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Expert Review

CYP Induction-Mediated Drug Interactions: *in Vitro* Assessment and Clinical Implications

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Abstract. Cytochrome P450 (CYP) induction-mediated interaction is one of the major concerns in clinical practice and for the pharmaceutical industry. There are two major issues associated with CYP induction: a reduction in therapeutic efficacy of comedications and an induction in reactive metabolite-induced toxicity. Because CYP induction is a metabolic liability in drug therapy, it is highly desirable to develop new drug candidates that are not potent CYP inducer to avoid the potential of CYP induction-mediated drug interactions. For this reason, today, many drug companies routinely include the assessment of CYP induction at the stage of drug discovery as part of the selection processes of new drug candidates for further clinical development. The purpose of this article is to review the molecular mechanisms of CYP induction and the clinical implications, including pharmacokinetic and pharmacodynamic consequences. In addition, factors that affect the degree of CYP induction and extrapolation of *in vitro* CYP induction data to *in vivo* situations will also be discussed. Finally, assessment of the potential of CYP induction at the drug discovery and development stage will be discussed.

KEY WORDS: aryl hydrocarbon receptor; constitutive androstane receptor; cross talk; glucocorticoid receptor; hepatic and intestinal CYP induction; *in vitro*/*in vivo* extrapolation; interindividual variability; pregnane X receptor; species differences; xenobiotic responsive elements.

INTRODUCTION

Cytochrome P450 (CYP) induction-mediated interaction is one of the major concerns in clinical practice and for the pharmaceutical industry because of potential involvement of multidrug therapy. There are two major issues associated with CYP induction. First, induction may cause a reduction in therapeutic efficacy of comedications. For drugs whose effect is produced primarily by the parent drug, induction would increase the drug's elimination, resulting in lower drug concentrations, and decrease the drug's pharmacological effect. For instance, rifampicin had caused acute transplant rejection in patients treated with cyclosporine, presumably because of induction of the CYP3A4-mediated metabolism of cyclosporine (1,2). Second, induction may create an undesirable imbalance between detoxification and activation as a result of increased formation of reactive metabolites, leading to an increase in the risk of metabolite-induced toxicity (3,4). For example, induction of CYP1A enzymes may activate some xenobiotics to their reactive metabolites, leading to toxicity (5,6). Induction of CYP2E1 is also considered undesirable because the enzyme level of CYP2E1 highly correlates with the susceptibility of hepatotoxicity (7). CYP2E1 induction is believed to increase the severity of hepatotoxicity not only by increasing the formation of reactive metabolites of numerous compounds, but also by generating reactive oxygen species (oxidative stress) during its catalytic cycle.

Unlike CYP inhibition, which is an almost immediate response, CYP induction is a slow regulatory process. It takes time to reach a higher steady-state enzyme level as a result of a new balance between the rate of biosynthesis and degradation (8). Similarly, it also takes time to return the enzyme basal level after discontinuing the treatment with inducer. Kinetic analysis of verapamil trough concentration

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ABBREVIATIONS: AF-2, activation factor 2; AhR, aryl hydrocarbon receptor; Arnt, AhR nuclear translocator; AUC_{iv}, area under plasma concentration–time curve after intravenous dosing; AUC_{po}, area under plasma concentration–time curve after oral dosing; CaMK, calcium/cAMP-dependent kinase; CAR, constitutive androstane receptor; CCRP, cytoplasmic CAR retention protein; C_{max}, maximal plasma concentration following oral dose; CYP, cytochrome P450; DNA, deoxyribonucleic acid; DBD, DNA binding domain; DMSO, dimethylsulfoxide; DR, direct repeat; EC_{50}, effective concentration for 50% maximal CYP induction; ER, everted repeat; EM, extensive metabolizer; E_{max}, maximal CYP induction; GR, glucocorticoid receptor; GSH, glutathione; HIV, human immunodeficiency virus; Hsp90, heat shock protein 90; INR, international normalized ratio; IR, inverted repeat; LBD, ligand binding domain; mRNA, messenger ribonucleic acid; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NF1, nuclear factor 1; PAS, Per-Arnt-Sim; PB, phenobarbital; PBREM, PB-responsive enhancer module; PCN, pregnenolone-16α-carbonite; PhIP, 2-amino-1-methyl-6-phenyl-imidazole[4,5-b]pyridine; PM, poor metabolizer; PXR, pregnane X receptor; RARE, retinoid acid response element; RXR, retinoid X receptor; SNP, single nucleotide polymorphism; SRC-1, steroid receptor cofactor-1; TCDD, 2,3,7,8-tetraxchlorodibenzo-*p*-dioxin; TCPOBOP, (1,4-bis-[2-(3,5,-dichloropyridyloxy)]benzene); UGT, UDP-glucuronosyltransferase; XRE, xenobiotic-responsive element; XREM, xenobiotic-responsive enhancer module.

revealed that a full induction was reached in roughly 1 week after starting rifampicin, and the recovery to baseline activity was attained in about 2 weeks after discontinuing rifampicin (9). Because of the nature of time-dependent process, CYP induction may complicate drug-dosing regimens in chronic drug therapy. Addition of any potent inducer to or withdrawal of a potent inducer from an existing drug-dosing regimen may cause pronounced changes of drug concentrations, leading to failure of drug therapy or adverse effects. It should be performed gradually with appropriate monitoring for therapeutic efficacy and adverse events.

Because CYP induction is a metabolic liability in drug therapy, it is highly desirable to develop new drug candidates that are not potent CYP inducer. Ideally, the information on whether a new drug candidate is a potent CYP inducer should be obtained at the drug discovery stage before the drug candidate is selected for clinical development. Several *in vitro* models have been established to assess the potential of CYP induction, including liver slices, immortalized cell lines, and primary hepatocytes (10–13). Among these models, primary culture of human hepatocytes is probably the most predictive model for evaluating CYP induction. However, a major problem associated with the use of human hepatocytes has been the erratic supply of human liver tissue. From an industrial perspective, a convenient and reliable method for routine screening for potential CYP induction at the stage of drug discovery is highly desirable.

Recent advances in molecular biology have led to a significant increase in our understanding of the regulation of CYP enzymes. Kliewer *et al.* (14,15) first identified an orphan nuclear receptor, pregnane X receptor (PXR), that transcriptionally activates the *CYP3A* genes by interacting with the PXR response elements in the genes. Since then, numerous studies by other investigators confirmed that PXR transcriptionally activates the *CYP3A4* gene in humans (16,17). The discovery of PXR has led to the development of PXR reporter gene assays for screening the induction potential of drugs (18–20). Today, many drug companies routinely include the PXR gene assay at the drug discovery stage as part of the selection processes of drug candidates for clinical development. Several different intracellular receptors have also been identified to be involved in induction of CYP enzymes, including the aryl hydrocarbon receptor (AhR), the constitutive androstane receptor (CAR), and the glucocorticoid receptor (GR) (21).

The purpose of this article is to review the molecular mechanisms of CYP induction and clinical implications, including pharmacokinetic and pharmacodynamic consequences. In addition, factors that affect the degree of CYP induction and extrapolation of *in vitro* induction data will also be discussed. Finally, assessment of the potential of CYP induction at the drug discovery and development stage will be discussed.

MOLECULAR MECHANISMS OF CYP INDUCTION

One of the intriguing aspects of CYPs is that some of these enzymes, but not all, are inducible. Human CYP1A, CYP2A, CYP2B, CYP2C, CYP2E, and CYP3A enzymes are known to be inducible. Although CYP induction has been known for almost half century, our understanding of the molecular mechanisms of the induction has just begun, which is attributed to the recent advances in molecular biology and biotechnology. Recent studies indicate that most *CYP* genes are induced via receptor-mediated mechanisms leading to an increase in gene transcription. *CYP* gene families 2 and 3 have a similar mechanism of gene activation through a ligand-activated nuclear receptor (CAR and/or PXR). Both CAR and PXR belong to the same gene family (family NR1) and share a common heterodimerization partner, retinoid X receptor (RXR) (14,22). On the other hand, the AhR-regulating *CYP1A* genes belong to the Per-Arnt-Sim (PAS) family of transcription factors and require AhR nuclear translocator (Arnt) as its heterodimerization partner (23).

Whereas in most cases CYP induction is the consequence of an increase in gene transcription, some nontranscriptional mechanisms also are known to be involved. For example, troleandomycin induces rat CYP3A resulting from a decrease in the rate of CYP3A protein degradation without increasing the rate of protein synthesis (24). Similarly, induction of CYP2E1 by alcohol, acetone, and isoniazid is caused by a posttranslational mechanism by stabilization of the enzyme protein, not involving a receptor-mediated mechanism (25,26). Moreover, increased protein level of CYP2E1 in spontaneously induced diabetic rats seems to be caused by a nontranscriptional mechanism by stabilization of mRNA (27).

CYP Induction by Nuclear Receptors

Aryl Hydrocarbon Receptor

The human CYP1A subfamily consists of two members, CYP1A1 and CYP1A2. Whereas CYP1A2 is one of the major CYPs in human liver, accounting for approximately 10% of total amount of hepatic CYPs, CYP1A1 is mainly expressed in human lung, placenta, and lymphocytes in a much less abundance (21,23). Although both CYP1A1 and CYP1A2 are inducible, CYP1A1 is generally much sensitive to inducers than CYP1A2. The induction of CYP1A enzymes by polycyclic aromatic hydrocarbons has been studied extensively since 1970s. Initial evidence of AhR involvement in the induction of CYP1A enzymes is the identification of a hepatic cytosolic protein that exhibits stereospecific and high affinity binding to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), an environmental toxin (28). The discovery led to the identification of a gene that encodes the AhR. Subsequently, the role of AhR in CYP1A induction was confirmed by the studies in inbred strains of mice. TCDD is about 10-fold more potent in inducing CYP1A1 in AhR-more-responsive strains (C57B/6) than in AhR-less-responsive strains (DBA/2) (29). In this study, both the TCDD inducibility and the expression level of AhR correlate well with the genetic polymorphism of AhR in mice.

Additional study in mouse hepatoma cell lines led to the identification of a second regulatory protein, Arnt, in CYP1A induction (30). Three hepatoma cell lines, namely, wild-type, AhR-defective, and Arnt-defective cell lines, were used in this study. The AhR-defective variant cells contained an altered AhR, which had low affinity for TCDD, and responded poorly to TCDD. Whereas TCDD binding to the AhR in Arnt-defective cells was almost identical to that in

wild-type cells, the Arnt-defective cells failed to respond to TCDD (30). Collectively, these data indicate that both AhR and Arnt are required for CYP1A induction. Both the AhR and Arnt are expressed various tissues, being abundant in liver and less abundant in intestine and lung (31). The unliganded AhR is almost exclusively located in the cytoplasma, whereas the Arnt is found to be exclusively nuclear with or without ligands (inducers).

Both AhR and Arnt are prototypical members of the basic helix–loop–helix (bHLH) proteins belonging to the PAS family of transcription factors (23). The structure is quite similar between the AhR and Arnt; the N-terminal regions of both the AhR and Arnt have a bHLH domain and a PAS region with two subdomains (PAS A and PAS B) juxtaposed to the bHLH domain. The basic region (b) contributes to DNA binding, and the HLH region is responsible for protein–protein dimerization. Protein-cross-linking studies suggest that the basic region of Arnt binds to the 5′GTG3′ sequence of xenobiotic-responsive element (XRE) of *CYP1A1* gene, whereas the basic region of AhR binds neighboring nucleotides. The PAS domains also contribute to protein–protein dimerization and influence the DNA recognition by bHLH/PAS heterodimer. Interestingly, despite their structural similarity, the PAS domains of AhR and Arnt do not function identically. For example, the PAS domain of AhR binds to ligand and heat-shock protein (hsp90), whereas Arnt PAS domain binds neither (23). The unliganded AhR is maintained in cytoplasma as a complex containing chaperone proteins, such as dimer of heat-shock protein Hsp90 and p23 that are required in the stabilization of AhR (31). Upon ligand binding, AhR sheds the chaperone proteins away and translocates into the nucleus, where it forms heterodimer with the Arnt through the HLH and PAS domains of both proteins.

Although it was originally believed that Arnt translocates AhR into the nucleus, the original belief has been challenged by intracellular distribution studies of Arnt. If Arnt does translocate AhR into the nuclei after inducer exposure, the distribution of Arnt is expected to be increased in the nuclei. Using anti-Arnt antibody, immunohistochemical studies revealed that there were no detectable changes in the Arnt distribution in the nucleus when comparing the nucleus in uninduced mouse hepatoma cells with that in induced cells (23,32). These results therefore strongly suggest that the Arnt does not involve in the translocation of AhR. In support of the notion that Arnt is not a nuclear translocator, the AhR can still translocate into the nucleus even in Arnt-deficient cells (32). One possible explanation for the misconception of the original belief is that the process of AhR/Arnt heterodimerization produces the appearance of AhR translocation because it leads to a shift in the distribution of the liganded AhR toward the nucleus.

The AhR/Arnt heterodimer (complex) activates *CYP 1A1* gene by interacting with responsive elements of the gene. Transfection experiments revealed that the DNA upstream of the *CYP1A1* gene contains two types of transcriptional elements. The more distal element has the functional properties of AhR-dependent transcriptional enhancer, spanning about 400 base pairs (bp) in about 1000 bp upstream of the CYP1A1 transcription start site, whereas the more proximal element functions as a transcriptional promoter, spanning about 150 bp immediately near the transcriptional start site (33,34). The enhancer contains at least three binding sites for the AhR/Arnt complex. In contrast, the promoter has no binding sites for the AhR/Arnt complex. Together with the findings of AhR and Arnt, the discovery of transcriptional elements in the *CYP1A* gene provides the basis for our understanding of how the cells recognize the inducers and how the induction signal is conveyed to the transcriptional machinery.

Transfection studies revealed that neither the enhancer nor the promoter, individually, is sufficient to mediate a transcriptional response to TCDD, suggesting that there must be a mechanism for transmitting the induction signal from enhancer to promoter after binding of the AhR/Arnt heterodimer to the AhR-responsive enhancer (34). However, the precise mechanism of enhancer–promoter communication is still not fully understood. Studies by Wu and Whitlock (35) suggest that the mouse *Cyp1a1* promoter is inaccessible to transcriptional factors that are constitutively present in uninduced cells, whereas the AhR/Arnt complex–enhancer interaction increases the accessibility of the promoter, allowing the transcriptional factors to bind and transcription to occur. Furthermore, genetic reconstitution experiments revealed that deletion of the C-terminal region of AhR abolished the ability of AhR/Arnt complex to induce changes in chromatin structure at the promoter, whereas the deletion had no effect on changes in chromatin structure at the enhancer. Collectively, these results suggest that the enhancer–promoter communication involves changes in chromatin structural in two steps. The first step is local chromatin structural changes at the enhancer after AhR/Arnt binding to the enhancer, which does not require the C-terminal region of AhR, and the second step is local chromatin structural changes at the promoter that does require the C-terminal region of AhR (23).

Constitutive Androstane Receptor

Compared with the well-studied AhR-mediated induction of *CYP1A* genes, the progress of our understanding of the molecular mechanism of CYP induction within subfamilies 2 and 3 has been relatively slow. Although the key nuclear receptors, including CAR, PXR, and GR, have been identified for more than a decade, their functional role in the induction of *CYP2B*, *CYP2C*, and *CYP3A* genes has just only begun to understand. These receptors form a tangle of networks and share the overlapping ligands and DNA response elements of target genes (36). Functional studies have shed light on the nature of interplay between these receptors. However, there are still many regulatory mechanisms that remain to be elucidated. For example, one of the unsolved issues of CYP induction is how can a single chemical compound induce more than one *CYP* gene. Phenobarbital (PB) is a potent inducer for human CYP2B6, and, to a lesser extent, it also induces human CYP2C9 and CYP3A4. Likewise, human CYP3A4 is efficiently induced by rifampicin, whereas CYP2B6 and CYP2C9 are also induced by rifampicin to a lesser extent (36).

CAR was first isolated in 1994 by Baes *et al.* (37) through screening of a cNDA library. The functional role of CAR in CYP2B induction has been clearly demonstrated in

transgenic mice. Although treatment with PB significantly induced *Cyp2b10* gene in $CAR^{(+/+)}$ mice, PB had no inductive effect in $CAR^{(-/-)}$ mice (38). The study of CAR null mice has provided direct evidence that CAR is an essential mediator of the transcriptional response to PB. In contrast to the classical nuclear receptors, which are activated by their cognate ligands, the CAR forms a heterodimer with RXR that binds to retinoid acid response elements (RAREs) and transactivates the target genes of RAREs in a constitutive manner in the absence of ligands. Because CAR can transactivate the target genes in a constitutive manner without ligands, CAR is referred to as "constitutive androstane receptor." The unique nature of CAR has led to an intensive search of endogenous ligands of the receptor. While searching for potential ligands of CAR, androstane metabolites, such as androstanol and androstenol, were found to effectively inhibit the constitutive activity of CAR (39). Forman *et al.* (40) showed that the intrinsic activity of CAR was completely inhibited by endogenous steroids androstanol and androstenol, with an IC_{50} value of about 400 nM. These androstane ligands are good examples of naturally occurring inverse agonists that reverse transcriptional activation by nuclear receptors.

The CAR/RXR heterodimer transcriptionally activates the *CYP2B* genes by interacting with the PB-responsive DNA elements (PB-responsive enhancer module; PBREM). A major breakthrough in CYP2B induction was made by Trottier *et al.* (41) who identified a 163-bp PBREM in the rat *CYP2B2* 5′-flanking region situated at −2318 to −2155 bp upstream of the transcription start site. The identification of PBREM made a very significant contribution to our understanding of the role of CAR in the induction of *CYP2B* genes by PB and a number of PB-like inducers. Sequence comparison of the rat *CYP2B2* PBREM and the subsequently identified mouse *Cyp2b10* and human *CYP2B6* PBREM revealed that the PBREM is a conserved arrangement of two nuclear binding sites (NR1 and NR2) and a nuclear factor 1 (NF1) binding site between NR1 and NR2 (42,43).

Both the NR1 and NR2 are direct repeat separated by 4-bp (DR-4) motifs. NR1 is identical between rats and mice, and only a single base pair difference is observed in human NR1. Only the NR binding sites are essential for the PB response activity, although the NF1 binding site may be required to confer full PBREM activity (22,44). Mutations of either NR1 or NR2 decreased PBREM activity to one third of the wild-type activity in transfected primary hepatocytes, whereas simultaneous mutations of both NR sites completely abolished the PBREM activity. The responsive elements that confer induction by PB have also been identified for *CYP2C* and *CYP3A* genes. In contrast to the highly conserved CYP2B PBREM, there are marked species differences in the amino acid sequence of PBREM of *CYP2C* and *CYP3A* genes (36).

CAR is predominately expressed in the liver and, to a lesser extent, in the intestine (37). Similar to AhR, CAR is located in the cytoplasma of hepatocytes of uninduced mice (45). Western blot as well as immunohistochemical analyses confirmed that CAR is completely absent in the liver nuclei of untreated mice. After the administration of PB in primary hepatocytes, CAR is accumulated rapidly in the nuclei, suggesting that CAR nuclear translocation is the first activation step in response to PB (45). However, even with sensitive *in vitro* binding assay, PB has not been shown to bind directly to either human CAR or mouse CAR. Therefore, ligand binding seems not to be critical for CAR nuclear translocation. The question of how PB translocates CAR into the nucleus leads to an extensive investigation. Although the mechanism involved in CAR nuclear translocation is still not fully understood, it seems to involve a specific and sensitive dephosphorylation event. In mouse primary hepatocyte cultures, the phosphatase inhibitor okadaic acid was found to effectively inhibit PB-mediated CAR translocation and *Cyp2b10* induction, providing evidence that dephosphorylation of mouse CAR is required for the nuclear translocation process (46). In addition, CAR nuclear translocation seems to be dependent on a leucine-rich region near the C-terminus of the receptor (47).

Immediately after being accumulated in the nucleus, CAR forms heterodimer with RXR and interacts with DNA (48). Studies with a reporter gene assay suggest that CAR alone without ligand is able to stimulate PBREM, supporting the notion that CAR is transcriptionally active in the absence of inducer (48). However, nuclear activation of CAR seems to be quite complicated and requires coregulators. It is believed that CAR adopts its active conformation by recruiting transcriptional coactivator, such as steroid receptor cofactor-1 (SRC-1), in the absence of ligand and is shifted toward an inactive conformation by binding to the naturally occurring inverse agonists, such as androstanol and androstenol through dissociation of the coactivators SRC-1 from CAR, resulting in the suppression of *CYP2B* gene transcription. This suppression is overcome by treatment with PB-like inducers, which abolish the inhibitory effects of inverse agonists from CAR. Interestingly, recent studies with PB-induced mouse primary hepatocytes revealed that calcium/cAMP-dependent kinase (CaMK) might be involved in the CAR activation process (49). This finding has added an additional dimension of the complexity to gene regulation by CAR.

Pregnane X Receptor

Although CYP3A enzymes have long been known to be inducible, the molecular mechanism remained unknown until the identification of PXR. Database searching and subsequent cDNA cloning resulted in the discovery of a new mouse orphan nuclear receptor designated PXR by Kliewer *et al.* in 1998 (14). Direct evidence that PXR mediates the induction of *CYP3A* genes was provided by transgenic mice. Although pregnenolone-16α-carbonite (PCN) and dexamethasone significantly induced *Cyp3a11* gene in wild-type $PXR^{(+/+)}$ mice, the $PXR^{(-/-)}$ mice failed to induce *Cyp3a11* gene expression when challenged with PCN or dexamethasone (50). Soon after the discovery of mouse PXR, human PXR was identified by Bertilsson *et al.* (17). Since then, PXR orthologs in rats, rabbits, dogs, and monkeys have also been cloned (51–54). PXR is expressed predominately in human liver and, to a lesser extent, in small intestine, and it mediates the induction of CYP3A4, CYP2B, and CYP2C enzymes. PXR is a promiscuous nuclear receptor, which can be activated by numerous structurally diverse xenobiotics and drugs, and is referred as the "master" regulator of CYP enzymes. The number of compounds identified as PXR li-

gands continues to grow (19,20). In addition to rifampicin, two high-affinity human PXR ligands, hyperforin and SR12813, have been identified (55). The former is a putative active component of St. John's wort, and the latter is an investigational cholesterol-lowering drug. However, there is no obvious quantitative structure/activity relationship of these ligands.

Activation of PXR seems to be less complicated compared to that of CAR. PXR is generally thought to be retained in the nucleus and can be activated by direct binding of ligand (22,36). However, Squires *et al.* (56) have recently shown that PXR is located in the cytoplasma of untreated mouse liver cells and is concentrated in the nucleus after treatment with PCN, suggesting translocation of PXR into the nucleus. Using hsp90 antibody, these investigators further demonstrated that PXR formed a complex with endogenous cytoplasmic CAR retention protein (CCRP) and hsp90 in HepG2 cells. Based on these results, the investigators concluded that nuclear translocation of PXR occurs, and the PXR–CCRP–Hsp90 complex maintains the receptor in the cytosol. Apparently, the finding of nuclear translocation of PXR in mice is not consistent with the general belief that PXR is expressed in the nucleus. Certainly, Squires *et al.*'s finding will stimulate future research on the nuclear localization and the mechanism of PXR activation following drug treatment.

