Pharmaceutical Research, Vol. 24, No. 5, May 2007 (© 2007)
DOI: 10.1007/s11095-006-9217-9

Expert Review

The Evolving Role of Drug Metabolism in Drug Discovery and Development

Lilian G. Yengi,[1,2] Louis Leung,[1] and John Kao[1]

Received May 10, 2006; accepted December 13, 2006; published online March 1, 2007

Abstract. Drug metabolism in pharmaceutical research has traditionally focused on the well-defined aspects of absorption, distribution, metabolism and excretion, commonly-referred to ADME properties of a compound, particularly in the areas of metabolite identification, identification of drug metabolizing enzymes (DMEs) and associated metabolic pathways, and reaction mechanisms. This traditional emphasis was in part due to the limited scope of understanding and the unavailability of *in vitro* and *in vivo* tools with which to evaluate more complex properties and processes. However, advances over the past decade in separate but related fields such as pharmacogenetics, pharmacogenomics and drug transporters, have dramatically shifted the drug metabolism paradigm. For example, knowledge of the genetics and genomics of DMEs allows us to better understand and predict enzyme regulation and its effects on exogenous (pharmacokinetics) and endogenous pathways as well as biochemical processes (pharmacology). Advances in the transporter area have provided unprecedented insights into the role of transporter proteins in absorption, distribution, metabolism and excretion of drugs and their consequences with respect to clinical drug–drug and drug–endogenous substance interactions, toxicity and interindividual variability in pharmacokinetics. It is therefore essential that individuals involved in modern pharmaceutical research embrace a fully integrated approach and understanding of drug metabolism as is currently practiced. The intent of this review is to reexamine drug metabolism with respect to the traditional as well as current practices, with particular emphasis on the critical aspects of integrating chemistry and biology in the interpretation and application of metabolism data in pharmaceutical research.

KEY WORDS: drug discovery and development; drug interactions clearance; drug metabolism; exposure; idiosyncratic drug toxicity; inhibition; integration of emerging sciences; pharmacogenetics; pharmacogenomics; transporters.

INTRODUCTION

In the complex and multidisciplinary process of drug discovery and development where groups with diverse backgrounds and expertise such as medicinal chemistry, pharmacology, preclinical development, safety assessment, clinical development and regulatory affairs are represented, drug metabolism (and pharmacokinetics) scientists play a delicate and important role in interfacing with the various disciplines (Fig. 1). In early discovery, drug metabolism input provides a basis for choosing chemical structures and lead compounds with desirable drug metabolism and pharmacokinetic (DMPK) or safety profiles and later, preclinical data aids in the development of clinical plans with regard to human drug exposures and safety. In fact in 1990 it was estimated that approximately 40% of drug attrition was due to undesirable DMPK properties and in 2000, this number was reduced to 10% (Fig. 2). This may be attributed to the increased efforts in applying DMPK principles for drug candidate optimization, selection and characterization during the drug discovery and development process. Traditional drug metabolism research focused on areas such as absorption, distribution, metabolism and excretion (ADME), with particular emphasis on *in vivo* and *in vitro* metabolite identification, enzymology of DMEs, and associated metabolic pathways and reaction mechanisms. However, there has been a shift in paradigm, as advances in fields such as pharmacogenetics, pharmacogenomics and transporters, have provided unprecedented insights into the biochemical processes that can affect the ADME properties of a drug. In order to fully maximize the impact of these emerging sciences on drug metabolism and the drug discovery and development process, the design and conduct of drug metabolism studies and interpretation of results must take into account these advances. Drug metabolism scientists must also in depth understanding of the discovery and development process, in order to design timely and appropriate studies that are in alignment with the traditional drug discovery and development process.

Drug metabolism approaches and methodologies employed during discovery and development can be vastly different, primarily because of the different needs and endpoints. In Discovery, the primary purpose is to screen large numbers of compounds in order to select ideal candidates for development, hence require technologies with

[1] Drug Metabolism Division, Drug Safety and Metabolism, Wyeth Research, 500 Arcola Road, Collegeville, Pennsylvania 19426, USA.
[2] To whom correspondence should be addressed. (e-mail: yengil@wyeth.com).

