. At lower concentrations, flavonoids function as AhR antagonists by binding to the receptor without activation of a transcription factor, while at higher concentrations the same flavonoids might function as AhR agonists, activating gene expression and resulting in the induction of CYP1 family enzymes (Hodek et al., 2002). Actually, extracts from numerous dietary herbal supplements, vegetables, fruits, and teas have shown the ability to activate or inhibit AhR
(Jeukens et al., 2003; Amakura et al., 2002, 2003). Ligands for PXR present in diet and herbal remedies have also been identified. St John’s wort promotes the metabolism of coadministered drugs, including the HIV protease inhibitor indinavir, the immunosuppressant cyclosporin, and oral contraceptives (Ernst et al., 1998; Piscitelli et al., 2000; Fugh-Berman, 2000) through the induction of hepatic and intestinal CYP3A4, an enzyme catalyzing the biotransformation of the drugs. The component in this herbal remedy responsible for the induction is thought to be hyperforin since it is a potent agonist of human PXR (Moore et al., 2000; Wentworth et al., 2000), leading to the induction of CYP3A4 and Pgp in human tissues (Moore et al., 2000; Dürr et al., 2002). Recently, some forms of vitamin E have been shown to activate CYP3A gene expression via the PXR. In particular, tocotrienols induce the expression of a PXR-driven reporter gene and the expression of endogenous CYP3A4 and CYP3A5 (Landes, et al., 2003a, 2003b; Brigelius-Flohé, 2003). In light of the highly variable human diet, it is conceivable that the concentrations of these naturally occurring ligands vary significantly among individuals, comprising an important factor in the scenario of variable P450 induction.

It has been well documented that many AhR ligands are man-made environmental chemicals including pesticides, air pollutants, and occupational chemicals (Ma and Lu, 2003). For instance, halogenated aromatic hydrocarbons can stimulate the induction of CYP1A through their agonistic action on the receptor. Recently, reports on environmental chemicals identified as PXR and/or CAR ligands are starting to emerge, while only based on animal data or in vitro human systems. Several organochlorine pesticides have been reported for the first time to activate human PXR and induce CYP3A4 expression by Coumoul et al (2002) using Northern blot and transient transfection assays in various cell lines. Similar observation has been made in rats treated with 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), a persistent metabolite of the notorious pesticide DDT. It
was found that the induction of rat hepatic CYP3A1 and 2B1 by DDE correlated with CAR and PXR activity. In addition, in receptor transactivation assays, both CAR and PXR transcriptional activities were significantly enhanced by DDE (Wyde et al., 2003). In principle, individuals may have different levels of P450 induction depending on their exposure to those chemicals. However, environmental chemicals are widespread and present as mixtures, and human exposure is often a long term process at low dose and through multiple routes. Therefore, their impact on P450 induction in humans is complex and difficult to assess.

**Future research**

In this commentary, we outlined the major factors responsible for the individual variability in P450 induction. It is clear that the main cellular components governing the induction process include drug transporters, drug metabolizing enzymes, and various receptors mediating the signal transduction of the inducer. Much progress has been made in recent years in the identification of genotypes (SNPs and haplotypes) of Pgp, P450, CAR, PXR and other proteins with altered functions. This area of research will undoubtedly be continued and intensified in the years to come. It is important that the significance of the variants of these proteins in P450 induction be assessed in the *in vivo* pharmacokinetics studies and only those variants showing major effect on induction are important in evaluating the induction-mediated drug-drug interactions.

Reliable drug substrates selective for each of the major human liver microsomal P450s are now available for in vivo phenotyping studies, either using a single substrate or a mixture of probes in a cocktail. This in vivo approach provides not only a valuable way to assess each individual’s capacity to metabolize drugs based on their P450 genotypes, but also a meaningful way to evaluate the significance of each phenotype in a human subject in P450 induction using a probe substrate and an inducer selective for the particular P450. Through this type of study, the major genotypes
of Pgp (maybe other transporters), P450, PXR, and CAR, in both the coding and non-coding regions, which cause large changes in P450 induction, can be identified in a population. Information from such studies would greatly facilitate the assessment of individual variability in induction-based drug-drug interactions.