Like many other nuclear receptors, PXR contains two functional domains: ligand binding domain (LBD) and highly conserved DNA binding domain (DBD). The highly conserved DBD, characterized by two C4-type zinc fingers, links the PXR to the specific regions of the target genes (57). Crystal structure analyses suggest that the LBD of the human PXR is highly hydrophobic and flexible. The unique structure of the ligand pocket not only allows PXR to bind a diverse set of substrates of different molecular size, but also permits a single molecule to dock in multiple orientations (58). This explains why PXR can be activated by various structurally diverse ligands. The LBD serves as the binding site for ligands and also contains the transcriptional activation domains such as the activation function-2 (AF-2) helix. The binding of a ligand to the LBD results in a conformational change in AF-2 helix, leading to the recruitment of coactivators and cointegrators, and, in turn, transactivation of the target genes (57).

PXR interacts with its cognate response elements in the 5′-flanking region of the target genes as a heterodimer with RXR. Three DNA binding sites have been identified for human *CYP3A4* gene. One site is located at approximately −150 bp region of the proximal promoter, and another two sites are found between −7733 and −7672 bp of the distal enhancer region of *CYP3A4* gene, termed as xenobiotic-responsive enhancer module (XREM) (22). The XREM is composed of two hexameric half-sites [AG(G/T)TCA] organized as a direct repeat with a three-nucleotide spacing (DR-3) and an everted repeat separated by 6 bp (ER-6), whereas the promoter element contains an ER-6 motif (59). Mutation studies suggested that all three DNA binding sites are required for PXR activation. A mutation of either one of these three sites alone decreased the response activity only by 30%, whereas mutation of all three sites completely abolished the response activity (22).

In conclusion, although the discovery of nuclear receptor represents a major breakthrough that contributes significantly to our understanding of the induction of *CYP* genes, much remains to be learned about the molecular mechanisms of CYP induction. For example, how does the AhR/Arnt, CAR/RXR, or PXR/RXR heterodimers access its binding sites within nucleosomes, and how does the enhancer–promoter communication take place after the binding of the heterodimers to their cognate responsive elements? Do these nuclear receptors require additional coregulators and cofactors to trigger the gene transcription, and what are their identities? Considering a new steady-state enzyme level by a new balance between the rate of biosynthesis and degradation of CYP enzymes during induction (60), does a feedback mechanism exist in equilibrium between the rate of biosynthesis and degradation of CYP enzymes? Could the rate of CYP degradation be altered during CYP induction?

Cross Talk between CAR and PXR

One of the intriguing aspects of CYP induction is the overlapping induction profiles of CYP2B, CYP2C, and CYP3A enzymes with respect to one single inducing agent. Rifampicin is known to be a good inducer of *CYP2B6*, *CYP2C9*, and *CYP3A4* genes in humans (36,57). Similar to the overlapping induction profiles of CYP enzymes, accumulating evidence from *in vitro* studies indicates that there is a remarkable redundancy between CAR and PXR with regard to the overlapping ligand spectrum. In addition, there is also a significant overlapping affinity between the binding of CAR and PXR to the DNA response elements of many genes. As will be discussed later, each of *CYP* genes contains multiple xenobiotic response elements, and each of the response elements can be recognized by more than one nuclear receptor. The process that individual gene can be activated by more than one nuclear receptors is often referred to as "cross talk." In fact, the cross talk (interplay) between CAR and PXR becomes one of the central themes in the field of CYP induction.

The cross talk between CAR and PXR is best illustrated by the use of CAR and PXR null mice. For example, both *Cyp2b10* and *Cyp3a11* genes were significantly induced by PB and 1,4-bis-[2-(3,5,-dichloropyridyloxy)]benzene (TCPOBOP) in $CAR^{(+/+)}$ mice, whereas the inducers failed to induce *Cyp2b10* and *Cyp3a11* genes in $CAR^{(-/-)}$ mice (61). In contrast, dieldrin and clotrimazole (PXR activators) greatly increased *Cyp3a11* gene, but not *Cyp2b10* gene in both $CAR^{(+/+)}$ and $CAR^{(-/-)}$ mice (61). These results suggest that *Cyp3a11* gene can be activated by not only PXR, but also CAR. The CAR-mediated induction of *Cyp3a11* gene is further supported by the study with $PXR^{(-/-)}$ mice. In the PXR null mice, *Cyp3a11* was efficaciously induced by clotrimazole and PB (62). Collectively, these results strongly suggest that *CYP3A* genes can be induced by both PXR and CAR through a cross talk.

Consistent with the results with transgenic mice, Xie *et al.* (62) reported that CAR/RXR heterodimer is able to bind and transactivate the response elements for PXR/RXR heterodimers in *CYP3A* genes in a transfected cell assay. These investigators provided compelling evidence that CAR was able to activate a response element (IR-6) from the human *CYP3A4* gene, when a synthetic CYP3A4/IR-6

containing reporter gene was introduced into CV-1 cells in the presence of CAR. Similarly, CAR can also bind to a PXR response element (DR-3) of rat *CYP3A23* gene and activate the gene (62). In addition, Goodwin *et al.* (63) have shown that *CYP3A4* gene can be induced by CAR. The CAR-mediated CYP3A4 induction was shown to be primarily mediated by two high affinity-binding sites located in the distal XREM and promoter proximal regions of the *CYP3A4* gene (63). Collectively, these *in vitro* results further support the *in vivo* observations that CAR can bind to the response elements of *CYP3A* genes.

Additionally, it has been demonstrated that PXR is able to activate *CYP2B* genes by binding to the response elements of *CYP2B* genes. In an *in vitro* study using an electrophoretic shift assay, it was clearly demonstrated that PXR bound to the NR1 of the PBREM of human *CYP2B6* gene in a fashion similar to the binding of CAR to the PBREM (62). In another study with primary human cultured hepatocytes, *CYP2B6* gene was highly inducible by a number of compounds known to be potent human PXR ligands, including rifampicin and hyperforin (64). In this study, PXR was also shown to be capable of activating the PBREM of *CYP2B6* gene, a 51-bp enhancer element that mediates induction of *CYP2B6* gene by CAR (64). Recently, a distal XREM was identified in the promoter of *CYP2B6* gene (65). Functional analyses demonstrated that the XREM could also be activated by PXR. Interestingly, maximal activation of *CYP2B6* gene expression was observed with a luciferase reporter construct containing both the PBREM and XREM (65). In addition, Smirlis *et al.* (66) reported that CAR and PXR competed with each other for binding to the same responsive element, when equimolar amounts of CAR and PXR expression vector were cotransfected with a PBREM-reporter construct. The ability of CAR and PXR to bind the common elements clearly indicates that the cross talk between these two nuclear receptors occurs.

In addition to *CYP3A* and *CYP2B*, many other genes of drug-metabolizing enzymes and transporters are also known to be activated by CAR and PXR (67–71). It is likely that the cross talk between CAR and PXR might also be involved in the induction of these enzymes and transporters. In fact, CAR/PXR cross talk has been reported for the CYP2C9 induction (52). Two functional responsive elements in the regulatory region of *CYP2C9* gene have been identified: a glucocorticoid-responsive element (−1648/−1684 bp) and a CAR-responsive element (a DR4 motif at −1803/−1818 bp). Interestingly, the CAR-responsive element of the *CYP2C9* gene is not only recognized by CAR, but also activated by PXR (52). The cross talk between CAR and PXR has also been reported for the induction of UDP-glucuronosyltransferase (UGT) 1A1 (69,70). Both CAR and PXR are able to activate *UGT1A1* gene by binding to a common 290-bp distal enhancer sequence (−3499/−3210) of the gene. Collectively, CAR and PXR are able to bind common response elements in the *CYP3A4*, *CYP3A23*, *Cyp3a11*, *CYP2B6*, *Cyp2b10*, *CYP2C9*, and *UGT1A1* genes.

Glucocorticoid Receptor

The role of GR on CYP induction has long been a controversial topic, and there are conflicting reports on this subject. Using $GR^{(+/+)}$ and $GR^{(-/-)}$ mice, Schuetz *et al.* (72) demonstrated that dexamethasone (a GR activator) failed to induce *Cyp2b10* gene in GR-null mice, whereas *Cyp2b10* gene was readily induced in $GR^{(+/+)}$ mice. Unlike *Cyp2b10* gene, *Cyp3a11* gene was induced by dexamethasone in either $GR^{(+/+)}$ and $GR^{(-/-)}$ mice. These results suggest that GR is essential for the induction of *Cyp2b10* gene, whereas the induction of *Cyp3a11* gene is independent of GR. The lack of GR effect on *Cyp3a11* gene suggests that GR has little effect on PXR activation in mice. In contrast, GR seems to play an important role in human PXR activation. In Huh7 cells, dexamethasone had no effect on PXR-mediated *CYP2B6* gene expression, when human PXR was only cotransfected with PBREM. Upon cotransfection of GR with PXR and PBREM, dexamethasone greatly enhanced PXR-mediated *CYP2B6* gene expression in Huh7 cells (73). These results suggest that dexamethasone enhances PXR-mediated activation of *CYP2B6* gene expression by a GR-dependent mechanism. Similarly, GR has a direct effect on human CAR-mediated induction. When human CAR was cotransfected with *CYP2B6* PBREM and GR into Huh7 cells, GR enhanced CAR-mediated *CYP2B6* gene expression (73). These results suggest that dexamethasone exhibits synergistic effect on gene expression through CAR- and PXR-dependent manner in humans, indicating that GR may function as a coregulator that facilitates the binding of CAR and PXR to the target response elements.

In addition to synergistic effect, GR may induce *CYP* genes expression by directly interacting with the responsive elements of *CYP* genes without interfering CAR- or PXR-mediated pathways. Deletion analysis of *CYP2C9* regulatory region suggested the presence of a glucocorticoid-responsive element (−1648/−1684 bp) in *CYP2C9* gene. Further studies showed that GR can interact directly with the glucocorticoid-responsive element (52). Identification of these functional elements provides molecular basis for CYP2C9 induction by dexamethasone. Like *CYP2C9* gene, *CYP3A4* gene can also be induced by glucocorticoids through a PXR-independent pathway (74). This pathway involves hepatocyte-nuclear-factor-3/CCAAT enhancer binding protein α (HNF-3/CEBP) binding site in the proximal promoter of the *CYP3A4* gene. Mutation of the HNF-3/CEBP resulted in a decrease in CYP3A4 induction by dexamethasone, but had no effect on the PXR-dependent CYP3A4 induction by rifampicin (74). Taken together, it is strongly suggested that the GR can enhance CYP induction through PXR- or CAR-dependent, and/or independent, pathways.

Recently, the role of GR on the gene regulation of CAR and PXR has been reported. Studies by Pascussi *et al.* (75) have demonstrated that PXR seems to be regulated by GR. Using human hepatocytes, both RXR and PXR mRNA expressions were increased by dexamethasone, but not by rifampicin and clotrimazole. Similarly, a significant increase in PXR and RXR mRNA by dexamethasone was also reported by Huss and Kasper (76). As for the PXR, dexamethasone also enhanced the CAR expression in human hepatocytes in a GR-dependent manner (77). Several lines of evidence support the hypothesis that the expressions of PXR and CAR are induced by GR: (1) dexamethasone did not affect the degradation of PXR and CAR mRNA; (2) increases in PXR and CAR mRNA by dexamethasone were

reduced by the glucocorticoid antagonist; and (3) increases in PXR and CAR mRNA by dexamethasone were inhibited by actinomycin D, an RNA synthesis inhibitor (75,77). Therefore, it is reasonable to conclude that the GR may function as a regulator for the regulation of both CAR and PXR.

A marked sexual dimorphism in CAR expression in Wistar Kyoto (WK) rats has been reported (78). Treatment of male WK rats with PB significantly increased the level of *CYP2B1* mRNA in a time-dependent manner, whereas PB had no inductive effect on the level of *CYP2B1* mRNA in female rats. Additional studies revealed extremely low levels of CAR expression in the female WK rats compared with male rats. Based on these results, the investigators concluded that the sex-dependent induction of *CYP2B1* gene in WK rats is attributable to the marked difference in the CAR expression between male and female rats. Because of the marked gender difference in the CAR expression, it is expected that GR will have differential effect on the CAR expression between male and female WK rats. Therefore, the WK rat may serve as a good animal model for the evaluation of the GR effect on the regulation of CAR.

Species Differences in Nuclear Receptors

To date, our understanding of the molecular mechanisms regarding the role of nuclear receptors in the induction of *CYP* genes is based mainly on *in vitro* models and animal studies. Although it is believed that CYP induction is regulated in humans in principle in the same fashion as in animals, inductive response to inducers is markedly different, both quantitatively and qualitatively, among animal species. For example, omeprazole, a gastric-acid-suppressing drug, is a good CYP1A enzyme inducer in humans, but has little inductive effect in mice or rabbits (79,80). Similarly, significant differences in CYP3A induction were also observed between animal species. Rat CYP3A enzymes are readily induced by PCN, whereas neither rabbit nor human CYP3A enzyme is induced by PCN. In contrast, rifampicin is a potent inducer for CYP3A enzymes in rabbits and humans, whereas it has little inductive effect on CYP3A enzymes in rats (81,82). Species-dependent induction of CYP1A1 and CYP1A2 by TCDD was also observed in cultured rat and human hepatocytes (83). TCDD induced predominately CYP1A1 in rat hepatocytes, whereas TCDD induced mainly CYP1A2 in human hepatocytes.

Marked species differences in CYP induction even occur between rodents. Degawa *et al.* (84) reported that significant species differences in CYP1A1/2 induction were observed among rodents, including mice, rats, hamsters, and guinea pigs, when treated with 2-amino-1-methyl-6-phenyl-imidazole[4,5-b]pyridine (PhIP). Treatment with PhIP caused significant increases in the levels of CYP1A1/2 in the liver of rats. However, CYP1A1/2 was not induced by PhIP in mice, hamsters, and guinea pigs. Species-dependent induction between mice and rats was also reported for CYP2B and CYP3A after treatment with tamoxifen (85). Treatment with tamoxifen resulted in significant increases in both CYP2B and CYP3A proteins as well as their enzyme activities in rats, whereas no induction was observed in mice. In contrast, treatment with 4-vinyl-1-cyclohexene caused significant induction of CYP2A and CYP2B in mice, but not in rats (86).

The molecular basis for the observed species differences in CYP induction has been intensively studied. A transfection study was conducted by Barwick *et al.* (87) to determine whether the observed species difference in CYP3A induction was a result of species differences in the sequences of the promoters in the *CYP3A* genes. The CYP3A promoters from rat and rabbit *CYP3A* genes were transfected separately into primary hepatocytes from rats and rabbits. The rat *CYP3A1* promoter was transcriptionally activated by PCN in rat hepatocytes, but not in rabbit hepatocytes. Conversely, the rabbit *CYP3A6* promoter was transcriptionally activated by rifampicin in rabbit hepatocytes, but not in rat hepatocytes. These results suggest that the observed species differences in CYP3A induction by xenobiotics are not caused by the structural differences in the gene promoter.

Subsequently, PXR was identified as the cellular factor responsible for species differences in CYP3A induction. Jones *et al.* (88) compared the CYP3A induction of a variety of different compounds, such as dexamethasone, RU486, clotrimazole, PB, and rifampicin, in rat and rabbit hepatocytes with their ability to activate the rat and rabbit PXR. There were marked differences in the induction of *CYP3A* gene expression between rat and rabbit hepatocytes. For example, rifampicin markedly increased CYP3A6 mRNA in rabbit hepatocytes, but had no effect on CYP3A1 mRNA in rat hepatocytes. In contrast, PCN significantly increased CYP3A1 mRNA in rat hepatocytes, but not in rabbit hepatocytes. Consistent with the observations in hepatocytes, a good inducer in hepatocytes of a given species was also a good activator of PXR for the same species. PCN is a good activator for rat PXR, but not for rabbit PXR, whereas rifampicin is an efficacious activator for rabbit PXR, but not for rat PXR (88). These results suggest that structural difference in PXR is the molecular basis for the observed species differences in CYP3A induction.

The notion that structural difference in PXR is responsible for the observed species differences in CYP3A induction is further supported by the study with humanized PXR mice (50). Although *Cyp3a11* gene was markedly induced in mice by PCN and dexamethasone, targeted disruption of the mouse PXR gene resulted in loss of PCN and dexamethasone-mediated *Cyp3a11* gene induction. The disruption of PXR alleles was confirmed by the absence of PXR expression in the liver and small intestine, two principal PXR-expressing tissues. The transgenic mPXR-null mice (absence of mouse PXR) were then humanized by introduction of human PXR. Interestingly, the humanized mice responded to human CYP3A4 specific-inducer, rifampicin, but not PCN. Therefore, humanization of PXR is sufficient to convert the induction characteristics from mice to humans. Similarly, the conversion of species-specific inducibility of CYP3A was observed *in vitro*, when human PXR was transfected into rat hepatocytes (50). In control rat hepatocytes, CYP3A23 was strongly induced by PCN, whereas rifampicin and clotrimazole had little or no inductive effect on CYP3A23 induction. However, there was a significant induction of CYP3A23 by rifampicin and clotrimazole, when human PXR was cotransfected into rat hepatocytes. These results provide direct evidence that species differences in CYP3A induction are caused mainly by the structural differences in PXR between species.

Structurally, PXR consists of a DBD and an LBD in the same fashion across animal species. Structural comparison of PXR from different species revealed that there is greater than 95% sequence homology in the DBD regions, but only 76–83% homology for LBD regions (51,57). Because the DBD is highly conserved among the PXR across species, the species differences in CYP3A induction are more likely a result of the structural differences in the LBD. Indeed, mutation studies indicated that alteration of four amino acids in the LBD of mouse PXR to human PXR (Arg 203 Leu, Pro 205 Ser, Gln 404 His, and Gln 407 Arg) was sufficient to switch the mouse PXR that responds to PCN to a human-like PXR that is activated effectively by SR-12813, but not by PCN (58).

Alignment analysis reveals that only nine amino acids substitutions (Asp/Gly_{178}, Val/Phe_{184}, Ser/Arg_{203}, Val/Leu_{210}, His/Tyr_{260}, Glu/Asp_{263}, Arg/His_{333}, Glu/Lys_{334}, and Thr/Ser_{414}) are different in the LBD between rat and mouse PXR, suggesting close similarity between rats and mice (51). Interestingly, although there are only minor structural differences in the LBD, significant species differences in CYP3A induction between rats and mice still occur. Rifampicin caused a significant increase in CYP3A enzyme in mice, when a dose of 40 mg/kg was given once daily for 3 days (89). However, no induction of CYP3A was observed in rats, even when a dose of 200 mg/kg was given once daily for 7 days (90). Collectively, these results suggest that the LBD in the PXR, rather than the promoter or DBD sequence of gene structure, is responsible for the species differences induction on *CYP3A* gene transcription.

Similar to the PXR, comparison of CAR amino acid sequence revealed that the human and rat CAR share only about 70% amino acid homology in their LBD and demonstrates marked species differences in responses to xenobiotics (91). For example, clotrimazole is an efficacious deactivator of human CAR, but has little or no effect on mouse CAR (57). Conversely, TCPOBOP is a potent deactivator for mouse CAR but lacks any activity on human CAR (57). Similarly, there is significant species difference in CYP2B induction between rats and mice. TCPOBOP, the most potent ligand for mouse CAR, has little effect on rat CAR (57). Like PXR, it is reasonable to assume that the structural difference in the LBD of nuclear receptors is the major cellular factor responsible for species differences in CYP2B induction (91). Collectively, it becomes evident that it is difficult to make the prediction of CYP induction in humans by using animal models because of the marked species differences.

Interindividual Variability in CYP Induction

Variability in the protein levels of CYP enzymes among individuals has been recognized as an important determinant in drug disposition and pharmacological response in humans. In a comprehensive study of human livers of 30 Japanese and 30 Caucasians, Shimada *et al.* (92) have found that the level of each individual CYP enzyme is highly variable. The interindividual differences in the protein levels are approximately 5-fold for CYP2C and CYP3A4, 12-fold for CYP2E1, 20-fold for CYP1A2, and >50-fold for CYP2A6, CYP2B6, and CYP2D6. Large interindividual variability also has been reported by other investigators. There was a 37-fold variation in the level of CYP3A4 protein in biopsy tissues of 21 liver donors (93). Interindividual variability in the content of CYP enzymes has also been found in the small intestine. Paine *et al.* (94) showed that the levels of intestinal CYP3A4 in duodenum, jejunum, and ileum were extremely variable among 20 human donors. The content of CYP3A protein ranged from 3.0 to 91, 2.1 to 98, and 1.9 to 60 pmol/mg protein for the duodenum, jejunum, and ileum, respectively.

Many environmental factors, such as diet, pollutants, and tobacco smoking, can contribute to the interindividual variability in the level of CYP enzymes. It has been demonstrated that dietary composition can modulate the level of CYP enzymes. For example, the enzyme activity of CYP1A enzymes was increased significantly following a 2-week high protein/low carbohydrate diet (95). Additionally, certain vegetables including Brussels sprouts, cabbage, broccoli, and cauliflower contain chemicals that can induce CYP1A enzymes (96,97). Consumption of charcoal-broiled meat is also known to increase the levels of CYP1A enzymes through the contamination of polycyclic hydrocarbons, resulting from the incomplete combustion of meat drippings (98). Like charcoal-broiled meat, tobacco smoke contains a variety of polycyclic hydrocarbons. Tobacco smoke has been reported to increase the levels of CYP1A enzymes. The average content of CYP1A2 in the liver biopsies from smokers (16.3 pmol/mg microsomal protein) was significantly higher than that from nonsmokers (4.7 pmol/mg microsomal protein) (99). In addition, infections or inflammatory stimuli can also change the expression levels and activities of CYP enzymes (100).

As for the interindividual variability in the expression level of CYP enzymes, there is also a large variability in the extent to which inducers can induce CYP enzymes in humans (101). In a clinical study, there was an 18-fold difference in the degree of CYP3A4 induction after the treatment with rifampicin (600 mg/day) for 4 days (102). Fourteen patients were included in this study, in which liver biopsies were collected before and after rifampicin treatment. The extent of CYP3A4 protein induction ranged from 1.6- to 29-fold increase in these patients. Similar to hepatic CYP3A4 induction, considerable interindividual variability in human intestinal CYP3A4 induction by rifampicin was also reported in healthy volunteers. The extent of increase in mRNA of CYP3A4 in enterocytes during rifampicin treatment (600 mg/day for 7 days) ranged from no change in one subject to a 12-fold change in another volunteer (103). A large interindividual variability was also seen in intestinal CYP1A induction by omeprazole. Endoscopic tissue specimens from six healthy volunteers were analyzed for mRNA and enzyme activity of CYP1A before and after omeprazole treatment. The extent of increases in both mRNA and activity was quite variable among these individuals and ranged from no change in one volunteer to a 6-fold increase in another subject (80).