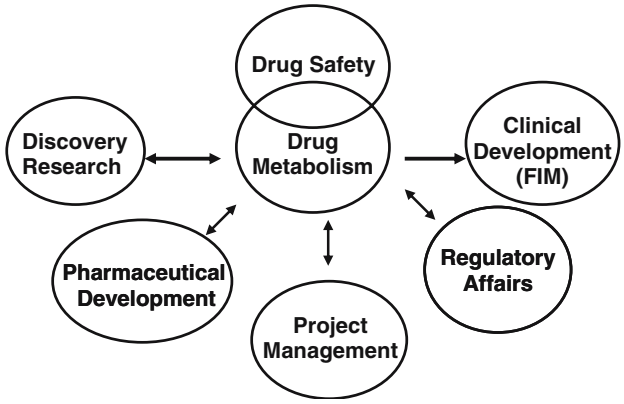

Fig. 1. A central role for drug metabolism (and drug safety) in pharmaceutical research: A schematic representation of DSM interaction with other departments throughout the drug discovery and development process.

high throughout capabilities. These methods can either be *in vitro*, *in silico*, *in situ and isolated organs* or *in vivo*, and vary greatly with regards to their physiological relevance, time and cost. On the other hand, development studies require more in depth analyses of a single compound, employing methods that have been thoroughly validated, from a good laboratory practice (GLP) perspective.

The intent of this review is to highlight advances in the fields of pharmacogenetics, genomics, and transporters, and how their integration with the traditional aspects of drug metabolism can impact the drug discovery and development process. A brief historical review of drug metabolism and how it has traditionally been applied in the pharmaceutical industry will also be discussed.

EARLY ASPECTS OF DRUG METABOLISM

While current drug metabolism departments typically comprise scientists with diverse backgrounds, the emergence of drug metabolism as a discipline, is intricately linked to the evolution of the field of organic chemistry. Hence the early stages of drug (xenobiotic) metabolism research were dominated by chemists such as the Millers (1–6) and later, RT Williams, who is credited as founder of the modern field of drug metabolism. Williams proposed the concept of a two-phase elimination of xenobiotics. He classed reactions such as oxidation, reduction and hydrolysis as phase 1 and activating, and reactions such as conjugation as phase 2 and detoxifying in nature (7,8). While this concept of Phase 1 and 2 reactions has and continues to be applied, it has recently been challenged as flawed because it groups mechanistically unrelated reactions together, while placing mechanistically similar reactions in different phases (9). It has instead been suggested that reactions be grouped into broad categories such as oxidations, reductions, conjugations and/or nucleophilic trapping processes, without making use of the phase 1 and 2 classification.

Metabolite identification, metabolic pathways and reaction mechanisms dominated early drug metabolism studies, with examples including the metabolism of aminoazo dyes (3–5), amphetamines and ephedrine (1), and hydroxylation of steroids (2,6). The concept of mixed-function oxidation stoichiometry and the discovery of P450 enzymes were aided by the research efforts of individuals such as Mason (10), Cooper (11), Sato (12), Estabrook (13), Lu and Coon, who demonstrated that the enzyme system from rabbit liver microsomes responsible for metabolic transformations consisted of NADPH-P450 reductase and cytochrome P450 (14).

The Role of Cytochrome P450s in Drug Metabolism

Although cytochrome P450s are not the only enzyme family responsible for phase 1 reactions, they are responsible for the phase 1 transformation of the majority of pharmaceutical drugs. Their role in drug metabolism, their catalytic mechanisms and the common reactions they catalyzed have been extensively reviewed (15,16). There are multiple forms of P450s, with isoforms in the same family sharing overlapping substrate requirements (17–20). As many as 57 human *P450* genes have been identified and characterized (21). The CYP1, CYP2 and CYP3 subfamilies are involved in the metabolism of xenobiotics, including pharmaceutical drugs. Examples of probe substrates for these CYPs are given in Table I. Of the xenobiotic metabolizing classes, the CYP3A subfamily is of critical importance in drug metabolism because in humans, it accounts for approximately 30–40% of total liver and intestinal CYP content and is