Another important area of research is to determine the major components in our diet and environment that are potent P450 inducers or potent inhibitors of the inductive process. Again, major efforts should be focused on the confirmation of many dietary components and environmental chemicals that have been shown to be active in vitro by pharmacokinetic studies using probes selective for each P450. It is our belief that with a better understanding of the major determinants in P450 induction and a profile of the phenotypes of these determinants in each individual, the individual variability in induction-mediated drug-drug interactions can be adequately evaluated.
Acknowledgment

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References


Legends for Figures

Fig. 1. Scheme of CYP3A induction and major factors contributing to variable induction

Agonists (rifampin or other endogenous/exogenous ligands) activate PXR (either present in nucleus or in cytoplasm and translocated into nucleus upon agonist binding), which then bind to the target response elements (RE) as heterodimers with RXRs, leading to transcription of CYP3A genes. The extent of induction can be affected by tissue and intracellular concentration of agonists (Pgp-mediated efflux, metabolism by enzymes), genetic variations of P450 genes and their regulatory regions, genetic variations of receptors and regulatory proteins required for induction, as well as physiological and environmental variables.
Table 1. Examples of inter-subject variability in induction

<table>
<thead>
<tr>
<th>Experimental System</th>
<th>Inducer &amp; Treatment</th>
<th>Endpoints Measured</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>Rifampin for 22 days, racemic mephenytoin at 0 and 22 days</td>
<td>Increase in urinary mephenytoin R/S ratio; from no increase to 8-fold increase</td>
<td>Zhou et al (1990)</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>Rifampin for 5 days, triazolam at 0 and 6 days</td>
<td>After induction, the plasma triazolam AUC in subjects decreased to 1% - 12%, a 12-fold variation</td>
<td>Villikka et al (1997)</td>
</tr>
<tr>
<td>Patients with normal hepatic and renal function</td>
<td>Rifampin for 4 days, $^{14}$C-erythromycin at 0 and 4 days</td>
<td>Increase in $^{14}$CO₂ by breath test; from 20% to 400% increase</td>
<td>Watkins et al (1989)</td>
</tr>
<tr>
<td>Biopsy of small bowel mucosa from healthy volunteers</td>
<td>Rifampin for 7 days</td>
<td>CYP3A4 mRNA, from no increase to 11-fold increase</td>
<td>Kolars et al (1992)</td>
</tr>
<tr>
<td>Liver microsomes from patients with digestive tumors</td>
<td>Omeprazole for 4 days, microsomes prepared from liver biopsy samples before and after treatment</td>
<td>Microsomal CYP1A2 contents increased by 2 to 8-fold, 1A2-related enzyme activities increased by 2 to 7-fold</td>
<td>Diaz et al (1990)</td>
</tr>
</tbody>
</table>
Table 2. Major Factors Responsible for the Individual Variability in P450 Induction by Drugs

<table>
<thead>
<tr>
<th>Factors</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable transport and metabolism of inducers in vivo</td>
<td>Variable tissue and intracellular inducer concentrations resulting in different extent of induction</td>
</tr>
<tr>
<td>Genetic variations of P450 genes and their regulatory regions</td>
<td>Altered pattern of induction</td>
</tr>
<tr>
<td>Genetic variations of receptors and regulatory proteins required for induction</td>
<td>Altered pattern of induction</td>
</tr>
<tr>
<td>Physiological factors: disease states (inflammation, infection, and liver dysfunction), gender, hormonal homeostasis</td>
<td>Altered response to inducers</td>
</tr>
<tr>
<td>Environmental factors: dietary components, environmental contaminants</td>
<td>Altered response to inducers</td>
</tr>
</tbody>
</table>
Variables:

**Physiological**
- Endogenous Ligand
- Disease, Gender, Age
- Endocrine Homeostasis

**Environmental**
- Dietary Component
- Environmental Ligand: Agonist, Partial Agonist, Antagonist

Polymorphism in PXR and RXR

Intracellular Concentration of Inducer

Rifampin

Ligands:
- Endogenous
- Xenobiotic

Polymorphism in CYP3A

Export by p-glycoprotein

Metabolism of ligand

P450 3A in ER

mRNA

Nuclear RNA

DR3 ER3 DR4

XRE

CYP3A

+1

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Figure 1.