Interestingly, the degree of CYP induction elicited by a given inducer seems to be dependent on the basal level of the enzyme in hepatocytes before inducer treatment. Chang *et al.* (104) reported that induction of oxazaphosphorine 4-hydroxylation activity by rifampicin in human hepatocyte cultures was inversely related to the basal activity of the hepatocytes. Similar results were also observed for taxol-mediated CYP3A4

induction (11). The lower the basal level, the higher the extent of CYP3A4 induction, and there was a negative correlation between the basal level of CYP3A4 in human untreated hepatocytes and fold increase of testosterone 6-β-hydroxylase activity after treatment with taxol (11). Similarly, a negative correlation between the basal CYP3A4 activity and rifampicin-mediated induction was reported by LeCluyse (13). In this study, the maximal CYP3A4 induction of 17 liver donors was determined by treating with high concentration of rifampicin. Although there was a marked variation in the basal activity (10-fold) of hepatocyte preparations from the 17 liver donors, the CYP3A4 activity at the maximal induction by rifampicin seemed to be quantitatively similar between different donors (13). These data demonstrated that the extent of CYP induction is dependent on the basal CYP3A4 activity. Moreover, the maximally induced enzyme levels tend to be quantitatively similar among individuals, suggesting a limit to which the CYP enzymes can be maximally induced.

Consistent with *in vitro* data, there was also a negative correlation between the degree of CYP3A4 induction by dexamethasone and the basal enzyme activity *in vivo* in humans (105). The effect of dexamethasone on CYP3A4 activity in 12 healthy volunteers was determined with the erythromycin breath test. The extent of CYP3A4 induction was inversely correlated with baseline erythromycin breath test. In this study, the effect of dexamethasone on CYP3A4 activity was also assessed in hepatocytes. The extent of CYP3A4 induction in hepatocytes was also inversely correlated with baseline activity. Collectively, the *in vivo* data support the notion that the extent of CYP induction is dependent on the baseline levels of CYP enzymes. Therefore, the large interindividual variability in CYP induction can be explained, at least partially, by the interindividual variation in the baseline levels of CYP enzymes. Individuals with low basal levels of CYP enzymes would be more susceptible to induction than those with high basal levels.

Although numerous environmental factors can contribute to the interindividual variability in CYP induction, a significant component of interindividual variability in CYP induction can be explained by the genetic polymorphism. Many polymorphisms of nuclear receptors have been identified. Four allelic forms of the AhR have been identified in mice, namely, Ahr^{b1}, Ahr^{b2}, Ahr^{b3}, and Ahr^{d} (106,107). TCDD is about 10-fold more potent in inducing *Cyp1a1* gene in C57B/6 mice than in DBA/2 mice (29). The difference in inductive sensitivity between C57B/6 and DBA/2 mice is caused mainly by the AhR polymorphism. DBA/2 mice with Ahr^{d} allele are much less sensitive to TCDD as compared with C57B/6 mice with Ahr^{b1} (106,107). This is because the Ahr^{d} allele has an A375V substitution in the LBD and exhibits a lower binding affinity for TCDD as compared with Ahr^{b1}. Because most of the biochemical and toxic effects of TCDD are mediated by the AhR, genetic variations in the AhR may lead to substantial differences in sensitivity to biochemical and toxic effects of TCDD and related compounds (108).

Similarly, there is a substantial difference in response to TCDD in rats. TCDD-sensitive Long-Evans and resistant Han/Wistar rats have more than 100-fold difference in CYP1A induction and lethality. The cDNA coding regions of *Ahr* genes of Long-Evans and Han/Wistar rats have been cloned. The Long-Evans rats were found to have the same cDNA sequence of *Ahr* as reported for Sprague–Dawley rats (wild-type allele designated as Ahr^{wt}), whereas the *Ahr* (designated as Ahr^{hw}) of Han/Wistar rats had a critical point mutation in the first base of intron 10, leading to three splicing variants with deletions and insertions (109). The point mutation in intron causes altered carboxyl-terminal structure and function of the *Ahr* of the TCDD-resistant rat strain. In addition to AhR, polymorphism of Arnt has also been reported. Several Arnt isomers have been identified in rats: deletions at exon 5, 3′ end of exon 6, or 5′ end of exon 11, or with an insertion at 5′ end of exon 20 (110). However, additional studies suggest that the variants of Arnt do not contribute to TCDD resistance (110).

Discovery of AhR polymorphisms in animals has spurred the search for genetic variants in humans. To date, at least six genetic variants of human AhR have been identified, including a proline-to-serine substitution at codon 517, an arginine-to-lysine substitution at codon 554, and a valine-to-isoleucine substitution at codon 570 (111). The effect of polymorphism AhR on CYP1A induction has been studied *ex vivo* using human cultured lymphocytes. In a study with Japanese subjects, Kawajiri *et al.* (112) reported that the codon 554 polymorphism had no significant effect on CYP1A induction in cultured lymphocytes as compared to the wild type. Similarly, there was no significant effect of the codon 554 polymorphism on CYP1A induction in lymphocytes from French subjects (113). In addition, there was no apparent effect of the codon 570 polymorphism on CYP1A induction (114). However, when the combined Lys 554 + Ile570 variant or the combined Lys 554 + Ile570 + Ser517 variant haplotype was introduced in Hepa-1 Group B mutant cells, they lost the ability to induce the CYP1A1 by TCDD (115). In conclusion, unlike in rodents, single AhR variant seems to have little effect on the CYP1A inducibility in humans, whereas the combination of AhR variants may have significant impact on the CYP1A induction. However, it should be noted that the combination of AhR variant genotypes is rare and seems to be confirmed primarily to persons of African descent (115).

Polymorphisms have also been reported for CAR and PXR (116–118). Zhang *et al.* (116) have identified 38 single nucleotide polymorphisms (SNPs) of PXR including 6 SNPs in the coding region. Three of the coding SNPs are non-synonymous resulting in new PXR alleles (PXR*2, P27S; PXR*3, G36R; PXR*4, R122Q). PXR*2 and PXR*3 are located in exon coding of the N-terminal region and the DNA binding. Transactivation properties of these variants are not distinguishable from the wild-type PXR. On the other hand, PXR*4 is located in the third helix of DBD. This variant is deficient in DNA binding, but it displays only a minimal loss of transcriptional activity in transiently transfected cells (116). These results suggest that these three PXR variants do not exhibit any obvious phenotypic alterations. Recently, another four human PXR variants (R98C, R148Q, R381W, and I403V) have been identified by Koyano *et al.* (117). Using a gene reporter assay in COS-7 and HepG2 cells, it was found that the R98C variant failed to transactivate the CYP3A4 reporter. The R381W and I403V variants showed varying degrees in transactivation. However, the R148Q variant had no effect on transactivation. These results suggest that R98C, R381W, and I403V may influence

the induction of *CYP3A4* gene. In addition to PXR, polymorphism of CAR has been identified. Recently, four splice variants of CAR have been identified, although their functional role remains to be determined (118).

As shown in Fig. 1, a large number of factors could contribute to the variability in CYP induction, including physiological and environmental variables (119). A better understanding of the mechanisms that underlie the variability in CYP induction will help to predict and tailor drug therapies to individual patients. Although many genetic variants have been identified for AhR, CAR, and PXR in humans, they cannot explain fully the wide interindividual variability in CYP inducibility in the population. Undoubtedly, this could reflect, at least partially, the complexity of CYP induction. It is also possible that important variants that might alter the functional activity of the nuclear receptors are still not yet identified.

CLINICAL IMPLICATIONS

Effects of CYP Induction on Pharmacokinetics of Drugs

In animal studies, CYP induction can be readily evidenced by direct measurement of the enzyme amount and activity in the liver. However, owing to ethical considerations and practical limitations, direct assessment of CYP induction *in vivo* by measuring enzyme amount and activity in the liver is difficult in humans. A simple but indirect way of assessing the effect of CYP induction in clinical settings is the comparison of the area under plasma concentration–time curve (AUC) of a drug before and after coadministration of an inducer. As will be discussed below, the magnitude of changes in the plasma AUC after inducer treatment is highly dependent on the route of administration and the kinetic characteristics of drugs. Therefore, the changes in plasma AUC of a drug after treatment with an inducer may not directly reflect the changes of enzyme activity. A 10-fold increase in the plasma AUC by an inducer does not necessarily reflect a 10-fold increase in enzyme activity.

In addition, changes in AUC after inducer treatment may reflect the sum of induction of multiple CYP enzymes in various tissues. Because CYP enzymes are not only expressed in the liver (they are also present in other tissues, including intestine, lung, and kidney), it is expected that CYP induction also occurs in various tissues besides the liver. For example, after treatment of mice with TCDD (10 μg/kg), a 20- to 40-fold CYP1A1 induction was observed in liver, lung, and skin in mice (120). The enzyme level of CYP1A1 in the lung was about 4% of that in the liver before the TCDD treatment, whereas the pulmonary CYP1A1 enzyme level was increased to about 10–15% of that in the liver after the TCDD treatment. Therefore, interpretation of the effect of CYP induction based on the kinetic analysis of AUC data is not as straightforward as generally believed. The situation can be further complicated by the fact that many drug transporters are also susceptible to induction through the same nuclear receptors that regulate CYP enzymes. Changes in the expression of transporters may have significant effects on the plasma AUC.

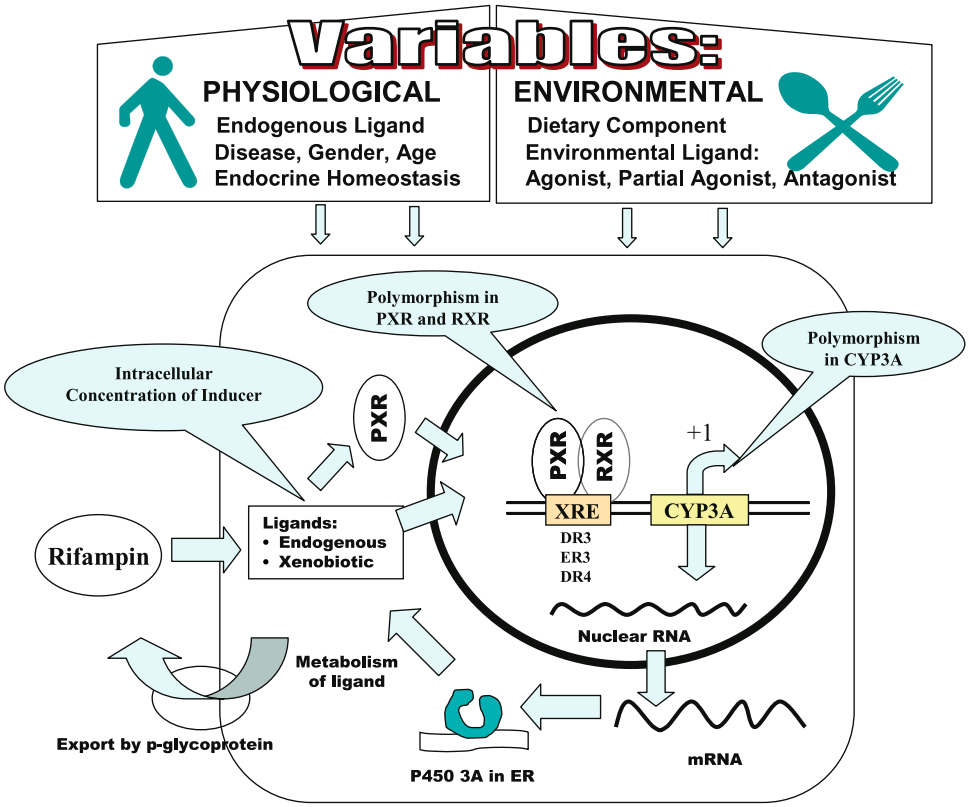

Fig. 1. Physiological and genetic variables contributing to interindividual variability in CYP3A4 induction [from Tang *et al.* (119), with permission].

Route of Administration and Drug's Characteristics

Depending on the pharmacokinetic characteristics of drugs, the route of drug administration may have a significant impact on the AUC changes caused by CYP induction (101,121,122). For high hepatic clearance drugs, a marked decrease in AUC during CYP induction is expected following oral administration, whereas CYP induction has little effect on the AUC after intravenous administration. Kinetically, the AUC (AUC_{iv}) of a drug after intravenous administration is determined mainly by the systemic clearance. Because the systemic clearance of high hepatic clearance drugs is limited by hepatic blood flow, it is not sensitive to the changes in enzyme activity. Therefore, for high hepatic clearance drugs, an increase of metabolic (intrinsic) clearance caused by CYP induction will have little effect on the AUC_{iv} after intravenous administration. In contrast, the AUC (AUC_{po}) after oral administration is determined by both systemic clearance and bioavailability. Although the systemic clearance is not sensitive to the changes in enzyme activity for high hepatic clearance drugs, their bioavailability is very sensitive to the changes in enzyme activity because of changes in first-pass metabolism.

The pentobarbital–alprenolol interaction is a good example in support of the notion that the magnitude of the AUC changes of a drug by CYP induction is highly dependent on the drug's pharmacokinetic characteristics and the route of drug administration. The metabolism of alprenolol, a high hepatic clearance drug with a clearance of 1200 mL/min (a value approaching to the hepatic blood flow of 1500 mL/min in humans), is known to be significantly induced by pentobarbital in humans. Alprenolol was administered orally or intravenously to five healthy subjects on two different occasions before and after 10–14 daily doses of 100 mg pentobarbital (123). The AUC_{po} of alprenolol after a 200-mg oral dose decreased dramatically from an average of 706 to 154 ng h/mL with barbiturate treatment. In contrast, the AUC_{iv} of alprenolol following an intravenous administration of 5-mg dose only slightly decreased from 67 to 58 ng h/mL with pentobarbital treatment. There was a 4.5-fold decrease in the AUC_{po} of alprenolol after oral dosing and only a 15% decrease in the AUC_{iv} of alprenolol after intravenous administration. There was no significant change in the elimination half-life (approximately 2 h) of alprenolol before and after pentobarbital treatment, independent of the route of administration, supporting the argument that CYP induction has little effect on the systemic clearance of high hepatic clearance drugs. The marked decrease in the AUC_{po} of alprenolol is mainly because of a decrease in bioavailability resulting from an increase in first-pass metabolism.

Similarly, treatment with rifampicin had little effect on the metabolism of nifedipine (a high hepatic clearance drug) after intravenous administration, whereas it greatly reduced the AUC of nifedipine following oral administration (124). After 7 days of rifampicin treatment (600 mg/day), the AUC_{po} of nifedipine decreased from 280 to 18 ng h/mL when a 20-mg oral dose of nifedipine was given. In contrast, the AUC_{iv} of nifedipine only slightly reduced from 38 to 27 ng h/mL, when the drug was dosed intravenously at 20-μg/kg body weight. There was a 15.5-fold decrease in the AUC_{po} of nifedipine after oral administration, whereas only 30% decrease in the AUC_{iv} after intravenous dosing. As with alprenolol, rifampicin had little effect on the elimination half-life (~2 hr) of nifedipine after either oral or intravenous administration.

The differential effect of route administration has also been reported for another high hepatic clearance drug, verapamil. Treatment with rifampicin had little on the AUC_{iv} of (*S*)- and (*R*)-verapamil after intravenous administration of verapamil, whereas it caused a dramatic decrease in the AUC_{po} of (*S*) and (*R*)-verapamil after oral administration of verapamil by 30- to 50-fold (9). After rifampicin treatment for 16 days (600 mg/day), the AUC_{po} of (*S*)-verapamil decreased from an average of 151 to 5 ng h/mL after oral administration (120 mg), whereas the AUC_{iv} of (*S*)-verapamil decreased from 77 to 61 ng h/mL following intravenous dosing (10 mg). Overall, these results from the above examples clearly demonstrated that for high hepatic clearance drugs, CYP induction has little effect on the AUC_{iv} after intravenous administration, whereas it causes a marked decrease in the AUC_{po} after oral dosing.

Unlike high hepatic clearance drugs, enzyme induction yields significant effects on both the systemic clearance and AUC for low hepatic clearance drugs, independent of the route of administration. The systemic clearance of low hepatic clearance drugs is enzyme-limited and sensitive to the changes in enzyme activity. Because low hepatic clearance drugs are generally not subject to significant first-pass metabolism, both the AUC_{iv} and AUC_{po} are determined mainly by the systemic clearance. Therefore, an increase in the intrinsic clearance caused by CYP induction will have significant effect on both the AUC_{iv} and AUC_{po} of the low hepatic clearance drugs. It is expected that the magnitude of the changes in the AUC of the low hepatic clearance drugs will be quantitatively similar between oral and intravenous administration. In addition, CYP induction will also have a significant effect on the elimination half-life of low hepatic clearance drugs, regardless of the route of administration.

The methadone–rifampicin interaction is a good example that both the AUC_{iv} and AUC_{po} of the low hepatic clearance drugs are sensitive to the changes of enzyme activity caused by CYP induction. In a study, the effect of rifampicin (600 mg/day for 5 days) on the pharmacokinetics of methadone has been studied with 12 healthy volunteers (125). Methadone, a low hepatic clearance drug with a clearance of 140 mL/min, is used to treat opiate addiction by preventing opiate withdrawal syndrome. After an intravenous dose (4.5 mg), the AUC_{iv} and half-life of methadone decreased from an average of 816 ng h/mL and 38 h before rifampicin treatment to 259 ng h/mL and 18 hr after rifampicin treatment, respectively. Similarly, the AUC_{po} and half-life of methadone decreased from an average of 1128 ng h/mL and 33 h before rifampicin treatment to 262 ng h/mL and 25 h, respectively, after oral administration of methadone (10 mg).

The elimination of alprazolam, a low hepatic clearance drug, was investigated in two groups of healthy volunteers with and without rifampicin treatment at dose 450 mg daily for 4 days (126). The AUC_{po} of alprazolam was about 8-fold lower in the rifampicin treatment group (28.4 ng h/mL) compared to the control group without rifampicin (224 ng h/mL) after an oral dose of 1 mg alprazolam. Similar to the

AUC, the half-life was also decreased dramatically from an average of 14 h in the control group to 2.6 h in the rifampicin-treated group. In another clinical study, the disposition of diazepam, a low hepatic clearance drug, was investigated in 21 healthy subjects before and after 7 days administration of rifampicin 600 mg. In this study, diazepam was given orally at a dose of 10 mg. After treatment with rifampicin, there was a significant decrease in the AUC and half-life of diazepam (127). The AUC of diazepam decreased from an average of 4430 ng h/mL before rifampicin treatment to 1040 ng h/mL after treatment. In addition, the elimination half-life of diazepam decreased from an average of 52 h before rifampicin treatment to 15.8 h after the treatment. Overall, these results from the above examples clearly demonstrate that for low hepatic clearance drugs, CYP induction has similar effect on the AUC_{iv} after intravenous administration and the AUC_{po} after oral dosing. In addition, CYP induction also has significant effect on the half-life of low hepatic clearance drugs. Unlike high hepatic clearance drugs, the changes in plasma AUC of low hepatic clearance drugs after treatment with an inducer may more accurately reflect the changes of enzyme activity.

Another intriguing aspect of the effect of CYP induction on the pharmacokinetics of drugs is that the magnitude of changes in the AUC_{po} after oral dosing tends to be greater for high hepatic clearance drugs compared to that for low hepatic clearance drugs. The clearance (<200 mL/min) of methadone, alprazolam, diazepam, zolpidem, and zolpiclone is substantially smaller than hepatic blood flow (1500 mL/min), whereas the clearance (>1000 mL/min) of nifedipine and verapamil is approaching to the hepatic blood flow. Thus, the drugs of former group are defined as low hepatic clearance drugs, and those in the latter group are considered as high hepatic clearance drugs (9,124,128,129). As shown in Table I, the magnitude of the decrease in the AUC tends to be smaller for the low hepatic clearance drugs (4- to 8-fold) than that for high hepatic clearance drugs (15- to 50-fold). Similar to the high clearance drugs, the moderate hepatic clearance drugs (~500 mL/min), such as midazolam and triazolam, are also subject to a significant decrease in the AUC_{po} after oral dosing during CYP induction (130,131).

CYP2C8 and CYP2C9 can also be induced by rifampicin (132,133). However, the effect of rifampicin on drugs that are predominately metabolized by CYP2C8 or CYP2C9 seems to be less significant compared to that on drugs metabolized by CYP3A4 (Table II). Rifampicin treatment only caused 2- to 4-fold changes in both the AUC and half-life of warfarin, which is metabolized predominately by CYP2C9 (134). After the treatment with rifampicin at 300 mg twice daily for 4 days, the AUCs and half-life of (*R*)-warfarin decreased from an average of 159 μg h/mL and 43 h to 48 μg h/mL and 17 h, respectively, whereas the AUCs and half-life of (*S*)-warfarin decreased from 220 μg h/mL and 26 h to 59 μg h/mL and 13 h, respectively, in healthy volunteers receiving an oral dose, 0.75 mg/kg body weight, of racemic warfarin.

Rosiglitazone, an insulin-sensitizing agent for the treatment of patients with type 2 diabetes, is eliminated predominately by CYP2C8 (135). After the treatment of rifampicin 600 mg once daily for 6 days in healthy volunteers, the AUC_{po} of rosiglitazone decreased from an average of 2676 ng h/mL to 988 ng h/mL after an oral dose of 8 mg rosiglitazone (136). Glimepiride is a new sulfonylurea antidiabetic agent, which is eliminated predominated by CYP2C9-mediated metabolism (137). There was only a 1.5-fold decrease in the AUC_{po} of glimepiride after the treatment of rifampicin 600 mg once daily for 5 days (138). Following an oral dose of 1 mg glimepiride, the AUC_{po} of glimepiride decreased from 287 ng h/mL before the rifampicin treatment to 190 ng h/mL after the treatment. Similarly, rifampicin has less profound effect on the AUC_{po} of gliclazide, glyburide, and glipizide, which are eliminated predominately via metabolism by CYP2C9 (139,140). The changes in the AUC_{po} after the rifampicin treatment ranged from 1.3- to 2.9-fold for gliclazide, glyburide, and glipizide (Table II).

Table I. Effect of Rifampicin on the Oral AUC of Drugs that are Metabolized Predominately by CYP3A4

Drug	Type of Clearance (CL)[a]	Rifampicin (mg/day)	AUC (ng h/mL)		Fold Induction[b]	Ref.
			Before RIF	After RIF		
Cyclosporine	Low	600 mg × 11 days	8986	2399	3.7	(2)
Tacrolimus	Low	600 mg × 18 days	351	112	3.1	(179)
Methadone	Low	600 mg × 5 days	1128	262	4.3	(125)
Alprazolam	Low	450 mg × 4 days	224	28	8.0	(126)
Diazepam	Low	600 mg × 7 days	4430	1040	4.2	(127)
Zolpidem	Low	600 mg × 5 days	1202	336	3.6	(128)
Zopiclone	Low	600 mg × 5 days	473	86	5.5	(129)
Quinidine	Moderate	600 mg × 7 days	8000	910	8.8	(185)
Midazolam	Moderate	600 mg × 5 days	612	25	24.0	(130)
Triazolam	Moderate	600 mg × 5 days	14.8	0.74	20.0	(131)
Nifedipine	High	600 mg × 7 days	280	18	15.5	(124)
Indinavir	High	600 mg × 8 days	18.8[c]	1.2[c]	16.0	(199)
(*S*)-Verapamil	High	600 mg × 12 days	152	5	30.0	(9)
(*R*)-Verapamil	High	600 mg × 12 days	724	14	52.0	(9)

AUC: area under plasma concentration–time curve; RIF: rifampicin.
[a] Type of clearance: low clearance < 200 mL/min; moderate clearance > 500 mL/min; high clearance > 1000 mL/min.
[b] Fold induction: the ratio of AUC before and after rifampicin.
[c] μg h/mL.