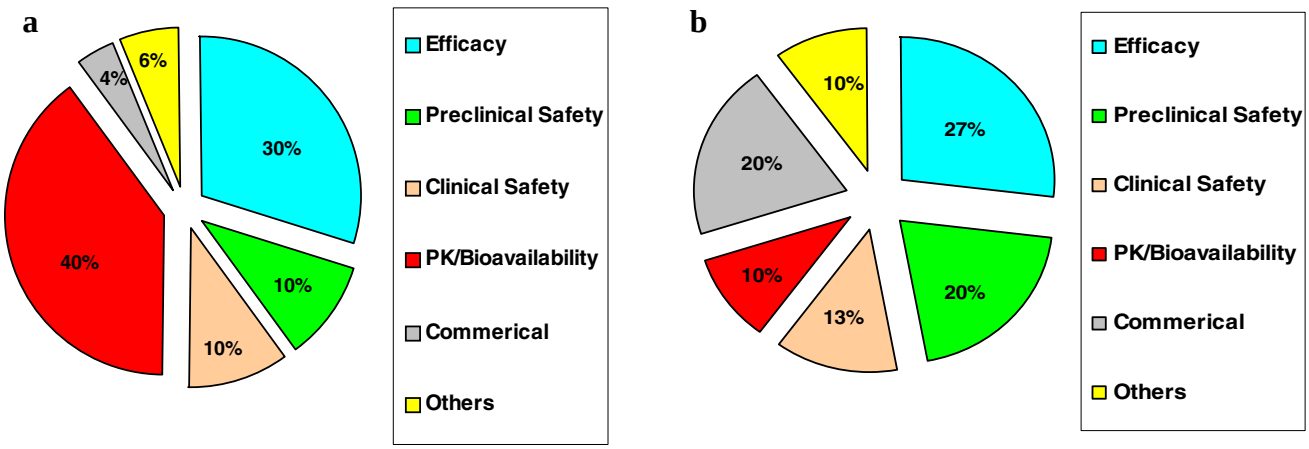

Fig. 2. (A) and (B): Reasons for attrition in drug development (196).

Table I. Examples of Commonly used Substrates, Inhibitors And Inducers of Cytochrome P450s (107)

CYP Isozyme	Substrate/Probe	Inhibitors	Inducers	Inducers Conc. μM)	Fold Induction
1A2	Ethoxyresorufin	Furafylline	Omeprazole	25–100	14–24
	Phenacetin	α-Naphthoflavone	β-Naphthoflavone	33–50	4–23
	Caffeine		3-methylcholanthrene	1–2	6–26
2A6	Coumarin	Tranylcypromine Methoxsalen	Dexamethasone	50	9.4
2B6	S-mephenytoin	Phencyclidine	Phenobarbital	500–1,000	5–10
		Sertraline Thiotepa	Rifampicin	10	
2C8	Taxol	Montelucast Quercetin	Rifampicin	10	2–4
2C9	Diclofenac S-warfarin Tolbutamide	Sulfaphenazole Fluconazole Fluvoxamine Fluoxetine	Rifampicin	10	3.7
2C19	S-mephenytoin	Ticlopidine Nootkatone	Rifampicin	10	20
2D6	Bufuralol Dextromethorphan	Quinidine			
3A4	Midazolam Testosterone	Ketoconazole Troleondomycin	Rifampicin	10	4–31

responsible for the metabolic transformation of 50–70% of commonly used pharmaceutical drugs (15,16,21–28). Many of the *P450* genes are polymorphic and the significance of these polymorphisms is exemplified by the individual variability associated with the hydroxylation of debrisoquine in man, which is attributed to polymorphisms at the *CYP2D6* gene locus (29–32). Many functional polymorphisms with clinical consequences have now been identified, including those at the CYP2C9 and CYP2C19 loci that affect the disposition of drugs such as warfarin and phenytoin (21,25,26). In addition to genetic polymorphisms, cytochrome P450 enzymes are also susceptible to inhibition, activation and induction by structurally diverse chemicals and the impact of these on drug metabolism will be discussed in later sections of this review.