Table II. Effect of Rifampicin (RIF) on the Oral AUC of Drugs that are Metabolized Predominately by CYP2C8 or CYP2C9 in Humans

Drug	Type of Clearance (CL)[a]	Rifampicin (mg/day)	AUC (ng h/mL)		Fold of Induction[b]	Ref.
			Before RIF	After RIF		
Rosiglitazone	Low	600 mg × 6 days	2676	988	2.7	(136)
Glimepiride	Low	600 mg × 5 days	287	190	1.5	(138)
Gliclazide	Low	600 mg × 6 days	44[c]	15[c]	2.9	(139)
Glyburide	Low	600 mg × 5 days	324	198	1.6	(140)
Glipizide	Low	600 mg × 5 days	801	621	1.3	(140)
(*S*)-Warfarin	Low	600 mg × 4 days	220[c]	59[c]	3.7	(134)
(*R*)-Warfarin	Low	600 mg × 4 days	159[c]	48[c]	3.3	(134)

[a] Type of clearance: low clearance < 200 mL/min/.
[b] Fold induction: the ratio of oral AUC before and after rifampicin treatment.
[c] μg h/mL.

All of the CYP2C8/CYP2C9 drugs listed in Table II can be defined as low hepatic clearance drug because their clearance value is less than 200 mL/min in humans. The relatively small changes in the AUC_{po} of these CYP2C8/CYP2C9 drugs after rifampicin treatment are consistent with the notion that the magnitude of changes in the AUC_{po} after CYP induction tends to be lower for low hepatic clearance drugs compared to that for high hepatic clearance drugs. However, even for the class of low hepatic clearance drugs, the effect of rifampicin seems to be less significant for CYP2C8/CYP2C9 drugs compared to CYP3A4 drugs. As shown in Tables I and II, the changes in AUC_{po} were generally smaller for CYP2C8/CYP2C9 drugs (1.3- to 3.5-fold) than that for low clearance drugs of CYP3A4 (3.5- to 8-fold). The differences in the magnitude of changes in the AUC_{po} between CYP3A4 drugs and CYP2C8/CYP2C9 drugs may be a result of differential induction between different *CYP* genes by rifampicin, suggesting that the *CYP2C8* and *CYP2C9* genes are less sensitive to rifampicin as compared to the *CYP3A4* gene.

The argument that *CYP2C8* and *CYP2C9* genes are less sensitive to rifampicin is supported by data from *in vitro* studies. In a study with human hepatocytes, the CYP3A4 mRNA increased about 25-fold, whereas the CYP2C8 and CYP2C9 mRNA only increased 3- to 4-fold after incubation for 24 h with rifampicin (141). In another study with cultured human hepatocytes, protein and activity of CYP2C8 and CYP2C9 were induced by 3- to 5-fold after rifampicin treatment, whereas the protein and activity of CYP3A4 were induced by 10-fold after rifampicin treatment (142). Similar observations of differential induction of CYP genes by rifampicin have been reported by other investigators (133,143). Although the PXR response elements of *CYP2C8* and *CYP2C9* genes have not been identified, the differential induction between *CYP2C* and *CYP3A* genes by rifampicin may reflect the differences in the affinity of PXR/RXR to the responsive elements between *CYP2C8*/*CYP2C9* genes and *CYP3A4* genes (144–146).

Time- and Dose-Dependent CYP Induction

Enzyme induction is a slow regulatory process, involving biosynthesis of mRNA and protein. Therefore, the CYP induction is expected to be a time- and concentration (dose)-dependent process. The time- and concentration-dependent induction of CYP1A1 and CYP1A2 by TCDD has been demonstrated in studies using human splenic lymphocytes and colon carcinoma cell line LS180 (147,148). In the human splenic lymphocytes and LS180, TCDD markedly induced mRNA, protein, and enzyme activity in a time- and concentration-dependent manner. In the LS180 cell cultures, maximal mRNA induction of CYP1A1 and CYP1A2 occurred between 6 and 24 h after the treatment with TCDD. CYP1A1 protein reached its maximum at 48 h, whereas the CYP1A2 protein reached its maximum between 48 and 72 h. These results clearly suggest that there is a lag time between the biosynthesis of mRNA and protein synthesis, reflecting an extra time required for protein synthesis.

Consistent with *in vitro* observations, time- and dose-dependent TCDD-mediated CYP1A1/CYP1A2 induction has also been demonstrated in rats (149). The protein expression of CYP1A1 and CYP1A2 was induced in a dose-dependent manner in rats over a dose range of TCDD (0.01–30 μg/kg). The ED_{50} for TCDD-induced CYP1A1 and CYP1A2 protein expression was estimated to be 0.22 and 0.4 μg/kg, respectively. Although a significant increase in the expression of TCDD-induced CYP1A1 and CYP1A2 protein was observed at 24 h, both CYP1A1 and CYP1A2 reached their maximal levels at 7 days after following a single oral dose of TCDD (10 μg/kg). After reaching the peak, the protein level declined slowly via protein degradation. Even at 35 days after TCDD administration, only a slight decrease was observed. The persistence of the expression of TCDD-induced CYP1A1 and CYP1A2 protein may be related to the slow hepatic elimination of TCDD. Hepatic concentration of TCDD reached a maximum about 8 h after TCDD administration followed by a slow elimination with a half-life of approximately 10 days. A physiologically based pharmacokinetic model incorporating tissue retention of TCDD and its affinity binding to AhR has been successfully developed to describe the persistence of CYP1A induction in rats and mice for TCDD and its analogs (150–152).

In addition to TCDD, the time- and dose-dependent induction of CYP1A1 and CYP1A2 by omeprazole has been demonstrated in human hepatoma cell line HepG2 (153,154). Consistent with *in vitro* observations, dose dependency of CYP1A1/CYP1A2 has also been observed in humans. Induction of intestinal CYP1A by omeprazole was studied in six healthy volunteers (80). In this study, endoscopic tissue specimens were analyzed for mRNA and enzyme activity measured by deethylation of ethoxyresorufin before and after the treatment with omeprazole 20 mg/day for 1 week.

Large interindividual variations of CYP1A induction were observed. The extent of CYP1A induction ranged from no change in one subject to a 6-fold increase in another subject. The individual who did not initially respond (20 mg/day) had a marked increase in both mRNA and enzyme activity after receiving 60 mg of omeprazole daily for 1 week, suggesting that enzyme induction is dose-dependent. Similarly, dose-dependent induction of CYP1A2 by omeprazole has been demonstrated between 40- and 120-mg doses, as measured by ^{13}C-[*N*-3-methyl]-caffeine breath test (155). In another clinical study, CYP1A2 was induced in poor metabolizers (PMs) of CYP2C19, but not in extensive metabolizers (EMs) after 7 days treatment with omeprazole at 40 mg/day (156). Because omeprazole is eliminated predominately by CYP2C19-mediated metabolism, the plasma AUC of omeprazole in PMs of CYP2C19 was approximately 5-fold higher than that in EMs after an oral dose of 40 mg. Therefore, the differential effect of omeprazole on CYP1A induction between PMs and EMs of CYP2C19 can be explained by concentration-dependent induction. Collectively, these results clearly demonstrate that omeprazole induces *CYP1A1* and *CYP1A2* genes in a concentration (dose)-dependent manner.

Cigarette smoking is known to induce CYP1A2 enzyme, and the smoking-induced CYP1A2 returns to the basal level after cessation of smoking via protein degradation. The degradation half-life of the smoking-induced CYP1A2 has been studied in heavy cigarette smokers after cessation of smoking (157). This study was conducted with eight men and four women who smoked 20 cigarettes or more per day. Subjects were phenotyped for CYP1A2 activity at 6, 4, and 1 day before smoking cessation and at 0, 1, 2, 3, 6, 8, 10, and 13 days thereafter by measuring caffeine clearance. The CYP1A2 activity decreased as a function of time, and a maximal decrease was observed at 6 or 8 days after cessation. The degradation half-life of CYP1A2 activity was estimated to be about 36 h. Similar to the degradation half-life of CYP1A2 enzyme in humans, the degradation half-life of the Aroclor-induced CYP1A1 and CYP1A2 has been estimated to be 20 and 37 h, respectively, in rats (158,159).

Rifampicin is one of the most potent inducers of human CYP3A4 isozyme. The time- and dose-dependent induction of CYP3A4 by rifampicin has also been demonstrated in primary cultures of human hepatocytes (11,13,160,161). In human hepatocytes treated with rifampicin, the induction of CYP3A4 activity (measured by testosterone 6β-hydroxylation) was concentration dependent, and the EC_{50} for rifampicin was estimated to be 0.3–0.5 μM (162). The dosage of rifampicin in the treatment of tuberculosis is usually between 450 and 600 mg once daily (163). In a clinical study, the effect of rifampicin on the pharmacokinetics of diazepam (a low hepatic clearance drug) was not significantly different between 600- and 1200-mg oral dose (127). The AUC of diazepam decreased from an average of 4430 to 1040 ng h/mL after the treatment with rifampicin at 600 mg, whereas it decreased from an average of 4170 to 1130 ng h/mL after 1200 mg rifampicin. Similarly, the effect of rifampicin daily dose of 600, 900, and 1200 mg on the disposition of propranolol (a high hepatic clearance drug) was also not significantly different (164). Together, these results suggest that the inducing effect of rifampicin at the dosage of 450–600 mg is near maximal. The notion of maximal inductive effect at 450–600 mg is further supported by the comparison of the EC_{50} and the systemic exposure of rifampicin at clinical dose. In a clinical study, the peak plasma concentration and AUC of rifampicin were 20 μg/mL and 100 μg h/mL, respectively, following an oral dose of 600 mg (165). The peak concentration and average exposure (measured by AUC/24 hr) are much greater than the EC_{50} for rifampicin (0.3–0.5 μM).

Time-dependent CYP3A4 induction by rifampicin has also been demonstrated in humans. In a clinical study, the time course of the CYP3A4 induction was evaluated by measuring the trough concentrations of verapamil before, during, and after a 12-day treatment with rifampicin 600 mg once daily (9). As shown in Fig. 2, the maximal effect of rifampicin on the CYP3A4 activity was observed at 8 days after starting the rifampicin treatment and returned to the baseline activity about 2 weeks after discontinuing rifampicin treatment. The half-life for increase in CYP3A4 activity was estimated about 0.9 day for (*R*)-verapamil and 1.0 day for (*S*)-verapamil, and the half-life for decrease in CYP3A4 activity was 1.5 days for (*R*)-verapamil and 2.1 days for (*S*)-verapamil. It is reasonable to assume that the increase in CYP3A4 activity reflects mainly the synthesis of CYP3A4 protein, and the decrease in CYP3A4 activity reflects mainly the degradation of CYP3A4 protein. If the assumption is valid, these results suggest that the process of protein degradation is somewhat slower than the process of protein synthesis.

Consistent with the half-life of CYP3A4 induction of approximately 1 day, a significant CYP3A4 induction was observed as early as 8 h after a single rifampicin treatment (166). Nifedipine (10 mg) was given orally 8 h after a single pretreatment of rifampicin (1200 mg) in healthy volunteers. The AUC_{po} of nifedipine decreased from an average of 573 ng h/mL before rifampicin treatment to 205 ng h/mL at 8 h after a single rifampicin treatment. Although the change in the AUC_{po} of nifedipine after 8 h (2.8-fold) is much less than that after a 7-day treatment (15.5-fold as

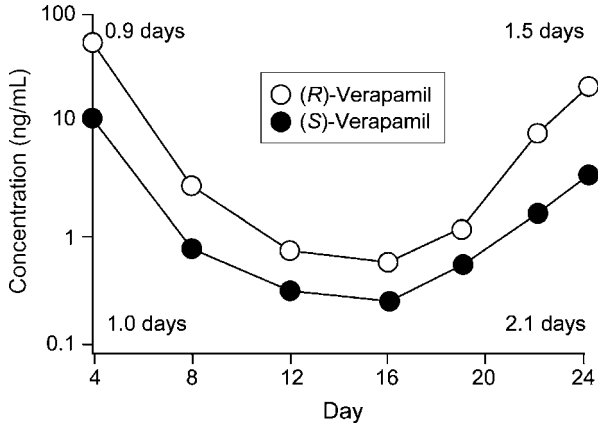

Fig. 2. Mean (*S*)- and (*R*)-verapamil trough concentrations before (day 4), during (days 8–16), and after (days 19–24) induction with rifampicin treatment. Half-life of induction phase (increase in enzyme activity) was determined between days 4 and 8, and half-life of recovery phase was measured between days 16 and 24 [from Fromm *et al.* (9), with permission].

shown in Table I), an 8-h pretreatment with rifampicin is a sufficient period to bring about significant increase in the activity of CYP3A4.

Intestinal and Hepatic CYP Induction

Because of its anatomical situation, the small intestine contributes to overall first-pass metabolism of many drugs. Some studies have even suggested that the role of intestinal metabolism is quantitatively more important than that of hepatic metabolism in the overall first-pass effect (9,124,167,168). Much of the evidence for such claims has derived indirectly from comparisons of the AUCs following intravenous and oral administration, before and after treatment of rifampicin. In a clinical study, Wu *et al.* (167) claimed that the intestinal first-pass metabolism (50%), expressed as hepatic extraction ratio, for cyclosporine is approximately twice the hepatic first-pass metabolism (24%). Similarly, Gorski *et al.* (168) claimed that the intestinal first-pass metabolism of midazolam is quantitatively more important than hepatic first-pass metabolism of midazolam in health volunteers. The intestinal and hepatic first-pass metabolism was estimated to be 53 and 29%, respectively. Moreover, in another clinical study, Holtbecker *et al.* (124) concluded that intestinal CYP3A4 is more sensitive to rifampicin than hepatic CYP3A4. After rifampicin treatment (600 mg/day for 7 days), the intestinal first-pass metabolism of nifedipine increased from 21.8 to 75.8% (a 3.5-fold increase), whereas the hepatic first-pass metabolism increased from 47.4 to 67.4% (a 40% increase). Based on these results, these investigators concluded that the contribution of intestinal metabolism to overall first-pass metabolism is quantitatively as important as hepatic metabolism, or even more important than hepatic metabolism. In addition, they claimed that the intestinal metabolism is induced preferentially by orally administered inducers compared with hepatic metabolism.

The notion that small intestine plays a quantitatively more important role in first-pass metabolism has been critically questioned (169). The quantitative importance of intestinal first-pass metabolism may have been exaggerated as a result of invalid assumptions and problems inherent in the kinetic analysis of the AUC data. The argument that the role of small intestine in first-pass metabolism is not as quantitatively important as liver is further supported by the fact that the protein levels (per milligram of microsomal protein) of CYP enzymes, such as CYP2C8, CYP2C9, and CYP3A4 in hepatocytes, are 5- to 10-fold greater than that in the enterocytes of mucosal epithelium of the small intestine (94,170,171). This difference is even greater when one considers the total mass of microsomal protein in the intestinal mucosa and liver. Similar to the observations in humans, the protein levels of CYP enzymes are much lower in the small intestine than in the liver in rats (172,173). Consistent with the low protein levels of intestinal CYP3A4, in a clinical study, Kleinbloesem *et al.* (174) have demonstrated that the intestinal first-pass metabolism of nifedipine in patients with a portacaval shunt was absent because the bioavailability of nifedipine was almost complete (100%) in these patients, whose portal blood circulation bypassed the liver. In another clinical study, the intestinal first-pass metabolism of verapamil in patients with a portacaval shunt was also reported to be absent (175). Collectively, these results clearly suggest that the role of small intestine in first-pass metabolism is not as important as some scientists claimed.

There is another widespread misconception that the intestinal CYP enzymes are preferentially induced by orally administered inducers compared to hepatic CYP enzymes. This misconception arises in part from the general belief that there is more direct availability of the orally administered inducers in the intestine as compared to the liver, and in part from the observations that the decreases in the oral AUC of drugs are generally greater than those in the intravenous AUC after induction. However, mere observation of a greater change in the oral AUC_{po} than the intravenous AUC_{iv} after induction does not necessarily reflect a greater degree of induction in the small intestine. As discussed earlier, CYP induction has more profound effect on the AUC_{po} than the AUC_{iv}, particularly for high hepatic clearance drugs (9,124,167,168). Moreover, although Holtbecker *et al.* (124) concluded that intestinal CYP3A4 is more sensitive to rifampicin than hepatic CYP3A4, the calculation of the intestinal and hepatic first-pass metabolism was performed with invalid assumptions and problems inherent in the data analysis.

In fact, the literature data suggest that the degree of CYP3A4 induction in the intestine is generally lower than the degree of hepatic CYP3A4 induction. In a clinical study, the protein level of hepatic CYP3A4 was increased by about 6- to 10-fold after the treatment with rifampicin (102). Fourteen patients were included in the study, in which liver biopsies were collected before and after rifampicin treatment (600 mg/day for 4 days). On the other hand, rifampicin administration (600 mg/day for 10 days) only resulted in a 2- to 3-fold induction of CYP3A4 protein in the small intestine, in a recent clinical study with six healthy volunteers (176). Similarly, in another study, the intestinal CYP3A4 mRNA was increased by approximately 3- to 4-fold in five healthy volunteers after rifampicin treatment (600 mg/day for 7 days) using endoscopic biopsies (103). The observed 3- to 4-fold increase in intestinal CYP3A4 mRNA is much lower than the reported 10- to 50-fold increases in hepatic CYP3A4 mRNA. These results are consistent with the fact that the expression levels of PXR are substantially lower in the small intestine than those in the liver (54). Similar to humans, a greater induction degree of CYP3A enzymes was observed in the liver compared with the small intestine after the treatment of rats with dexamethasone (172).

Effects of CYP Induction on Pharmacodynamics of Drugs

For drugs whose elimination is cleared primarily by CYP-mediated metabolism, CYP induction will decrease the therapeutic efficacy as a result of a decrease of systemic exposure. In some cases, changes in drug dosage are required to attain and maintain a therapy during the initiation, maintenance, and discontinuation of the coadministration of a potent CYP inducer. In addition, CYP induction may create an undesirable imbalance between detoxification and activation, leading to an increase in metabolite-induced toxicity. Although the research on the potential risk of CYP induction-mediated toxicity has received much less attention than the pharmacokinetic and pharmacodynamic consequences, there

is increasing evidence that CYP induction could be a serious cause for drug-induced toxicity.

Reduction in Therapeutic Efficacy

Rifampicin–cyclosporine interaction is a good example that CYP induction causes a reduction in therapeutic efficacy. There is a high incidence of tuberculosis among renal transplant recipients. In a retrospective study, analysis of the records of 880 renal transplant recipients in Turkey revealed that 36 patients were infected and developed tuberculosis after renal transplantation (177). In another study, a high incidence of tuberculosis (42%) was reported among 305 renal transplant recipients in India (178). Because rifampicin still remains as an effective antituberculosis agent, it is often concomitantly used with cyclosporine (or tacrolimus) in transplant recipients. Therefore, clinically significant drug interaction between rifampicin and cyclosporine is expected. In a clinical study, treatment with rifampicin 600 mg once daily for 11 days caused a more than 3-fold decrease in the AUC_{po} of cyclosporine, presumably because of CYP3A4 induction (2). As a result of the significant decrease in cyclosporine blood concentrations, rifampicin had caused acute transplant rejection in patients treated with cyclosporine (1). In another clinical study, an 18-day treatment with rifampicin 600 mg daily markedly reduced the AUC_{po} of tacrolimus (a good substrate of CYP3A4) by about 3-fold (179). Therefore, an increase in the tacrolimus dosage was required to attain the effective blood concentration of tacrolimus to avoid graft rejection in transplant recipients (180).

Warfarin–rifampicin interaction is a well-documented but underappreciated drug interaction. Warfarin, a prototypic CYP2C9 substrate, remains as the first-line anticoagulant therapy. The unfavorable narrow range of therapeutic concentration makes warfarin prone to potentially life-threatening drug–drug interactions (181). In a clinical study with eight healthy volunteers, the AUC_{po} of warfarin was decreased by about 3-fold after a 21-day treatment with rifampicin 600 mg daily (182). The reduction of warfarin plasma concentration is believed to be a result of the rifampicin-mediated CYP2C9 induction. Consistent with a 3-fold decrease in warfarin plasma concentration, rifampicin treatment caused a 3-fold decrease in the anticoagulant effect of warfarin. In a case report (183), a patient receiving concomitant rifampicin and warfarin to treat a mycobacterial infection and intraventricular thrombus, respectively, required a more than 2-fold increase in the dosage of warfarin to attain the therapeutic efficacy, measured by international normalized ratio (INR). Because of temporal changes of enzyme level, the dose of warfarin should be reduced gradually after discontinuing rifampicin treatment to maintain a balance between therapeutic efficacy and adverse effect. A gradual 70% reduction in warfarin dosage over 4–5 weeks was necessary to maintain the therapeutic INR after rifampicin discontinuation (183). In another case report, a 72-year-old patient who was taking warfarin was concurrently administered with rifampicin for several months (184). During this period, satisfactory anticoagulation was achieved only when a high dose of warfarin (20 mg) was given. After discontinuation of rifampicin therapy, warfarin dose was adjusted gradually, until the stabilization of the prothrombin time at warfarin 7.5 mg.

Because of narrow therapeutic index of antiarrhythmic agents, potential interaction with other drugs is of major therapeutic importance in the clinical practice. Quinidine, a substrate of CYP3A4, is used for the treatment of cardiac arrhythmias. After 7 days of daily treatment with rifampicin 600 mg in six healthy volunteers, the AUC_{po} of quinidine decreased dramatically by 9-fold (185). In another clinical study, a 7-day treatment with rifampicin 600 mg daily caused a 5-fold decrease in the AUC_{po} of quinidine (186). Although no published studies or case reports have described the pharmacodynamic changes with respect to the quinidine –rifampicin interaction, it is likely that the interaction could be clinically significant. A clinically significant reduction in the QRS prolongation has been reported as a result of rifampicin–propafenone interaction. Propafenone, a sodium channel-blocking antiarrhythmic drug, is metabolized by CYP2D6 to 5-hydroxy-propafenone and by CYP3A4 to *N*-dealkylation metabolite (187). Both 5-hydroxy-propafenone and *N*-dealkylation-propafenone are active metabolites with activity as potent as the parent drug. The oxidative metabolites are subsequently eliminated by phase II metabolism, glucuronidation and sulfation. The effect of rifampicin on the pharmacokinetics and pharmacodynamics of propafenone was studied in six EMs and six PMs of CYP2D6 (188). After a 9-day rifampicin treatment (600 mg/day), the AUC_{po} of propafenone decreased from an average of 6.9 to 1.8 μM h in the EMs and from 54 to 16 μM h in PMs. Interestingly, rifampicin also had similar effect on the pharmacodynamics of propafenone (a 1.6-fold decrease in QRS prolongation) in both EMs and PMs of CYP2D6. The maximum QRS prolongation decreased from 21 to 13% in EMs and from 15 to 9% in PMs. Because of the formation of active metabolites, interpretation of clinical consequences would be somewhat complicated in this case.

Similarly, the pharmacokinetic interaction of rifampicin with codeine is quite complex. Codeine is a widely used opiate analgesic agent that is converted through *O*-demethylation to morphine by CYP2D6 in humans (189,190). Although the formation of morphine accounts for less than 10%, morphine is responsible for most of the analgesic effect of codeine. Unlike morphine, which is eliminated mainly by UGTs, codeine is metabolized by multiple enzyme systems, including CYP2D6, CYP3A4, and UGTs (191,192). The effect of rifampicin on the pharmacokinetics and pharmacodynamics of codeine has been studied in EMs and PMs of CYP2D6 (192). After treatment of rifampicin (600 mg/day) for 3 weeks, rifampicin had little effect on pharmacodynamics of codeine in PMs of CYP2D6. Because CYP2D6 is not inducible by rifampicin, it is expected that rifampicin will have no significant effect on the pharmacodynamics of codeine in EMs as well. Surprisingly, the codeine's pharmacodynamic effects were attenuated in EMs of CYP2D6 after rifampicin treatment. This is because rifampicin significantly decreased the concentration of codeine through an increase in the clearance of the major elimination pathways of codeine, namely, CYP3A4-dependent *N*-demethylation and UGT-dependent glucuronidation, which were induced by rifampicin. Thus, the conversion of morphine from codeine was significantly reduced in the EMs of CYP2D6 because of the decreased codeine concentration during rifampicin treatment. In addition to the decreased morphine formation, the

elimination of the converted morphine can also be enhanced via glucuronidation by rifampicin-induced UGTs.

Morphine is eliminated exclusively by UGTs to form glucuronide-3-morphine and glucuronide-6-morphine in humans (193). A significant drug interaction has been observed between morphine and rifampicin. In a clinical study with ten healthy volunteers receiving an oral dose of morphine (10 mg), the serum concentrations of morphine, glucuronide-3-morphine, and glucuronide-6-morphine were measured before and after the treatment with rifampicin 600 mg once daily for 13 days (194). There was only a moderate decrease in the serum concentrations of morphine after the treatment with rifampicin. The AUC and C_{max} of morphine, respectively, decreased from an average of 132 nM h and 34.5 nM before rifampicin treatment to 97 nM hr and 18 nM after rifampicin treatment, respectively. Similarly, there was also a moderate decrease in the AUC of glucuronide-3-morphine and glucuronide-6-morphine (194). Although rifampicin treatment only caused a moderate decrease in concentrations of morphine and its active metabolite, a complete loss of analgesic effect of morphine was observed in these volunteers after the treatment of rifampicin. Apparently, the complete loss of analgesic effect of morphine cannot be attributed to the moderate UGT induction by rifampicin.

The human immunodeficiency virus (HIV) protease inhibitors are used for the treatment of patients infected with HIV. Because of the increasing incidence of tuberculosis among patients infected with HIV, rifampicin is often concomitantly used with HIV protease inhibitors. Therefore, rifampicin-mediated CYP induction becomes a serious concern in the treatment of HIV infection (195). After a 14-day rifampicin treatment (600 mg/day), both the AUC_{po} and C_{max} of saquinavir decreased by more than 4-fold in healthy volunteers (196). Similarly, treatment with rifampicin (600 mg/day) for 7 days caused a 5-fold decrease in both the AUC_{po} and C_{max} of nelfinavir (197). Significant reduction in the AUC_{po} and C_{max} of ritonavir and indinavir after the rifampicin treatment has also been reported (198,199). Because the antiretroviral effect is highly dependent on the systemic exposures of the HIV protease inhibitors, the use of rifampicin is contraindicated to avoid treatment failure. In some cases, a dosage adjustment may be required to achieve the antiretroviral effect. For example, the dosage of efavirenz, a nonnucleotide transcriptase inhibitor, needs to be adjusted from 400 to 800 mg daily, when rifampicin is concurrently administered (200). Interestingly, efavirenz is also a CYP3A4 inducer. In a clinical study, the AUC_{po} of indinavir was decreased by 35% when coadministered with efavirenz (201). To compensate the efavirenz-mediated induction, the dosage of indinavir was suggested to increase from 800 mg three times daily to 1000 mg three times daily.

The effect of rifampicin on the central nervous system drug action has also been studied. A 5-day rifampicin treatment (600 mg/day) caused a more than 3-fold decrease in the AUC_{po} of zolpidem (128). In parallel, a significant reduction in the effects of zolpidem was seen in all psychomotor tests (digit symbol substitution, critical flicker fusion test, and Maddox wing test). In another clinical study, rifampicin treatment at 600 mg daily for 5 days resulted in a 5.5-fold decrease in the AUC_{po} of zopiclone and a significant reduction in the hypnotic effects (129). Similarly, a 5-day rifampicin treatment (600 mg/day) caused a 10- to 20-fold decrease in the AUC_{po} of midazolam and triazolam (130,131). As a result of the substantial decrease in systemic exposure, both midazolam and triazolam are ineffective in patients taking rifampicin. Therefore, it is advisable to use hypnotic agents that are not predominantly metabolized by CYP3A4 during treatment with rifampicin.

Induction in Toxicity

Epidemiological and animal studies have suggested that many xenobiotics and drugs cause toxicity by generating reactive metabolites or reactive oxygen species (202–204). CYP1A enzymes are responsible for metabolic activation and detoxification of some xenobiotics, such as polycyclic aromatic hydrocarbons. Although it is still highly controversial whether CYP1A induction is beneficial because of detoxification or is detrimental because of metabolic activation forming reactive metabolites, there is increasing evidence that CYP1A induction could be a risk factor that may cause toxicity and cancer (205–207). For example, cigarette smoke has been found to be associated with pulmonary, cardiovascular diseases, and cancer via CYP1A induction (208–211). Recently, *in vitro* and animal studies suggest that some xenobiotics exert their toxicity, not only by generating reactive metabolites, but also by altering expression of specific genes through AhR activation (204,205). Therefore, from an industrial perspective (for fear of possible toxic or carcinogenic effects), it may be desirable to select a drug candidate that is not a potent CYP1A inducer.

Induction of CYP2E1 by ethanol is believed to be responsible for hepatotoxicity by generating oxidative stress species (212,213). Microsomes from ethanol-treated rats, in which CYP2E1 was significantly induced, increased the rates of formation of superoxide and hydrogen peroxide (214,215). *In vitro* studies with human CYP2E1-expressed HepG2 cells suggest that human CYP2E1 is also capable of forming reactive oxygen intermediates and catalyzing lipid peroxidation (216). There is increasing evidence that ethanol-induced hepatotoxicity is linked to the increased production of oxidative stress species. For instance, the ethanol-induced hepatotoxicity has been shown to correlate with the expression levels of CYP2E1 as well as the elevated lipid peroxidation in rats (217). Furthermore, treatment of diallyl sulfide, an inhibitor of CYP2E1, prevented the elevation of lipid peroxidation and partially blocked the ethanol-induced hepatotoxicity. Similarly, increases in formation of reactive oxygen species after ethanol treatment are prevented by anti-CYP2E1 IgG (218).

Hepatotoxicity caused by CYP2E1 induction in humans has been suggested (219). A male subject was in the habit of consuming three glasses of wine regularly with dinner. He stopped ingesting ethanol when he contracted influenza and began taking acetaminophen for treatment. Several days later, he had a complete liver failure. It is believed that the hepatotoxicity is caused by an increased formation of reactive metabolite of acetaminophen, *N*-acetyl-*p*-benzoquinone imine (NAPQI), as a result of CYP2E1 induction caused by heavy alcohol drinking. *In vitro* studies with human liver microsomes have demonstrated that CYP2E1 can

modulate the formation of NAPQI (220). Interestingly, ethanol is able not only to induce but also to inhibit CYP2E1 activity (221). Based on the dual effect of ethanol, Slattery *et al.* (219) have developed a kinetic model to explain the complex ethanol–acetaminophen interaction. Through the model simulations, it becomes clear that the time interval between the last consumption of alcohol and ingestion of acetaminophen is very important in terms of the risk of hepatotoxicity. If acetaminophen is taken in the morning because of a headache as a result of the heavy drinking the night before, there is a high risk of hepatotoxicity. This is because at the next morning after heavy drinking, the low level of ethanol concentration is insufficient to inhibit the formation of NAPQI in the liver where CYP2E1 enzyme activity was induced, resulting in an increased formation of the toxic metabolite. However, if acetaminophen and ethanol are taken together at the same time, the formation of NAPQI is not expected to increase because of the opposite effects of CYP2E1 inhibition and induction by ethanol.

In addition to CYP2E1, NAPQI can also be formed by human CYP1A2 and CYP3A4 (221,222). Therefore, it has been suggested that CYP1A2 and CYP3A4 may be also related with the acetaminophen-induced hepatotoxicity. The role of CYP1A2 in acetaminophen-induced hepatotoxicity has been studied in wild-type, *Cyp2e1*$^{(-/-)}$, and *Cyp1a2*$^{(-/-)}$ mice (223–225). Following the administration of acetaminophen at 250 mg/kg, there were no significant differences in the serum alanine aminotransferase between *Cyp1a2*$^{(+/+)}$ and *Cyp1a2*$^{(-/-)}$ mice. In addition, there was no difference in the urinary metabolites excreted over a 24-h period, including those derived from glutathione (GSH) conjugation of the major reactive metabolite NAPQI of acetaminophen, between the *Cyp1a2*$^{(+/+)}$ and *Cyp1a2*$^{(-/-)}$ mice. With these results, the investigators concluded that CYP1a2 does not play a significant role in acetaminophen hepatotoxicity in mice. Consistent with the findings in mice, induction of human CYP1A2 seems to have little effect on the formation of the reactive metabolite NAPQI of acetaminophen. In a clinical study with EMs and PMs of CYP2C19, omeprazole was administered orally at 40 mg daily for 7 days (226). A significant CYP1A2 induction was observed only in the PMs, but not in EMs of CYP2C19. Despite induction of CYP1A2 activity in the PMs, there was no significant difference in the formation of thioether conjugates, which are terminal metabolites of NAPQI.

Recently, the role of CYP3A enzymes on the acetaminophen-induced hepatotoxicity has also been studied in PXR-null mice (227). Pretreatment with PCN, a potent inducer of mice Cyp3a11, markedly enhanced acetaminophen-induced hepatotoxicity, as revealed by increased serum concentration of liver enzymes and hepatic centrilobular necrosis in wild-type mice, but not in PXR null mice following an intraperitoneal dose of acetaminophen (350 mg/kg). Furthermore, PCN treatment significantly increased the GSH-derived metabolites of NAPQI in wild-type mice, but not in PXR null mice. These results suggest that Cyp3a11 plays an important role in acetaminophen-induced hepatotoxicity in mice. In contrast to mice, the contribution of CYP3A4 to the formation of NAPQI of acetaminophen seems to be negligible as compared to CYP2E1 in humans (228). The involvement of CYP2E1 was assessed through pretreatment of healthy volunteers with disulfiram to inhibit the activity of CYP2E1 enzyme, and the role of CYP3A4 was measured by pretreatment with rifampicin (600 mg/day) for a week. The recovery of the thiol metabolites formed by NAPQI with GSH was significantly decreased by 69%, and the formation of NAPQI was decreased by 74% when treated with disulfiram. However, rifampicin treatment had no effect on the formation of NAPQI or the recovery of thiol metabolites formed by conjugation of NAPQI with GSH. These results strongly suggest that CYP2E1, rather than CYP3A4, is the major enzyme responsible for the acetaminophen-induced hepatotoxicity in humans.

ASSESSMENT OF CYP INDUCTION

From an industrial perspective, CYP induction is a highly undesirable drug property. This is not only because of metabolic liability with respect to the potential of decreased therapeutic efficacy and increased toxicity, but also because of the concern of marketing competition (229). Therefore, it is desirable to develop new drug candidates that are not potent CYP inducers to avoid the potential of CYP induction-mediated drug interactions. Ideally, information pertaining to CYP induction should be obtained at the stage of drug discovery before the selection of new drug candidates for drug development. For this reason, today, many drug companies routinely include early evaluation of CYP induction at the drug discovery stage as part of the selection processes of drug candidates (230,231).

Although many of the CYP enzymes are known to be inducible, CYP3A4 induction is probably the most important cause of the documented induction-based interactions (232,233). This is because CYP3A4 accounts for roughly 40% of the total CYP in human liver and catalyzes the metabolism of more than 60% of clinically used drugs. Therefore, most drug companies focus mainly on the assessment of CYP3A4 induction. The induction potential for other CYP enzymes is evaluated only when there is a need. Although animal models may provide some useful information on the factors that affect CYP induction, significant species differences in the inductive response preclude the use of animal models for the assessment of human CYP3A4 induction for new drug candidates. Therefore, the use of *in vitro* systems is the only means by which the potential of human CYP3A4 induction can be assessed.

In Vitro Methods for the Assessment of CYP3A4 Induction

Several *in vitro* models have been established for assessing the potential of CYP3A4 induction for new drug candidates, including liver slices, immortalized cell lines, and primary hepatocytes. Each method has its advantage and disadvantage, and the readers are referred to other review articles for further information on this topic (10–13,234). Among these models, primary cultures of human hepatocytes have been used extensively by academic and industrial laboratories for evaluating CYP3A4 induction. It is generally accepted that the primary hepatocyte culture is the most predictive *in vitro* model for assessing CYP induction. Thus, primary cultures of human hepatocytes have become the

"gold standard" for *in vitro* testing of CYP3A4 induction and widely accepted by drug companies in assessing the potential CYP3A4 induction of new drug candidates (10,230,231). It should be noted, however, that hepatocytes in culture are not always a faithful model for predicting CYP induction, unless optimal experimental conditions have been established.

Conditions of cell culture can have a significant impact on the extent of CYP induction. For example, coating dishes with Matrigel resulted in marked improvements in rat hepatocyte morphology and *CYP* gene expression (235). In addition, overlaying the hepatocyte monolayer with an additional layer of extracellular matrix, the so-called collagen-sandwiched hepatocytes, enhances the CYP induction response in rat hepatocyte cultures. Although the extracellular matrix seems to be critical for CYP induction in "rodent" hepatocytes, the extracellular matrix has little effect on CYP3A4 induction in "human" hepatocytes (235–237). The CYP3A4 induction by rifampicin was not significantly different between human hepatocytes maintained on a substratum of either Matrigel or collagen (237). Similarly, studies by Silva *et al.* (10) showed that extracellular matrix had little effect on CYP3A4 induction in human hepatocytes. Similar to extracellular matrix, *in vitro* studies revealed that the medium formulation seems to have little effect on the inductive response of human hepatocytes. For example, the induction of CYP3A4 by rifampicin was almost identical in human hepatocytes when different medium conditions (modified Chee's medium, Williams' E medium, and hepatocyte medium) were used (13).

Although the extracellular matrix and medium conditions seem to have little effect on CYP3A4 induction, the plating density of human hepatocytes (number of cells per unit area of substratum) seems to have an important impact on CYP3A4 induction. For example, plating at low cell density resulted in a lower inductive response to rifampicin treatment in human hepatocytes (13). Significant alterations in the morphology and integrity of hepatocytes were observed at low plating density. Many of the individual cells exhibited fibroblast-like morphological features with decreased cell contacts at low plating density. In addition, it has been shown that the cytoskeletons, especially microtubules and actin microfilaments, are present in hepatocytes maintained at high plating density, but not at low density (13). These results suggest that cell–cell interactions may play an important role in hepatocyte cell function, possibly through adhesion receptors. Based on these results, it is concluded that the optimal plating density of hepatocytes is around 120,000 and 180,000 cells/cm^2 (13).

During the cell culture, there is a time-dependent decrease in the expression of CYP3A4 mRNA, the corresponding protein level and enzyme activity (measured by testosterone 6β-hydroxylation) in hepatocytes, reaching the lowest levels within 24 h (13,237). After reaching the nadir, the CYP3A4 mRNA, protein level, and enzyme activity return to the basal levels within the next 24 h. Therefore, the temporal changes in the CYP3A4 mRNA, protein level, and enzyme activity should be taken into account when conducting CYP3A4 induction in human hepatocytes. Another important factor that may affect CYP3A4 induction in hepatocytes is the effect of solvents that are commonly used in the preparation of drug stock solution. LeCluyse *et al.* (238) have shown that dimethylsulfoxide (DMSO) can induce CYP3A4 in a concentration-dependent manner over a concentration range between 0.1 and 1% (v/v). The DMSO-mediated CYP3A4 induction seems to be related to the activation of PXR. In a separate study, Lehmann *et al.* (15) have demonstrated that DMSO can activate human PXR in a concentration-dependent manner over a concentration range of 0.1–1% (v/v). These results suggest that DMSO may induce CYP3A4 in human hepatocytes. Therefore, the effect of solvent on CYP induction should be carefully evaluated and incorporated into the experimental design for the assessment of CYP induction.

Although it is generally accepted that primary culture of human hepatocytes is the "gold standard" for *in vitro* testing of CYP3A4 induction, a major problem associated with using human hepatocytes has been the erratic supply of human liver tissue. Even from commercial sources, the timing and availability of human hepatocytes are not always convenient. In addition, the quality of the cells is often compromised in shipping from the vendor to the laboratory, and the quantity of hepatocytes is often limited. Therefore, a routine and robust screen for potential CYP3A4 induction is needed, particularly at the early stage of drug discovery. Recently, several *in vitro* reporter gene assays have been developed for assessing the induction of the *CYP3A4* gene (19,20).

In a study, 14 commercially available compounds were evaluated and compared for their ability to induce CYP3A4 in human hepatocytes and to activate human PXR in a reporter gene assay (20). Sandwiched primary cultures of human hepatocytes from six donors were used, and CYP3A4 activity was assessed by measuring microsomal testosterone 6β-hydroxylase activity. Hepatic CYP3A4 mRNA and protein levels were also analyzed using branched DNA technology/Northern blotting and Western blotting, respectively. A reasonably good correlation between human PXR activation and CYP3A4 induction in human hepatocytes was observed for these 14 compounds. In another study, 17 compounds, including known CYP3A4 inducers, were evaluated for their PXR activation, using a reporter gene assay cotransfected PXR and GR (19). The rank order of the potency of the known CYP3A4 inducers was generally in agreement with the results from the PXR/GR cotransfected reporter gene assay. Collectively, these results suggest that the PXR reporter gene assay can be used as a reliable *in vitro* model for CYP3A4 induction. Because of its robustness and reliability, the PXR reporter assay is particularly useful at the stage of drug discovery for high-throughput screen. However, it is important to note that the PXR reporter assay should be considered as a supplement, but not a replacement of human hepatocytes for assessment of CYP3A4 induction.

Assessment of CYP3A4 Induction and Data Presentation

Whereas enzyme induction is defined as an increase in mRNA and enzyme protein levels, the associated change in the enzyme activity is probably more clinically relevant than the changes in mRNA and protein level in assessing the potential for CYP3A4 induction. For example, ritonavir induced CYP3A4 mRNA and protein levels in human hepatocytes in a concentration-dependent manner, but there

was a decrease in CYP3A4 enzyme activity because of its strong CYP3A4 inhibition activity (239). In the clinical setting, ritonavir inhibits the CYP3A4-mediated metabolism of drugs, and the induction of CYP3A4 activity is offset by its strong inhibitory effect. Therefore, it is desirable to have all three endpoint measurements (mRNA, protein levels, and enzyme activity) at the same time for a compound's ability to induce CYP3A4 to accurately interpret the data. Kinetically, the intrinsic parameters EC_{50} (effective concentration for 50% maximal induction) and E_{max} (maximal CYP induction) of a drug are probably more useful for the interpretation of *in vitro* induction data. The EC_{50} for an inductive agent allows a direct comparison to be drawn between the plasma concentration of the agent and its ability of induction following drug administration, whereas E_{max} predicts the maximal extent of CYP induction of the agent in patients.

The EC_{50} and E_{max} values have been used to compare the capacity of troglitazone and rifampicin to induce CYP3A4 activity in primary human hepatocyte cultures and to predict the induction potential of troglitazone in humans (162). Whereas E_{max} for troglitazone induction (measured as testosterone 6β-hydroxylation, 6 nmol/mg protein/min) was comparable to that of rifampicin, troglitazone's EC_{50} (5–10 μM) was 10- to 30-fold higher than that of rifampicin (0.3–0.5 μM). Thus, the intrinsic induction activity (E_{max}/EC_{50}) of troglitazone is only about 10% of the rifampicin value. In addition, the derived EC_{50} of troglitazone for CYP3A4 induction is much higher than the steady-state plasma concentration of troglitazone in clinical studies (2 μM). Taken together, these results suggest that troglitazone is a weak CYP3A4 inducer at the clinical dose range. In another study with human hepatocytes, the EC_{50} and E_{max} for CYP3A4 induction by SR12813 were estimated to be 0.6–1.0 μM and 5 nmol/min/mg protein, respectively, and the corresponding values for rifampicin were 0.3 μM and 10 nmol/min/mg protein (13,88). Because the E_{max} of SR12813 is about 50% of the value of rifampicin, significant induction by the compound would occur *in vivo* only when plasma concentrations of SR12813 are much greater than 1 μM. From the above two examples, it is clear that the potential of a drug to induce CYP3A4 depends not only on its intrinsic activity (E_{max}/EC_{50}), but also on the relationship between its plasma concentration and EC_{50} value.

However, determination of the EC_{50} and E_{max} may not be very practical for routine screening purpose during the drug discovery or early development stage because of the limited availability of human hepatocytes and the requirement of including more concentration points to estimate the kinetic parameters. Sometimes, it may be difficult to obtain an E_{max} value because of the cell toxicity of the test compound at high concentrations. Alternatively, the induction potential (fold change in relation to the basal level) or induction potency (as a percentage of the rifampicin value at 10 μM) can be used to assess CYP3A4 induction in hepatocytes at the drug discovery stage (10). The primary advantage of the use of fold-change method is that this approach can provide useful information on interindividual variability of CYP3A4 induction in hepatocyte preparations from different donors.

However, the use of the fold-change method without proper reference may sometimes lead to inappropriate conclusions (10). There are two reasons for this. First, the basal levels of CYP3A4 in some individuals are so low that they are difficult to accurately quantify, leading to an inaccurate estimation of the fold change. Thus, based on the fold change of enzyme activity, one may inaccurately conclude that the drug candidate is a potent CYP3A4 inducer, although it would only be a moderate inducer based on the percentage induction relative to rifampicin. Second, the basal CYP3A4 level and associated enzyme activity can be highly variable between hepatocyte preparations obtained from different donors. As discussed earlier, the fold change in CYP3A4 induction elicited by a given inducing agent is highly dependent on the basal level of CYP3A4 in hepatocyte preparations; the lower the basal level, the higher the fold induction (11,104). Because of limited availability of liver donors (normally $N = 3–4$), it is quite possible that hepatocytes may be obtained from the liver donors that all have low basal CYP3A4 level (or high basal CYP3A4 level).

In Vitro/*in Vivo* Extrapolation

The ultimate goal of *in vitro* induction studies is to predict induction-based drug interactions in the clinical settings. However, the prediction of CYP induction by *in vitro* data is quite complicated. As discussed above, CYP induction by drugs is not only a concentration-dependent but also a time-dependent process. In addition, the magnitude of CYP induction depends on the net balance of enzyme biosynthesis and degradation in the time course during the chronic dosing of inducing agent. It would be very difficult to predict the extent of CYP induction as function of time. However, after a sufficient period of chronic dosing, CYP induction reaches a steady-state condition, and the process becomes time invariant. In theory, at the steady state, the magnitude (E) of *in vivo* induction can be predicted for a drug candidate using the following equation with *in vitro* EC_{50} and E_{max} values at the steady-state concentration of a drug (C).

$$E = \frac{E_{max} \times C}{EC_{50} + C} \quad (1)$$

Theoretically, the drug concentration (C) used for predicting CYP3A4 induction should be the unbound drug concentration at the active site of the PXR (or CAR) receptor. It is believed that only the unbound drug would activate PXR (or CAR) and induce the biosynthesis of CYP3A4 in hepatocytes. Because direct measurement of unbound drug concentration at the active site is almost impossible, the unbound drug concentration in plasma is used in lieu of unbound drug concentration at the active site, based on the assumption that drug binds reversibly to plasma and tissue proteins, and that equilibrium of unbound drug occurs readily between plasma and hepatocytes. Although it is generally accepted that plasma protein binding plays an important role in determining the degree of CYP inhibition (240–242), there is no information on the effect of plasma protein binding on CYP3A4 induction. In fact, the effect of plasma protein binding on CYP3A4 induction is still a

debatable topic. Some scientists believe that the role of plasma protein binding in CYP3A4 induction may not be as important as in CYP3A4 inhibition. This is because the process of CYP3A4 induction is a cumulative effect, whereas that of CYP3A4 inhibition is an immediate effect. However, other scientists believe that the role of plasma protein binding in CYP induction is as important as CYP inhibition because there is always a clear relationship between the magnitude of CYP induction and drug concentration in the *in vitro* hepatocyte system after a sufficient period of incubation time. Without a full understanding of the role of plasma protein binding in CYP induction, it is difficult to predict the degree of CYP induction *in vivo*. Therefore, more efforts should be made to evaluate the effect of plasma and tissue protein binding on the *in vivo* CYP3A4 induction.

In spite of the difficulties, attempt has been made to predict *in vivo* induction by comparing *in vitro* and *in vivo* E_{max}. As shown in Eq. (1), the *in vitro* E_{max} of an inducer can be obtained when the drug concentration of the inducer is much greater than its EC_{50} value, and the *in vivo* E_{max} of the inducer can also be obtained when a high dose of the inducer is given. As aforementioned, E_{max} becomes a time-invariant parameter when a steady-state condition is reached after sufficient period of dosing. In addition, the issues of plasma protein binding and biologic variability become less significant, when the E_{max} approach is applied. There are several good examples of the use of the E_{max} approach to predict CYP induction. For example, the *in vitro* E_{max} of CYP3A4 induction in rat hepatocytes by dexamethasone is in good agreement with the *in vivo* E_{max} determined in rats (243). The mean *in vitro* E_{max} was estimated to be 6-fold over its basal activity after 72-h incubation in rat hepatocytes at a high concentration of dexamethasone (15 μM). On the other hand, the mean *in vivo* E_{max} was determined to be about 5-fold in rats following administration of dexamethasone at 50 mg/kg/day for 4 days. It should be noted that the EC_{50} of dexamethasone was determined to be about 1.0 μM (243). Similarly, a good correlation between the maximal *in vitro* and *in vivo* CYP3A induction for 13 compounds has been reported by Silva *et al.* (10). In this study, rat hepatocytes were incubated with the test compounds at concentration of 50 μM for 4 days (96 h), whereas the compounds were given orally to rats at 400 mg/kg/day for 4 days. These results suggest that the *in vivo* E_{max} can be predicted reasonably well by using *in vitro* E_{max} data.

The *in vitro*/*in vivo* E_{max} approach has also been applied for rifampicin in humans. The *in vitro* E_{max} of rifampicin was estimated to be in the range of 10- to 20-fold of its basal CYP3A4 protein level in human hepatocytes at 10 μM (145,244,245). The *in vitro* E_{max} (fold change) of CYP3A4 by rifampicin is in good agreement with the *in vivo* E_{max} reported by Ged *et al.* (102). In Ged *et al.*'s study, there was an 18-fold increase in protein levels of CYP3A4 in liver biopsies after rifampicin treatment (600 mg/day for 4 days). The peak plasma concentrations of rifampicin are in the range of 5–15 μM in patients following an oral dose of 600 mg, and that the plasma protein binding of rifampicin is about 70–80% in human plasma (246). Thus, a maximal CYP3A4 induction of rifampicin would occur *in vivo* when given at 600-mg oral dose.

CONCLUSION

Although our understanding of CYP induction has advanced significantly over the last 10 years through the discovery of key nuclear receptors, much still remains to be learned about the molecular mechanisms of CYP induction. One of the unsolved issues is the wide interindividual variability in CYP induction. There are a large number of factors that could contribute to the variability, including genetic and environmental variables. The relative contribution between the genetic and environmental variables is not readily assessed because, in part, of the complexity of CYP induction and insufficiency of proper experimental tools. Another critical issue that may complicate the prediction and interpretation of CYP induction is the interplay between efflux transporters and CYP enzymes. Although there is now an increasing evidence to suggest the interplay between CYPs and efflux transporters because of a striking overlap in substrates and inducers with CYP enzymes and efflux transporters, our knowledge about this interplay is very limited (247–250). This is because it is very difficult to accurately estimate the relative contribution of CYP enzymes and transporters to drug absorption and disposition. This is particularly true for drug interactions that are caused by CYP and transporter induction.

Because of the complexity of the contributing factors, quantitative prediction of CYP induction is very difficult, if not impossible. Although numerous *in vitro* systems have been developed to assess CYP induction, these systems are only useful for semiquantitative assessment whether a new drug candidate has a potential for CYP induction. Information obtained from *in vitro* induction studies is still limited in its ability to predict whether there is a "probability" of induction-based drug interactions. If the *in vitro* data suggest that a drug candidate could have a potential for CYP induction, clinical studies should be conducted earlier to assess the degree of induction. Therefore, the induction data obtained from *in vitro* systems should not serve as a "No-Go" decision for drug development without a proper clinical assessment. Finally, it should be noted that in many cases, the CYP induction is manageable by adjusting dosage regimen.

REFERENCES

1. D. L. Modry, E. B. Stinson, and P. E. Oyer. Acute rejection and massive cyclosporine requirements in heart transplant recipients treated with rifampin. *Transplantation* **39**:313–314 (1985).
2. M. F. Hebert, J. P. Roberts, T. Prueksaritanont, and L. Z. Benet. Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. *Clin. Pharmacol. Ther.* **52**:453–457 (1992).
3. J. H. Lin and A. Y. H. Lu. Inhibition and induction of cytochrome P450 and the clinical implications. *Clin. Pharmacokinet.* **35**:361–390 (1998).
4. A. P. Beresford. CYP1A1: friend or foe? *Drug Metab. Rev.* **25**:503–517 (1993).
5. J. Digiovanna, D. L. Berry, M. R. Juchau, and T. J. Slaga. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: potent anticarcinogenic activity in CD-1 mice. *Biochem. Biophys. Res. Commun.* **86**:577–584 (1979).
6. H. V. Gelboin. Benzo[*a*]pyrene metabolism, activation and carcinogenesis: role of mixed function oxidases and related enzymes. *Pharmacol. Rev.* **60**:1107–1166 (1980).

7. H. Jaeschke, G. J. Gores, A. I. Cederbaum, J. A. Hinson, D. Pessayre, and J. J. Lemasters. Mechanism of hepatotoxicity. *Toxicol. Sci.* **65**:166–176 (2002).
8. O. Pelkonen, J. Manenpaa, P. Taavitsainen, A. Rautio, and H. Rautio. Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotics* **28**:1203–1253 (1998).
9. M. F. Fromm, D. Busse, H. K. Kroemer, and M. Eichelbaum. Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin. *Hepatology* **24**:796–801 (1996).
10. J. M. Silva, P. E. Morin, S. H. Day, B. P. Kennedy, P. Payette, T. Rushmore, J. A. Yergey, and D. A. Nicoll-Griffith. Refinement of an *in vitro* cell model for cytochrome P450 induction. *Drug Metab. Dispos.* **26**:490–496 (1998).
11. V. E. Kostrubsky, V. Ramachandran, R. Venkataramanan, K. Dorko, J. E. Esplen, S. Zhang, J. F. Sinclair, S. A. Wrighton, and S. C. Strom. The use of human hepatocyte cultures to study the induction of cytochrome P-450. *Drug Metab. Dispos.* **27**: 887–894 (1999).
12. P. Maurel. The use of adult human hepatocytes in primary culture and other *in vitro* systems to investigate drug metabolism in man. *Adv. Drug Deliv. Rev.* **22**:105–132 (1996).
13. E. L. LeCluyse. Human hepatocyte culture systems for the *in vitro* evaluation of cytochrome P450 expression and regulation. *Eur. J. Pharm. Sci.* **13**:343–368 (2001).
14. S. A. Kliewer, J. T. Moore, L. Wade, J. L. Staudinger, M. A. Watson, S. A. Jones, D. D. McKee, B. B. Oliver, T. M. Wiltson, R. H. Zetterstrom, T. Pellerman, and J. M. Lehmann. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92**:73–82 (1998).
15. J. M. Lehmann, D. D. McKee, M. A. Watson, T. M. Wilson, T. J. Moore, and S. A. Kliewer. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* **102**:1016–1023 (1998).
16. B. Blumberg, W. Sabbagh, H. Juguilon, J. Bolado, C. M. van Meter, E. S. Ong, and R. M. Evans. SXR, a novel steroid and xenobiotic sensing nuclear receptor. *Genes Dev.* **12**: 3195–3205 (1998).
17. G. Bertilsson, J. Heidrich, K. Svensson, M. Asman, L. Jendeberg, M. Sydow-Backman, R. Ohlsson, H. Postlind, P. Blomquist, and A. Berkenstam. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Natl. Acad. Sci. USA* **95**:12208–12213 (1998).
18. L. B. Moore, D. J. Parks, S. A. Jones, R. K. Bledsoe, T. G. Consler, J. B. Stimmel, B. Goodwin, C. Liddle, S. G. Blanchard, and T. M. Wilson. Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J. Biol. Chem.* **275**: 15122–15127 (2000).
19. W. El-Sankary, G. G. Gibson, A. Ayrton, and N. Plant. Use of a reporter gene assay to predict and rank the potency and efficacy of CYP3A4 inducers. *Drug Metab. Dispos.* **29**:1499–1504 (2001).
20. G. Luo, M. Cunningham, S. Kim, T. Burn, J. Lin, M. Sinz, G. Hamilton, C. Rizzo, S. Jolley, D. Gilbert, A. Downey, D. Mudra, R. Grham, K. Carroll, J. Xie, A. Madan, A. Parkinson, D. Christ, B. Selling, E. Lecluyse, and L. Gan. CYP3A4 induction by drugs: correlation between a pregnane x receptor reporter gene assay and CYP3A4 expression in human hepatocytes. *Drug Metab. Dispos.* **30**:795–804 (2002).
21. M. J. J. Roins and M. Ingelman-Sunderg. Induction of human drug-metabolizing enzymes: mechanisms and implications. In T. F. Woolf (ed.), *Handbook of Drug Metabolism*, Marcel Dekker, New York, 1999, pp. 239–262.
22. T. Sueyoshi and M. Negishi. Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu. Rev. Pharmacol. Toxicol.* **41**:123–143 (2001).
23. J. P. Whitlock Jr. Induction of cytochrome P4501A1. *Annu. Rev. Pharmacol. Toxicol.* **39**:103–125 (1999).
24. P. B. Watkins, S. A. Wrighton, E. G. Schuetz, P. Maurel, and P. S. Guzelian. Macolide antibiotics inhibit the degradation of the glucocorticoid-responsive cytochrome P450 in rat hepatocytes *in vivo* and in primary monolayer culture. *J. Biol. Chem.* **261**:6264–6271 (1986).
25. D. R. Koop, B. C. Crump, G. D. Nordblom, and M. J. Coon. Immunochemical evidence for induction of alcohol-oxidizing cytochrome P450 of rabbit liver microsomes by diverse agents: ethanol, imidazole, trichloroethylene, acetone, pyrazole and isoniazid. *Proc. Natl. Acad. Sci. USA* **82**:4065–4069 (1985).
26. B. J. Song, R. L. Veech, S. S. Park, H. V. Gelboin, and J. F. Gonzalez. Induction of rat hepatic nitrosodimethylamine demethylase by acetone is due to protein stabilization. *J. Biol. Chem.* **264**:3568–3572 (1989).
27. B. J. Song, T. Matsunaga, J. P. Hardwick, S. S. Park, R. L. Veech, C. S. Yang, H. V. Gelboin, and F. J. Gonzalez. Stabilization of cytochrome P450j messenger ribonucleic acid in the diabetic rat. *Mol. Endocrinol.* **1**:542–547 (1987).
28. A. P. Poland, E. Glover, and A. S. Kende. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol: evidence that the binding species is the receptor for induction of aryl hydrocarbon hydroxylase. *J. Biol. Chem.* **251**:4936–4946 (1976).
29. A. P. Poland and J. C. Knutson. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**:517–554 (1982).
30. A. G. Miller, D. I. Israel, and J. P. Whitlock Jr. Biochemical and genetic analysis of variant mouse hepatoma cells defective in the induction of benzo(*a*)pyrene-metabolizing enzyme activity. *J. Biol. Chem.* **258**:3523–3527 (1983).
31. O. Pelkonen, J. Hukkanen, P. Honkakoski, J. Hakkola, P. Viitala, and H. Raunio. *In vitro* screening of cytochrome P450 induction potential. In O. Pelkonen, A. Baumann, and A. Reichel (ed.), *Pharmacokinetic Challengers in Drug Discovery*, Springer-Verlag, Berlin, 2002, pp. 105–137.
32. R. C. Pollenz, C. A. Sattler, and A. Poland. The arylhydrocarbon receptor and arylhydrocarbon receptor nuclear translocator protein show distinct subcellular localizations in Hepa 1c1c 7 cells by immunofluorescence microscopy. *Mol. Pharmacol.* **45**:428–438 (1994).
33. M. S. Denison, J. M. Fisher, and J. P. Whitlock Jr. The DNA recognition site for the dioxin–Ah receptor complex. Nucleotide sequence and functional analysis. *J. Biol. Chem.* **263**: 17221–17224 (1988).
34. K. W. Jones and J. P. Whitlock Jr. Functional analysis of the transcriptional promoter for the CYP 1A1 gene. *Mol. Cell. Biol.* **10**:5098–5105 (1990).
35. L. Wu and J. P. Whitlock Jr. Mechanism of dioxin action: Ah receptor-mediated increase in promoter accessibility *in vivo*. *Proc. Natl. Acad. Sci. USA* **89**:4811–4815 (1992).
36. C. Handschin and U. A. Meyer. Induction of drug metabolism: the role of nuclear receptor. *Pharmacol. Rev.* **55**:649–673 (2003).
37. M. Baes, T. Gulick, H.-S. Choi, M. G. Martinoli, D. Simha, and D. D. Moore. A new orphan nuclear member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. *Mol. Cell. Biol.* **14**:1544–1551 (1994).
38. P. Wei, J. Zhang, M. Egan-Hafley, S. Liang, and D. D. Moore. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. *Nature* **407**:920–923 (2000).
39. D. J. Waxman. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. *Arch. Biochem. Biophys.* **369**:11–23 (1999).
40. B. M. Forman, I. Tzameli, H. S. Choi, J. Chen, D. Simha, W. Seol, R. M. Evans, and D. D. Moore. Androstane metabolites bind to and deactivated the nuclear receptor CARβ. *Nature* **395**:612–615 (1998).
41. E. Trottier, A. Belzil, C. Stoltz, and A. Anderson. Localization of a phenobarbital-responsive element (PBRE) in the 5′ flanking region of the rat CYP2B2 gene. *Gene* **158**:263–268 (1995).
42. P. Honkakoski, R. Moore, J. Gynther, and M. Negishi. Characterization of phenobarbital-inducible mouse Cyp2b10 gene transcription in primary hepatocytes. *J. Biol. Chem.* **271**: 9746–9753 (1996).
43. T. Sueyoshi, T. Kawamoto, I. Zelko, P. Honkakoski, and M. Negishi. The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. *J. Biol. Chem.* **274**:6043–6046 (1996).

44. C. Stoltz and A. Anderson. Positive regulation of the rat CYP2B2 phenobarbital response unit by the nuclear receptor hexamer half-site-nuclear factor 1 complex. *Biochem. Pharmacol.* **57**:1073–1076 (1999).
45. T. Kawamoto, T. Sueyoshi, I. Zelko, R. Moore, K. Washburn, and M. Negishi. Phenobarbital-responsive nuclear translocation of the receptor CAR in induction of the CYP2B gene. *Mol. Cell. Biol.* **19**:6318–6322 (1999).
46. P. Honkakoski and M. Negishi. Protein serine/threonine phosphatase inhibitors suppress phenobarbital-induced Cyp2b10 gene transcription in mouse primary hepatocytes. *Biochem. J.* **330**:889–895 (1998).
47. I. Zelko, T. Sueyoshi, T. Kawamoto, R. Moore, and M. Negishi. The peptide near the C terminus regulator CAR nuclear translocation induced by xenochemicals in mouse liver. *Mol. Cell. Biol.* **21**:2838–2846 (2001).
48. P. Honkakoski, I. Zelko, T. Sueyoshi, and M. Negishi. The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital responsive enhancer module in the Cyp 2b10 gene. *Mol. Cell. Biol.* **18**:5652–5658 (1998).
49. N. Marc, M. Galisteo, D. Lagadic-Gossmann, A. Fautrel, F. Joannard, A. Guillouzo, and L. Corcos. Regulation of phenobarbital induction of the cytochrome P450 2b9/10 genes in primary mouse hepatocyte culture: involvement of calcium- and cAMP-dependent pathways. *Eur. J. Biochem.* **267**:963–970 (2000).
50. W. Xie, J. L. Barwick, M. Downes, B. Blumberg, C. M. Simon, M. C. Nelson, B. A. Neuschwander-Teri, E. M. Brunt, P. S. Guzelian, and R. M. Evans. Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature* **406**:435–439 (2000).
51. H. Zhang, E. L. LeCulyse, L. Liu, M. Hu, L. Matoney, W. Zhu, and B. Yan. Rat pregnane X receptor: molecular cloning, tissue distribution, and xenobiotic regulation. *Arch. Biochem. Biophys.* **368**:14–22 (1999).
52. S. Gerbal-Chaloin, M. Daujat, J. M. Pascussi, L. Pichard-Garcia, M. J. Vilarem, and P. Maurel. Transcriptional regulation of CYP2C9 gene. Role of glucocorticoid receptor and constitutive androstane receptor. *J. Biol. Chem.* **277**:209–217 (2002).
53. U. Savas, M. R. Weester, K. J. Griffin, and E. F. Johnson. Rabbit pregnane X receptor is activated by rifampicin. *Drug Metab. Dispos.* **28**:529–537 (2000).
54. L. B. Moore, J. M. Maglich, D. D. McKee, B. Wisely, T. M. Willson, S. A. Kliewer, M. H. Lambert, and J. T. Moore. Pregnane X receptor (PXR), constitutive androstane receptor (CAR), and benzoate X receptor (BXR) define three pharmacologically distinct classes of nuclear receptors. *Mol. Endocrinol.* **16**:977–986 (2002).
55. L. B. Moore, B. Goodwin, S. A. Jones, G. B. Wisely, C. J. Serabjit-Singh, T. M. Wilson, J. L. Coliins, and S. A. Kliewer. St. John's wort induces hepatic drug metabolism through activation of pregnane X receptor. *Proc. Natl. Acad. Sci. USA* **97**:7500–7502 (2000).
56. E. J. Squires, T. Sueyoshi, and M. Negishi. Cytoplasmic localization of pregnane X receptor and ligand-dependent nuclear translocation in mouse liver. *J. Biol. Chem.* **279**: 49307–49314 (2004).
57. H. Wang and E. L. LeCluyse. Role of orphan nuclear receptors in the regulation of drug-metabolising enzymes. *Clin. Pharmacokinet.* **42**:1331–1357 (2003).
58. R. E. Watkins, G. B. Wisely, L. B. Moore, J. L. Collins, M. H. Lambert, S. P. Williams, T. M. Willson, S. A. Kliewer, and M. R. Redinbo. The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. *Science* **292**:2329–2333 (2001).
59. B. Goodwin, M. R. Redinbo, and S. A. Kliewer. Regulation of CYP3A gene transcription by the pregnane X receptor. *Annu. Rev. Pharmacol. Toxicol.* **42**:1–23 (2002).
60. M. A. Correia. Hepatic cytochrome P450 degradation: mechanistic diversity of the cellular sanitation brigade. *Drug Metab. Rev.* **35**:107–143 (2003).
61. P. Wei, J. Zhang, D. H. Dowhan, Y. Han, and D. D. Moore. Specific and overlapping functions of the nuclear hormone receptors CAR and PXR in xenobiotic response. *Pharmacogenomics J.* **2**:117–126 (2002).
62. W. Xie, J. L. Barwick, C. M. Simon, A. M. Pierce, S. Safe, B. Blumberg, P. S. Guzelian, and R. M. Evans. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev.* **14**:3014–3023 (2000).
63. B. Goodwin, E. Hodgson, D. J. D'Costa, G. R. Robertson, and C. Liddle. Transcriptional regulation of human CYP3A4 gene by the constitutive androstane receptor. *Mol. Pharmacol.* **62**:359–365 (2002).
64. B. Goodwin, L. B. Moore, C. M. Stoltz, D. D. McKee, and S. A. Kliewer. Regulation of the human *CYP2B6* gene by the nuclear receptor PXR. *Mol. Pharmacol.* **60**:427–431 (2001).
65. H. Wang, S. Faucette, T. Sueyoshi, R. Moore, S. Ferguson, M. Negishi, and E. L. LeCluyse. A novel distal enhancer module regulated by pregnane X receptor/constitutive androstane receptor is essential for the maximal induction of CYP2B6 gene expression. *J. Biol. Chem.* **278**:14146–14152 (2003).
66. D. Smirlis, R. Muangmoonchai, M. Edwards, I. R. Phillips, and E. A. Shephard. Orphan receptor promiscuity in the induction of cytochromes P450 by xenobiotics. *J. Biol. Chem.* **276**: 12822–12826 (2001).
67. G. A. Kullak-Ublick and M. B. Becker. Regulation of drug and bile salt transporters in liver and intestine. *Drug Metab. Rev.* **35**:305–317 (2003).
68. C. Li and A. N. Tong. Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch. Pharm. Res.* **28**: 249–268 (2005).
69. J. Sugatani, H. Kojima, A. Ueda, S. Kakizaki, K. Yoshinari, Q. H. Gong, I. S. Owens, M. Negishi, and T. Sueyoshi. The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase UGT1A1 gene and regulation by the nuclear receptor CAR. *Hepatology* **33**:1232–1238 (2001).
70. J. Sugatani, S. Nishitani, K. Yamakawa, K. Yoshinari, T. Sueyoshi, M. Negishi, and M. Miwa. Transcriptional regulation of human *UGT1A1* gene expression: activated glucocorticoid receptor enhances constitutive androstane receptor/pregnane X receptor-mediated UDP-glucuronosyltransferase 1A1 regulation with glucocorticoid receptor-interacting protein 1. *Mol. Pharmacol.* **67**:845–855 (2005).
71. J. Sugatani, K. Yamakawa, E. Tonda, S. Nishitani, K. Yoshinari, M. Degawa, I. Abe, H. Noguchi, and M. Miwa. The induction of human UDP-glucuronosyltransferase 1A1 mediated through a distal enhancer module by flavonoids and xenobiotics. *Biochem. Pharmacol.* **67**:989–1000 (2004).
72. E. G. Schuetz, W. Schmid, G. Schutz, C. Brimer, K. Yasuda, T. Kamataki, L. Bornheim, K. Myles, and T. J. Cole. The glucocorticoid receptor is essential for induction of cytochrome P-4502B by steroid but not for drug or steroid induction of CYP3A or P-450 reductase in mouse liver. *Drug Metab. Dispos.* **28**:268–278 (2000).
73. H. Wang, S. R. Faucette, D. Gilbert, S. L. Jolley, T. Sueyoshi, M. Negishi, and E. L. LeCluyse. Glucocorticoid receptor enhancement of pregnane X receptor mediated CYP2B6 regulation in primary human hepatocytes. *Drug Metab. Dispos.* **31**:620–630 (2003).
74. W. El-Sankary, V. Bombail, G. G. Gibson, and N. Plant. Glucocorticoid-mediated induction of CYP3A4 is decreased by disruption of a protein: DNA interaction distinct from the pregnane X receptor response element. *Drug Metab. Dispos.* **30**:1029–1034 (2002).
75. J.-M. Pascussi, L. Drocourt, J.-M. Fabre, P. Maurel, and M.-J. Vilarem. Dexamethasone induces pregnane X receptor and retinoid X receptor-α expression: synergic increase of CYP3A4 induction by pregnane X receptor activators. *Mol. Pharmacol.* **58**:361–372 (2000).
76. J. M. Huss and C. B. Kasper. Two-stage glucocorticoid induction of CYP3A23 through both the glucocorticoid and pregnane X receptors. *Mol. Pharmacol.* **58**:48–57 (2000).
77. J.-M. Passcussi, S. Gerbal-Chaloin, J.-M. Fabre, P. Maurel, and M.-J. Vilarem. Dexamethasone enhances constitutive androstane receptor expression in human hepatocytes: consequences on cytochrome P450 gene regulation. *Mol. Pharmacol.* **58**:1441–1450 (2000).
78. K. Yoshinari, T. Sueyoshi, R. Moore, and M. Negishi. Nuclear receptor CAR as a regulatory factor for the sexually dimorphic

induction of CYP2B1 gene by phenobarbital in rat livers. *Mol. Pharmacol.* **59**:278–284 (2001).
79. D. Diaze, I. Fabre, M. Daujat, B. Saint Aubbert, P. Bories, H. Michel, and P. Maurel. Omeprazole is an aryl hydrocarbon-like inducer of human hepatic cytochrome P-450. *Gastroenterology* **99**:737–747 (1990).
80. W. M. McDonnell, J. M. Scheiman, and P. G. Traber. Induction of cytochrome P450 1A genes (CYP1A) by omeprazole in human alimentary tract. *Gastroenterology* **103**:1509–1516 (1992).
81. T. A. Kocarek, E. G. Schuetz, S. C. Strom, R. A. Fisher, and P. S. Guzelian. Comparative analysis of cytochrome P4503A induction in primary cultures of rat, rabbit and human hepatocytes. *Drug Metab. Dispos.* **23**:415–421 (1995).
82. D. W. Nebert and F. J. Conzalez. The P450 gene superfamily. In K. Ruckpaul and H. Rein (ed.), *Principles, Mechanisms and Biological Consequences of Induction*, Taylor & Francis, London, 1990, pp. 35–61.
83. L. Xu, A. P. Li, D. L. Kaminski, and M. F. Ruh. 2,3,7,8 Tetrachlorodibenzo-*p*-dioxin induction of cytochrome P4501A in cultured rat and human hepatocytes. *Chem. Biol. Interact.* **124**:173–189 (2000).
84. M. Degawa, K. Kobayashi, S. Miura, H. Arai, H. Esumi, T. Sugimura, and Y. Hashimoto. Species difference among experimental rodents in induction of P450IA family enzymes by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. *Jpn. J. Cancer Res.* **83**:1047–1051 (1992).
85. I. N. White, A. Davies, L. L. Smith, S. Dawson, and F. Matteis. Induction of CYP2B1 and CYP3A1, and associated monooxygenase activities by tamoxifen and certain analogues in the livers of female rats and mice. *Biochem. Pharmacol.* **7**:21–30 (1993).
86. S. M. Fontaine, P. B. Hoyer, J. R. Halpert, and I. G. Sipes. Role of induction of specific hepatic cytochrome P450 isoforms in epoxidation of 4-vinylcyclohexene. *Drug Metab. Dispos.* **29**: 1236–1242 (2001).
87. J. L. Barwick, L. C. Quattrochi, A. S. Mills, C. Potenza, R. H. Tukey, and P. S. Guzelian. Trans-species gene transfer for analysis of glucocorticoid-inducible transcriptional activation of transiently expressed human CYP3A4 and rabbit CYP3A6 in primary cultures of adult rat and rabbit hepatocytes. *Mol. Pharmacol.* **50**:10–16 (1996).
88. S. A. Jones, L. B. Moore, J. L. Shenk, G. B. Wisely, G. A. Hamilton, D. D. McKee, N. C. O. Tomkinson, E. L. LeCluyse, M. H. Lambert, T. M. Wilson, S. A. Kliewer, and J. T. Moore. The pregnane X receptor, a promiscuous xenobiotic receptor that has diverged during evolution. *Mol. Endocrinol.* **14**:27–39 (2000).
89. J. M. Tredger, H. M. Smith, P. R. Powell-Jackson, M. Davis, and R. Williams. Effect of rifampicin on the mouse hepatic mixed-function oxidase system. *Biochem. Pharmacol.* **30**: 1043–1051 (1981).
90. Y. Adachi, T. Nanno, M. Yamashita, S. Ueshima, and T. Yamamoto. Induction of rat liver bilirubin-conjugating enzymes and glutathione S-transferase by rifampicin. *Gastroenterol. Jpn.* **20**:104–110 (1985).
91. H.-S. Choi, M. Chung, I. Tzameli, D. Simha, Y.-K. Lee, W. Seol, and D. D. Moore. Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. *J. Biol. Chem.* **272**:23565–23571 (1997).
92. T. Shimada, H. Yamazaki, M. Mimura, Y. Inui, and F. P. Guengerich. Interindividual variations in human liver cytochromes P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.* **270**:414–423 (1994).
93. K. E. Thummel, D. D. Shen, T. D. Podoll, K. L. Kunze, and W. F. Trager. Use of midazolam as a human cytochrome P4503A probe: II. Characterization of inter and intraindividual hepatic CYP3A variability after liver transplantation. *J. Pharmacol. Exp. Ther.* **271**:557–566 (1994).
94. M. F. Paine, M. Khalighi, J. M. Fisher, D. D. Shen, K. L. Kunze, C. L. Marsh, J. D. Perkins, and K. E. Thummel. Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J. Pharmacol. Exp. Ther.* **283**:1552–1562 (1997).
95. D. Juan, D. M. Worwag, D. A. Schoeller, A. N. Kotake, R. L. Huges, and M. C. Frederisken. Effects of dietary protein on theophyllin pharmacokinetics and caffeine and aminopyrine breath test. *Clin. Pharmacol. Ther.* **40**:187–194 (1989).
96. M. A. Kall, O. Vang, and J. Clausen. Effects of dietary broccoli on human *in vivo* drug metabolizing enzymes: evaluation of caffeine, oestrone and chlorzoxane metabolism. *Carcinogenesis* **17**:793–799 (1996).
97. E. J. Pantuck, C. B. Pantuck, B. H. Min, L. W. Wattenberg, and K. E. Anderson. Stimulatory effect of Brussel sprouts and cabbage on human drug metabolism. *Clin. Pharmacol. Ther.* **25**:88–95 (1979).
98. M. A. Kall and J. Clausen. Dietary effect on mixed function P4501A2 activity assayed by estimation of caffeine metabolism in human. *Human Exp. Toxicol.* **14**:801–807 (1995).
99. S. Zevin and N. L. Benowitz. Drug interactions with tobacco smoking. *Clin. Pharmacokinet.* **36**:425–438 (1999).
100. E. T. Morgan. Regulation of cytochrome P450 by inflammatory mediators: why and how? *Drug Metab. Dispos.* **29**:207–212 (2001).
101. J. H. Lin and A. Y. H. Lu. Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *Annu. Rev. Pharmacol. Toxicol.* **41**:535–567 (2001).
102. C. Ged, J. M. Rouillon, L. Pichard, J. Combalbert, and N. Bressot. The increase in urinary excretion of 6-hydoxycortisol as a maker of human hepatic cytochrome P450IIIA induction. *Br. J. Clin. Pharmacol.* **28**:373–387 (1989).
103. J. C. Kolars, P. Schmiedlin-Ren, J. D. Sceutz, C. Fang, and P. B. Watkins. Identification of rifampicin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J. Clin. Invest.* **90**:1871–1878 (1992).
104. T. K. Chang, L. Yu, P. Maurel, and D. J. Waxman. Enhance cyclophosphamide and ifosfamide activation in primary human hepatocyte cultures: response of cytochrome P-450 inducers and autoinduction by oxazaphosphorines. *Cancer Res.* **57**: 1946–1954 (1997).
105. J. S. McCune, R. L. Hawke, E. L. LeCluyse, H. H. Gillenwater, G. A. Hamilton, J. Ritchie, and C. Lindley. *In vivo* and *in vitro* induction of human cytochrome P4503A4 by dexamethasone. *Clin. Pharmacol. Ther.* **68**:356–366 (2000).
106. R. S. Thomas, S. G. Penn, K. Holden, C. A. Bradfield, and D. R. Rank. Sequence variation and phylogenetic history of the mouse *Ahr* gene. *Pharmacogenetics* **12**:151–162 (2002).
107. A. Poland and E. Glover. Characterization and strain distribution pattern of the murine Ah receptor specified by the Ahd and Ahb-3 alleles. *Mol. Pharmacol.* **38**:306–312 (1990).
108. L. S. Birnbaum, M. M. McDonald, P. C. Blair, A. M. Clark, and M. W. Harris. Differential toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6 mice congenic at the Ah locus. *Fundam. Appl. Toxicol.* **15**:186–200 (1990).
109. R. Pohjanvirta, J. M. Y. Wong, W. Li, P. A. Harper, J. Tuomisto, and A. B. Okey. Point mutation in intron sequence causes altered carboxyl terminal structure in the aryl hydrocarbon receptor of most 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-resistant rat strain. *Mol. Pharmacol.* **54**:86–93 (1998).
110. M. Korkalainen, J. Tuomisto, and R. Pohjanvirta. Identification of novel splice variants of ARNT and ARNT2 in the rat. *Biochem. Biophys. Res. Commun.* **303**:1095–1100 (2003).
111. P. A. Harper, J. M. Y. Wong, M. S. M. Lam, and A. B. Okey. Polymorphisms in the human AH receptor. *Chem. Biol. Interact.* **141**:161–187 (2002).
112. K. Kawajiri, J. Watanabe, H. Eguchi, K. Nakachi, C. Kiyohara, and S. Hayashi. Polymorphism of human Ah receptor gene are not involved in lung cancer. *Pharmacogenetics* **5**:151–158 (1995).
113. S. Cauchi, I. Stucker, C. Solas, P. Laurent-Puig, D. Hemon, M. Jacquet, P. Kremers, P. Beaune, and L. Massaad-Massade. Polymorphisms of human aryl hydrocarbon receptor (AhR) gene in a French population: relationship with CYP1A1 inducibility and lung cancer. *Carcinogenesis* **22**:1819–1824 (2001).
114. J. Smart and A. K. Daly. Variation in induced CYP1A1 levels: relationship to CYP1A1 Ah receptor and GSTM1 polymorphisms. *Pharmacogenetics* **10**:11–24 (2000).
115. J. M. Y. Wong, A. O. Okey, and P. A. Harper. Human aryl hydrocarbon receptor polymorphisms that result in loss of CYP1A1 induction. *Biochem. Biophys. Res. Commun.* **288**:990–996 (2001).

116. J. Zhang, P. Kuehl, E. D. Green, J. W. Touchman, P. B. Waltkin, A. Daly, S. D. Hall, P. Maurel, M. Relling, C. Brimer, K. Yasuda, S. A. Wrighton, M. Hancock, R. B. Kim, S. Strom, K. Thummel, C. G. Rusell, J. R. Hudson Jr., E. G. Schuetz, and M. S. Boguski. The human pregnane X receptor: gene structure and identification and functional characterization of natural allelic variants. *Pharmacogenetics* **11**:555–572 (2001).
117. S. Koyano, K. Kurose, Y. Saito, S. Ozawa, R. Hasegawa, K. Komamura, K. Ueno, S. Kamakura, M. Kitakaze, T. Nakajima, K. Matsumoto, A. Akasawa, H. Saito, and J. Sawada. Functional characterization of four naturally occurring variants of human pregnane X receptor (PXR): one variant causes dramatic loss of both DNA binding activity and the transactivation of the CYP3A4 promoter/enhancer region. *Drug Metab. Dispos.* **32**:149–154 (2004).
118. H. Jinno, T. Tanaka-Kagawa, N. Hanioka, S. Isgida, M. Saeki, A. Soyama, M. Itoda, T. Nishimura, Y. Saito, S. Ozawa, M. Ando, and J. Sawada. Identification of novel alternative splice variant of human constitutive androstane receptor and characterization of their expression in the liver. *Mol. Pharmacol.* **65**:496–502 (2004).
119. C. Tang, J. H. Lin, and A. Y. H. Lu. Metabolism-based drug–drug interactions: what determines individual variability in cytochrome P450 induction? *Drug Metab. Dispos.* **33**: 603–613 (2005).
120. J. J. Diliberto, P. I. Akubue, R. W. Luebke, and L. S. Birnbaum. Dose-response relationships of tissue distribution and induction of CYP1A1 and CYP1A2 enzymatic activities following acute exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in mice. *Toxicol. Appl. Pharmacol.* **130**:197–208 (1995).
121. G. R. Wilkinson. Clearance approaches in pharmacology. *Pharmacol. Rev.* **39**:1–47 (1987).
122. G. R. Wilkinson and D. G. Shand. A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther.* **18**:377–390 (1975).
123. G. Alvan, K. Piafsky, M. Linda, and C. Bahrvon. Effect of pentobarbital on the disposition of alprenolol. *Clin. Pharmacol. Ther.* **22**:316–321 (1977).
124. N. Holtbecker, M. F. Fromm, H. K. Kroemer, E. E. Ohnhaus, and H. Heidemann. The nifedipine–rifampin interaction: evidence for induction of gut wall metabolism. *Drug Metab. Dispos.* **24**:1121–1123 (1996).
125. E. D. Kharasch, C. Hoffer, D. Whittington, and P. Sheffels. Role of hepatic and intestinal cytochrome P450 3A and 2B6 in the metabolism, disposition, and miotic effects of methadone. *Clin. Pharmacol. Ther.* **76**:250–269 (2004).
126. J. Schmider, J. Brockmoller, G. Arold, S. Bauer, and I. Roots. Simultaneous assessment of CYP3A4 and CYP1A2 activity *in vivo* with alprazolam and caffeine. *Pharmacogenetics* **9**:725–734 (1999).
127. E. E. Ohnhaus, N. Brockmeyer, P. Dylewiez, and H. Habicht. The effect of antipyrin and rifampin on the metabolism of diazepam. *Clin. Pharmacol. Ther.* **42**:148–156 (1987).
128. K. Villikka, K. Kivisto, H. Luurila, and P. J. Neuvonen. Rifampin reduces plasma concentrations and effects of zolpidem. *Clin. Pharmacol. Ther.* **62**:629–634 (1997).
129. K. Villikka, K. Kivisto, T. S. Lamberg, T. Kantola, and P. J. Neuvonen. Concentrations and effects of zopiclone are greatly reduced by rifampicin. *Br. J. Clin. Pharmacol.* **43**:471–474 (1997).
130. J. T. Backman, K. T. Plkkola, and P. J. Neuvonen. Rifampin drastically reduces plasma concentrations and effects of oral midazolam. *Clin. Pharmacol. Ther.* **59**:7–13 (1996).
131. K. Villikka, K. Kivisto, J. T. Backman, K. T. Olkkola, and P. J. Neuvonen. Triazolam is ineffective in patients taking rifampin. *Clin. Pharmacol. Ther.* **61**:8–14 (1997).
132. T. W. Synold, I. Dussault, and B. M. Forman. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat. Med.* **7**:584–590 (2001).
133. S. Gerbal-Chaloin, J.-M. Pascussi, L. Pichard-Garcia, M. Daujat, F. Waechter, J.-M. Fabre, N. Carrere, and P. Maurel. Induction of CYP2C genes in human hepatocytes in primary cultures. *Drug Metab. Dispos.* **29**:242–251 (2001).
134. L. D. Heimark, M. Gibaldi, W. F. Tragger, R. A. O'Reilly, and D. A. Goulart. The mechanism of the warfarin–rifampin drug interaction in humans. *Clin. Pharmacol. Ther.* **42**:388–394 (1987).
135. S. J. Baldwin, S. E. Clarke, and R. J. Chenery. Characterization of the cytochrome P450 enzymes involved in the *in vitro* metabolism of rosiglitazone. *Br. J. Clin. Pharmacol.* **48**:424–432 (1999).
136. J.-Y. Park, K.-A. Kim, M.-H. Kang, S.-L. Kim, and J.-G. Shin. Effect of rifampin on the pharmacokinetics of rosiglitazone in healthy subjects. *Clin. Pharmacol. Ther.* **75**:157–162 (2004).
137. H. D. Langtry and J. A. Balfour. Glimepiride. A review of its use in the management of type-2 diabetes mellitus. *Drugs* **55**:563–584 (1998).
138. M. Niemi, K. T. Kivisto, J. T. Backman, and P. J. Neuvonen. Effect of rifampicin on the pharmacokinetics and pharmacodynamics of glimepiride. *Br. J. Clin. Pharmacol.* **50**:591–595 (2000).
139. J.-Y. Park, K.-A. Kim, P.-W. Park, C.-W. Park, and J.-G. Shin. Effect of rifampin on the pharmacokinetics and pharmacodynamics of gliclazide. *Clin. Pharmacol. Ther.* **74**:334–340 (2003).
140. M. Niemi, J. T. Backman, M. Neuvonen, P. J. Neuvonen, and K. T. Kivisto. Effects of rifampin on the pharmacokinetics and pharmacodynamics of glyburide and glipizide. *Clin. Pharmacol. Ther.* **69**:400–406 (2001).
141. Z. Dvorak, M. Modriansky, L. Pichard-Garcia, P. Balaguer, M.-J. Vilarem, J. Ulrichova, P. Maurel, and J.-M. Pascussi. Colchicine down-regulates cytochrome P450 2B6, 2C8, 2C9, and 3A4 in human hepatocytes by affecting their glucocorticoid receptor-mediated regulation. *Mol. Pharmacol.* **64**:160–169 (2003).
142. A. Madan, R. A. Graham, K. M. Carroll, D. R. Mudra, L. A. Burton, L. A. Krueger, A. D. Downey, M. Czerwinski, J. Forster, M. D. Ribadeneira, L.-S. Gan, E. L. LeCluyse, K. Zech, P. Robertson Jr., P. Koch, L. Antonian, G. Wagner, L. Yu, and A. Parkinson. Effects of prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. *Drug Metab. Dispos.* **31**:421–431 (2003).
143. J. Sahi, R. H. Stern, M. A. Milad, K. A. Rose, G. Gibson, X. Zheng, L. Stilgenbauer, N. Sadagopan, S. Jolley, D. Gilbert, and E. L. LeCluyse. Effects of avasimibe on cytochrome P450 2C9 expression *in vitro* and *in vivo*. *Drug Metab. Dispos.* **32**: 1370–1376 (2004).
144. J. M. Pascussi, S. Gerbal-Chaloin, L. Drocourt, P. Maurel, and M. J. Vilarem. The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. *Biochim. Biophys. Acta* **1619**:243–253 (2003).
145. L. Drocourt, J.-C. Ourlin, J.-M. Pascussi, and P. Maurel. Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by vitamin D receptor pathway in primary human hepatocytes. *J. Biol. Chem.* **277**:25125–25132 (2002).
146. L. Drocourt, J.-M. Pascussi, E. Assenat, J.-M. Fabre, P. Maurel, and M.-J. Vilarem. Calcium channel modulators of the dihydropyridine family are human pregnane X receptor activators and inducers of CYP3A, CYP2B, and CYP2C in human hepatocytes. *Drug Metab. Dispos.* **29**:1325–1331 (2001).
147. H. G. Jeong and K.-H. Yang. Expression of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible cytochrome P4501A1 in human splenic lymphocyte cultures. *Cancer Lett.* **98**:193–198 (1996).
148. W. Li, P. A. Harper, B.-K. Tang, and A. B. Okey. Regulation of cytochrome P450 enzymes by arylhydrocarbon receptor in human cells: CYP1A2 expression in the LS180 colon carcinoma cell line after treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or 3-methylcholanthrene. *Biochem. Pharmacol.* **56**:599–612 (1998).
149. M. J. Santostefano, D. G. Ross, U. Savas, C. R. Jefcoate, and L. S. Birnbaum. Differential time-course and dose-response relationships of TCDD-induced CYP1B1, CYP1A1, and CYP1A2 proteins in rats. *Biochem. Biophys. Res. Commun.* **233**:20–24 (1997).
150. B. Kedderis, J. J. Mills, M. E. Andersen, and L. S. Birnbaum. A physiologically based pharmacokinetic model of 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) in the rat: tissue distribution and CYP1A induction. *Toxicol. Appl. Pharmacol.* **121**:87–98 (1993).
151. H.-W. Leung, R. H. Ku, D. J. Paustenback, and M. E. Andersen. A physiologic-based pharmacokinetic model for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in C57BL/6J and DBA/2J mice. *Toxicol. Lett.* **42**:15–28 (1988).
152. H.-W. Leung, D. J. Paustenback, F. J. Murray, and M. E.

Andersen. A physiological pharmacokinetic model of tissue distribution and enzyme-inducing properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Toxicol. Appl. Pharmacol.* **103**:399–410 (1990).
153. S. Krusekopf, U. Kleeberg, A. G. Hildebrandt, and K. Ruckpaul. Effects of benzimidazole derivatives on cytochrome P450 1A1 expression in a human cell line. *Xenobiotica* **27**:1–9 (1997).
154. S. Krusekopf, I. Roots, A. G. Hildebrandt, and U. Kleeberg. Time-dependent transcriptional induction of CYP1A1, CYP 1A2 and CYP1B1 mRNAs by H^+/K^+-ATPase inhibitors and other xenobiotics. *Xenobiotica* **33**:107–118 (2003).
155. K. L. Rost, H. Brosick, G. Heinemeyer, and I. Roots. Specific and dose-dependent enzyme induction by omeprazole in human beings. *Hepatology* **20**:1204–1212 (1994).
156. K. L. Rost, H. Brosick, J. Brockmoller, G. Heinemeyer, and I. Roots. Increase of P450IA2 activity by omeprazole: evidence by the 13-C[N3-methyl]caffeine breath test in poor and extensive metabolizers of S-mephenytoin. *Clin. Pharmacol. Ther.* **52**:170–180 (1992).
157. M. S. Faber and U. Fuhr. Time response of cytochrome P450 1A2 activity on cessation of heavy smoking. *Clin. Pharmacol. Ther.* **76**:178–184 (2004).
158. A. Parkinson, P. E. Thomas, D. E. Ryan, and W. Levin. The *in vivo* turnover of rat liver microsomal epoxide hydrolase and both the apoprotein and heme moieties of species cytochrome P-450 isozymes. *Arch. Biochem. Biophys.* **225**:216–236 (1983).
159. A. Parkinson, P. E. Thomas, D. E. Ryan, L. M. Reik, S. H. Safe, L. W. Robertson, and W. Levin. Differential time course of induction of rat liver microsomal cytochrome P-450 isozymes and epoxide hydrolase by aroclor 1254. *Arch. Biochem. Biophys.* **225**:203–215 (1983).
160. V. E. Kostrubsky, L. D. Lewis, S. C. Strom, S. C. Wood, E. G. Schetz, J. D. Schuetz, P. S. Sinclair, S. A. Wrighton, and J. F. Sinclair. Induction of cytochrome P4503A by taxol in primary cultures of human hepatocytes. *Arch. Biochem. Biophys.* **355**: 131–136 (1998).
161. S. C. Nallami, T. A. Glauser, N. Hariparsad, K. Setchell, D. J. Buckley, A. R. Buckley, and P. B. Desai. Dose-dependent induction of cytochrome P450 (CYP) 3A4 and activation of pregnane X receptor by topiramate. *Epilepsia* **44**:1428–1521 (2003).
162. J. Sahi, G. Hamilton, M. Sinze, S. Barros, S.-M. Huang, L. J. Lesko, and E. L. Lecluyse. Effects of troglitazone on cytochrome P450 enzymes in primary cultures of human and rat hepatocytes. *Xenobiotica* **30**:273–284 (2000).
163. J. G. Dougles and M. J. McLeod. Pharmacokinetic factors in the modern drug treatment of tuberculosis. *Clin. Pharmacokinet.* **37**:127–146 (1999).
164. R. J. Herman, K. Nakamura, G. R. Wilkinson, and A. J. Wood. Induction of propanolol metabolism by rifampicin. *Br. J. Clin. Pharmacol.* **16**:565–569 (1983).
165. O. E. Orisakwe, D. N. Akunyili, P. U. Agbasi, and N. A. Ezejiofor. Some plasma and saliva pharmacokinetics parameters of rifampicin in the presence of pefloxacin. *Am. J. Ther.* **11**:283–287 (2004).
166. B. U. Ndanusa, A. Mustapha, and I. Abdu-Aguye. The effect of single dose of rifampicin on the pharmacokinetics of oral nifedipine. *J. Pharm. Biomed. Anal.* **15**:1571–1575 (1997).
167. C. Y. Wu, L. Z. Benet, M. F. Hebert, S. K. Gupta, M. Rowland, D. Y. Gomez, and V. J. Wacher. Differential of absorption and first-pass gut and hepatic metabolism in humans: studies with cyclosporine. *Clin. Pharmacol. Ther.* **58**:492–497 (1995).
168. J. G. Gorski, S. Vannaprasaht, M. A. Hamman, W. T. Ambrosius, M. A. Bruce, B. Haehner-Daniels, and S. D. Hall. The effect of age, sex, and rifampin administration on intestinal and hepatic cytochrome P450 3A activity. *Clin. Pharmacol. Ther.* **74**:275–287 (2003).
169. J. H. Lin, M. Chiba, and T. A. Baillie. Is the role of the small intestine in first-pass metabolism overemphasized? *Pharmacol. Rev.* **51**:135–157 (1999).
170. F. Lapple, O. van Richter, M. F. Fromm, T. Richter, K. P. Thon, H. Wisser, E.-U. Griese, M. Eichelbaum, and K. T. Kivisto. Differential expression and function of CYP2C isoforms in human intestine and liver. *Pharmacogenetics* **13**:565–575 (2003).
171. I. Waziersde, P. H. Cugnene, C. S. Yang, J. P. Leroux, and P. H. Beaune. Cytochrome P450 isozymes, epoxide hydrolase and glutathione transferase in rat and human hepatic and extrahepatic tissues. *J. Pharmacol. Exp. Ther.* **253**:387–394 (1990).
172. J. H. Lin, M. Chiba, J. A. Nishime, F. A. DeLuna, M. Yamazaki, and Y. Lin. Effect of dexamethasone on the intestinal first-pass metabolism of indinavir in rats: evidence of cytochrome P-450 3A and P-glycoprotein induction. *Drug Metab. Dispos.* **27**:1187–1193 (1999).
173. D. Sesardic, K. J. Cole, R. J. Edwards, D. S. Davis, P. E. Thomas, W. Levin, and A. R. Boobis. The inducibility and catalytic activity of cytochrome e (P450 1A1) and P450d (P450 1A2) in rat tissues. *Biochem. Pharmacol.* **39**:499–506 (1990).
174. C. H. Kleinbloesem, J. van Hartan, J. P. H. Wilson, M. Danhof, P. van Brummek, and D. D. Breimer. Nifedipine: kinetics and hemodynamic effects in patients with liver cirrhosis after intravenous and oral administration. *Clin. Pharmacol. Ther.* **40**: 21–28 (1986).
175. M. Eichelbaum, M. Albrecht, G. Kliems, K. Schafer, and A. Somogyi. Influence of meso-caval shunt surgery on verapamil kinetics, bioavailability and response. *Br. J. Clin. Pharmacol.* **10**:527–530 (1980).
176. H. Glaeser, S. Drescher, M. Eichelbaum, and M. F. From. Influence of rifampicin on the expression and function of human intestinal cytochrome P450 enzymes. *Br. J. Clin. Pharmacol.* **59**:199–206 (2005).
177. A. Sayiner, T. Ece, S. Duman, A. Yildiz, M. Ozkahya, Z. Kilicaslan, and Y. Tokat. Tuberculosis in renal transplant recipients. *Transplantation* **68**:1268–1271 (1999).
178. V. Sakhuja, V. Jha, P. P. Varma, K. Joshi, and K. S. Chugh. The high incidence of tuberculosis among renal transplant recipients in India. *Transplantation* **61**:211–215 (1996).
179. M. F. Hebert, R. M. Fisher, C. L. Marsh, D. Dressler, and I. Bekersky. Effects of rifampin on tacrolimus in healthy volunteers. *J. Clin. Pharmacol.* **39**:91–96 (1999).
180. R. Y. Chenhsu, C. C. Loong, M. H. Chou, M. F. Lin, and W. C. Yang. Renal allograft dysfunction associated with rifampin–tacrolimus interaction. *Ann. Pharmacother.* **34**:27–31 (2000).
181. R. A. O'Reilly. Warfarin metabolism and drug–drug interactions. *Adv. Exp. Med. Biol.* **214**:205–212 (1987).
182. R. A. O'Reilly. Interaction of chronic daily warfarin therapy and rifampin. *Ann. Intern. Med.* **83**:506–508 (1975).
183. C. R. Lee and K. A. Thrasher. Difficulties in anticoagulation management during coadministration of warfarin and rifampin. *Pharmacotherapy* **21**:1240–1246 (2001).
184. T. H. Self and R. B. Mann. Interaction of rifampin and warfarin. *Chest* **67**:490–491 (1975).
185. P. Damkier, L. L. Hansen, and K. Brosen. Rifampicin treatment greatly increases the apparent oral clearance of quinidine. *Pharmacol. Toxicol.* **85**:257–262 (1999).
186. Y. Twum-Barima and S. G. Carruthers. Quinidine–rifampin interaction. *N. Engl. J. Med.* **304**:1466–1469 (1981).
187. A. Hemeryck, C. DeVriendt, and F. M. Belpaire. Effect of selective serotonin reuptake inhibitors on the oxidative metabolism of propafenone: *in vitro* studies using human liver microsomes. *J. Clin. Psychopharmacol.* **20**:428–434 (2000).
188. K. Dilger, B. Greiner, M. F. Fromm, U. Hofmann, H. K. Kroemer, and M. Eichelbaum. Consequences of rifampicin treatment on propafenone disposition in extensive and poor metabolizers of CYP2D6. *Pharmacogenetics* **9**:551–559 (1999).
189. Q. Y. Yue, J. O. Sevesson, C. Alm, F. Sjoqvist, and J. Sawe. Codeine *O*-demethylation co-segregates with polymorphic debrisoquine hydroxylation. *Br. J. Clin. Pharmacol.* **28**:639–645 (1989).
190. Q. Y. Yue, J. O. Sevesson, C. Alm, F. Sjoqvist, and J. Sawe. Pharmacokinetics of codeine and its metabolites in Caucasian healthy volunteers: comparisons between extensive and poor hydroxylators of debrisoquine. *Br. J. Clin. Pharmacol.* **31**: 635–642 (1991).

191. S. C. Armstrong and K. L. Cozza. Pharmacokinetic drug interactions of morphine, codeine, and their derivatives: theory and clinical reality, Part II. *Psychosomatics* **44**: 515–520 (2003).
192. Y. Caraco, J. Sheller, and A. J. Wood. Pharmacogenetic determinants of codeine induction by rifampin: the impact on codeine's respiratory, psychomotor and miotic effects. *J. Pharmacol. Exp. Ther.* **281**:330–336 (1997).
193. M. W. H. Coughtrie, B. Ask, A. Rane, B. Burchell, and R. Hume. The enantioselective glucuronidation of morphine in rats and humans. Evidence for the involvement of more than one UDP-glucuronosyltransferase isoenzyme. *Biochem. Pharmacol.* **38**:3273–3280 (1989).
194. M. F. Fromm, K. Eckhardt, S. Li, G. Schanzle, U. Hofmann, G.Mikus, and M. Eichelbaum. Loss of analgesic effect of morphine due to coadministration of rifampin. *Pain* **72**: 261–267 (1997).
195. L. I. Malaty and J. J. Kupper. Drug interactions of HIV protease inhibitors. *Drug Safety* **20**:147–169 (1999).
196. S. Grub, H. Bryson, T. Goggin, E. Ludin, and K. Jorga. The interaction of saquinavir (soft gelatin capsule) with ketoconazole, erythromycin and rifampicin: comparison of the effect in healthy volunteers and in HIV-infected patients. *Eur. J. Clin. Pharmacol.* **57**:115–121 (2001).
197. Nelfinavir Mesylate (Viracept®) Prescribing Information. Agouron Pharmaceuticals, La Jolla, CA, 2001.
198. Norvir® Package Insert. Abbott Laboratories, Illinois, 1997.
199. Crixivan® Package Insert. Merck & Co, Pennsylvania, 1997.
200. Efavirenz (Sustiva®) Package Insert. Bristol-Myers Squibb Company, New Jersey, 2002.
201. W. D. Fiske, D. Mayers, and K. Wagner. Pharmacokinetics of DMP 266 and indinavir multiple oral doses in HIV-1 infected individuals [abstract no. 568]. 4th Conference on Retroviruses and Opportunistic Infections. 1996, Jan 28–Feb 1. Washington, DC.
202. C. Ju and J. Uetrecht. Mechanism of idiosyncratic drug reaction: reactive metabolite formation, protein binding and the regulation of immune system. *Curr. Drug Metab.* **3**:367–377 (2002).
203. J. Urtrecht. Screening for the potential of a drug candidate to cause idiosyncratic drug reaction. *Drug Discov. Today* **18**:832–837 (2003).
204. A. B. Okey, M. A. Franc, I. D. Moffat, N. Tijet, P. C. Boutros, M. Korkalainen, J. Tuomisto, and R. Pohjanvirta. Toxicological implications of polymorphisms in receptors for xenobiotic chemicals: the case of the aryl hydrocarbon receptor. *Toxicol. Appl. Pharmacol.* **207**:43–51 (2005).
205. D. W. Nebert, T. P. Dalton, A. B. Okey, and F. J. Gonzalez. Role of aryl hydrocarbon receptor-mediated induction of CYP1 enzymes in environmental toxicity and cancer. *J. Biol. Chem.* **279**:23847–23850 (2004).
206. A. B. Okey. Enzyme induction in the cytochrome P-450 system. *Pharmacol. Ther.* **45**:241–298 (1990).
207. A. H. Conney. Enzyme induction and dietary chemicals as approaches to cancer chemoprevention: the seventh DeWitt S. Goodman lecture. *Cancer Res.* **63**:7005–7031 (2003).
208. P. Tonnesen, C. Pisinger, S. Hvidberg, P. Wennike, L. Bremann, A. Westin, C. Thomsen, and F. Nilsson. Effects of smoking cessation and reduction in asthmatics. *Nicotine Tob. Res.* **7**:139–148 (2005).
209. A. Cantani and M. Micera. Epidemiology of passive smoke: a prospective study in 589 children. *Eur. Rev. Med. Pharmacol. Sci.* **9**:23–30 (2005).
210. D. R. Brooks, J. H. Austin, R. T. Heelan, M. S. Ginsberg, V. Shin, S. H. Olson, and J. E. Muscat. Influence of type of cigarette on peripheral *versus* central lung cancer. *Cancer Epidemiol. Biomark. Prev.* **14**:576–581 (2005).
211. H. Witschi, I. Espiritu, J. L. Peake, K. Wu, R. R. Maronpot, and K. E. Pinkerton. The carcinogenicity of environmental tobacco smoke. *Carcinogenesis* **18**:575–586 (1997).
212. C. S. Lieber. Cytochrome P-4502E1: its physiological and pathological role. *Physiol. Rev.* **77**:517–544 (1997).
213. A. I. Cederbaum. Microsomal generation of reactive oxygen species and their role in alcohol hepatotoxicity. *Alcohol Alcohol.* (Suppl):291–296 (1991).
214. G. Ekstrom and M. Ingelman-Sundberg. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P450 (P-450IIEI). *Biochem. Pharmacol.* **38**:1313–1319 (1989).
215. J. Rashba-Step, N. J. Turro, and A. I. Cederbaum. Increased NADPH-dependent production of superoxide and hydroxyl radical by microsomes after chronic ethanol treatment. *Arch. Biochem. Biophys.* **300**:401–408 (1993).
216. Y. Dai, J. Rashba-Step, and A. I. Cederbaum. Stable expression of human cytochrome P4502E1 in HepG2 cells: characterization of catalytic activities and production of reactive oxygen intermediates. *Biochemistry* **32**:6928–6937 (1993).
217. M. Morimoto, A.-L. Hagbjork, Y.-J. Yvonne Wan, P. C. Fu, P. Clot, E. Albano, M. Ingelman-Sundberg, and S. W. French. Modulation of experimental alcohol-induced liver disease by cytochrome P450 2E1 inhibitors. *Hepatology* **21**:1610–1617 (1995).
218. E. Kukielka and A. I. Cederbaum. DNA strand cleavage as a sensitive assay for the production of hydroxyl radicals by microsomes: role of cytochrome P4502E1 in the increased activity after ethanol treatment. *Biochem. J.* **302**:773–779 (1994).
219. J. T. Slattery, S. D. Nelson, and K. E. Thummel. The complex interaction between ethanol and acetaminophen. *Clin. Pharmacol. Ther.* **60**:241–246 (1996).
220. J. L. Raucy, J. M. Lasker, C. S. Lieber, and M. Black. Acetaminophen activation by human liver microsomes P450IIE1 and P450IA2. *Arch. Biochem. Biophys.* **271**:270–283 (1989).
221. R. Zand, S. D. Nelson, J. T. Slattery, K. E. Thummel, T. F. Kalhorn, S. P. Adams, and J. M. Wright. Inhibition and induction of cytochrome P4502E1-catalyzed oxidation by isoniazid in humans. *Clin. Pharmacol. Ther.* **54**:142–149 (1993).
222. K. E. Thummel, C. A. Lee, K. L. Kunze, S. D. Nelson, and J. T. Slattery. Oxidation of acetaminophen to *N*-acetyl-*p*-benzoquinone imine by human CYP3A4. *Biochem. Pharmacol.* **45**: 1563–1569 (1993).
223. S. S. T. Lee, J. T. M. Buters, T. Pineaus, P. Fernandez-Salguero, and F. J. Gonzalez. Role of CYP2E1 in hepatotoxicity of acetaminophen. *J. Biol. Chem.* **271**:12063–12067 (1996).
224. H. Zaher, J. T. M. Buters, J. M. Ward, M. K. Bruno, A. M. Lucas, S. T. Stern, S. D. Cohen, and F. J. Gonzalez. Protection against acetaminophen toxicity in CYP1A2 and CYP2E1 double-null mice. *Toxicol. Appl. Pharmacol.* **152**:193–199 (1998).
225. R. P. Tonge, E. J. Kelly, S. A. Bruschi, T. Kalhorn, D. L. Eaton, D. W. Nebert, and S. D. Nelson. Role of CYP1A2 in the hepatotoxicity of acetaminophen: investigations using CYP1a2 null mice. *Toxicol. Appl. Pharmacol.* **153**:102–108 (1998).
226. T. Sarich, T. Kalhorn, S. Magee, F. Al-Sayegh, S. Adams, J. Slattery, J. Goldstein, S. D. Nelson, and J. Wright. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin. *Clin. Pharmacol. Ther.* **62**:21–28 (1997).
227. G. L. Guo, J. S. Moffit, C. J. Nicol, J. M. Ward, L. A. Aleksunne, A. L. Slitt, S. A. Kliewer, J. E. Manautou, and F. J. Gonzalez. Enhanced acetaminophen toxicity by activation of the pregnane X receptor. *Toxicol. Sci.* **82**:374–380 (2004).
228. P. T. Manyike, E. D. Kharasch, T. F. Kalhorn, and J. T. Slattery. Contribution of CYP2E1 and CY3A to acetaminophen reactive metabolite formation. *Clin. Pharmacol. Ther.* **67**:275–282 (2000).
229. K. J. Petty and J. M. Vega. Drug–drug interactions: marketing perspectives. In A. D. Rodrigues (ed.), *Drug–Drug Interactions*, Marcel Dekker, New York, 2002, pp. 633–643.
230. D. A. Smith. Induction and drug development. *Eur. J. Pharm. Sci.* **11**:185–189 (2000).
231. J. H. Lin. Challenges in drug discovery: lead optimization and prediction of human pharmacokinetics. In R. T. Borchardt, E. H. Kerns, C. A. Lipinski, D. R. Thakker, and B. Wang (eds.), *Pharmaceutical Profiling in Drug Discovery for Lead Selection*, AAPS Press, Arlington, VA, 2004, pp. 293–325.
232. J. P. Whitlock and M. S. Dension. Induction of cytochrome P450 enzymes that metabolize xenobiotics. In P. R. Ortize de

Montellano (ed.), *Cytochrome P450 Structure, Mechanism and Biochemistry* (2nd edn.), Plenum Press, New York, 1995, pp. 367–390.
233. S. E. Clarke and B. C. Jones. Human cytochromes P450 and their role in metabolism-based drug–drug interactions. In A. D. Rodrigues (ed.), *Drug–Drug Interactions*, Marcel Dekker, New York, 2002, pp. 55–88.
234. J. H. Lin and A. D. Rodrigues. *In vitro* models for early studies of drug metabolism. In B. Testa, H. Waterbeemd, G. Folkers, and R. Guy (eds.), *Pharmacokinetic Optimization in Drug Research*, Wiley-VCH, New York, 2001, pp. 217–243.
235. E. L. LeCluyse, P. Bullock, P. Parkinson and J. H. Hochman. Cultured rat hepatocytes. In R. T. Borchard, G. Wilson, and P. Smith (ed.), *Model Systems for Biopharmaceutical Assessment of Drug Absorption and Metabolism*, Plenum Press, New York, 1996, pp. 125–128.
236. E. L. LeCluyse, P. Bullock, and P. Parkinson. Strategies for restoration and maintenance of normal hepatic structure and function in long-term cultures of rat hepatocytes. *Adv. Drug Deliv. Rev.* **22**:133–186 (1996).
237. C. Rodriguez-Antona, M. T. Donato, A. Boobis, R. J. Edwards, P. S. Watts, and M.-J. Gomez-Lechon. Cytochrome P450 expression in human hepatocytes and hepatoma cell lines: molecular mechanisms that determine lower expression in culture cells. *Xenobiotica* **32**:505–520 (2002).
238. E. L. LeCluyse, A. Madan, G. Hamilton, K. Carroll, R. Dehaan, and A. Parkinson. Expression and regulation of cytochrome P450 enzymes in primary cultures of human hepatocytes. *J. Biochem. Toxicol.* **14**:177–188 (2000).
239. A. Hsu, G. R. Grannemain, and R. J. Bertz. Ritonavir, clinical pharmacokinetics and interactions with other HIV agents. *Clin. Pharmacokinet.* **35**:275–291 (1998).
240. J. H. Lin. Sense and nonsense in the prediction of drug–drug interaction. *Curr. Drug Metab.* **1**:305–331 (2000).
241. K. Ito, T. Iwatsubo, S. Kanamitsu, K. Ueda, H. Suzuki, and Y. Sugiyama. Prediction of pharmacokinetic alterations caused by drug–drug interactions: metabolic interaction in the liver. *Pharmacol. Rev.* **50**:387–411 (1998).
242. H. Takahashi, T. Kashima, S. Kimura, N. Murata, T. Takaba, K. Iwade, T. Yasumori, and H. Echizen. Pharmacokinetic interaction between warfarin and a uricosuric agent, bucolome: application of *in vitro* approaches to predicting *in vivo* reduction of (*S*)-warfarin clearance. *Drug Metab. Dispos.* **27**:1179–1186 (1999).
243. A. Madan, R. Dehaan, D. Mudra, K. Carroll, E. L. LeClyuyse, and A. Parkinson. Effect of cryopreservation on cytochrome P450 enzyme induction in cultured rat hepatocytes. *Drug Metab. Dispos.* **27**:327–335 (1999).
244. C. Lu and A. P. Li. Species comparison in P450 induction: effects of dexamethasone, omeprazole, and rifampin on P450 isoforms 1A and 3A in primary cultures from man, Sprague-Dawley rat, minipig, and beagle dog. *Chem. Biol. Interact.* **134**:271–281 (2001).
245. J. L. Raucy, L. Mueller, K. Duan, S. W. Allen, S. Strom, and J. M. Lasker. Expression and induction of CYP2C P450 enzymes in primary cultures of human hepatocytes. *J. Pharmacol. Exp. Ther.* **302**:475–482 (2002).
246. L. Z. Benet and R. L. Williams. Design and optimization of dosage regimens: pharmacokinetic data. In A.F. Giman, T. W. Rall, A. S. Nies, and P. Taylor (eds.), *The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, 1990, pp. 1650–1735.
247. J. H. Lin and M. Yamazaki. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin. Pharmacokinet.* **42**:59–98 (2003).
248. J. H. Lin. Drug–drug interaction mediated by inhibition and induction of P-glycoprotein. *Adv. Drug Deliv. Rev.* **55**:53–81 (2003).
249. L. Z. Benet, C. L. Cummins, and C. Y. Wu. Transporter –enzyme interactions: implications for predicting drug–drug interactions from *in vitro* data. *Curr. Drug Metab.* **4**:393–398 (2003).
250. L. Z. Benet, C. L. Cummins, and C. Y. Wu. Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int. J. Pharm.* **277**:3–9 (2004).