Metabolic Activation in Drug Toxicity

As early as the 1940s, the potential role of xenobiotic metabolism and bioactivation in drug toxicity was postulated. The Millers first discovered that chemical carcinogens such as p-dimethyl-aminoazobenzene were biotransformed to electrophilic metabolites that covalently bound macromolecules, including DNA and proteins (33). In the 1970s, hemoglobin adducts were implicated in the toxicity of xenobiotics such as aromatic amines (34). Classically, it was demonstrated that covalent binding of acetaminophen metabolites to hepatic proteins was responsible for its hepatotoxicity (35,36). In the past two decades, a large amount of data linking bioactivation of certain functional groups to form protein adducts and their relevance to toxicity, such as idiosyncratic reactions, has emerged (37–41). For example, formation of electrophilic acyl glucuronides or sulfates of many drug compounds has been associated with drug toxicity. The drug–protein adducts cause toxicity either by impairing physiological functions of the modified proteins or through immune-mediated mechanisms. Table II gives examples of drugs whose toxicities are attributed to covalent binding of metabolites to proteins. Bioactivation of xenobiotics which contain certain functional groups such as a tertiary amine, furan ring or acetylene function, can also cause mechanism-based inactivation of P450s, leading to adverse clinical drug–drug interactions (42–44). Advances in analytical instrumentations, notably LC/MS/MS and LC/NMR, have markedly expanded our capability to detect and identify adduct formation via reactive intermediates. It remains difficult, however, to accurately predict potential adverse drug reactions due to formation of these protein adducts, since not all of them result in drug toxicity.

EVOLVING ASPECTS OF DRUG METABOLISM

Pharmacogenetics, genomics and drug transporters have profoundly impacted drug metabolism research by providing plausible mechanisms for interindividual variability in drug response and metabolism-related toxicity. They provide tools with which to understand enzyme regulation, identify factors that affect drug exposure, the potential for drug–drug interactions and species differences in drug disposition. Knowledge from these areas can form the scientific basis for designing appropriate clinical studies and data interpretation, which can lead to development of safer and more efficacious drugs.

Pharmacogenetics and Pharmacogenomics

Pharmacogenetics is the study of the effects of genetic differences between individuals on inter-individual responses to medicines (45,46). It is an old discipline which has been invigorated mainly due to an increased understanding of molecular biology and development of associated technological tools with which to conduct studies (47). Pharmacogenomics is a more recent term, coined to define a more 'holistic' or global approach in which the expression levels, regulation, functions and interactions of multiple genes are simultaneously studied, and their effects on overall variability in drug response determined (48). It is sometimes used interchangeably with pharmacogenetics, despite these subtle differences. These fields are continually being integrated in various aspects of the life sciences, including drug discovery and development, with the expectations that they would lead to the development of personalized medicines (49,50). Genome wide screening is used to identify single nucleotide polymorphisms (SNPs) that co-segregate with certain diseases with a view to using these SNP regions as potential targets for drug development (51,52). Global gene expression analysis is used preclinically and clinically, to identify surrogate markers of toxicity (toxicogenomics) and/or efficacy (53–55). That individuals differ in response to drug is not

Table II. Examples of Drugs that Form Reactive Intermediates and Exhibit Toxicity (41)

Drugs	Reactive Intermediate	Toxicity
Acetaminophen	Quinone-imine	Hepatotoxicity
Carbamazepine	2-Hydroxy, Quinone-imine	Agranulocytosis, Aplastic anemia
Clarithromycin	Nitroalkane	Hypersensitivity
Clozapine	Desmethylcloazpine	Agranulocytosis
Dapsone	Hydroxylamine, Nitroso	Hemolysis, hypersensitivity
Diclofenac	Acyl glucuronide, Benzoquinone imine	Hepatotoxicity
Halothane	Trifluoroacetyl	Hepatitis
Indomethacin	Iminoquinone	Hypersensitivity, Agranulocytosis, hepatotoxicity
Isonizaid	Isonicotinic acid, acetylating species	Hypersensitivity
Phenacetin	p-Nitrosophenetole	Hepatotoxicity
Procainamide	Hydroxylamine, Nitroso	Agranulocytosis
Tacrine	7-OH-tacrine	Hepatotoxicity
Tamoxifen	N-oxide, N-oxide-epoxide	Carcinogenicity
Ticlodipine	Keto, S-oxide	Agranulocytosis, aplastic anemia
Tienilic acid	Thiopene S-oxide	Hypersensitivity, hepatitis
Troglitazone	Conjugates, Benzoquinone, Quinone epoxide	Hepatotoxicity
Valproic acid	Acyl glucuronides, 2-N-propyl-4-pentenoic acid	Hypersensitivity, Hepatotoxicity

new and there have always been suggestions that a genetic component could be responsible, although it could not be directly proven due to lack of appropriate technologies. Nevertheless, differences in plasma levels of antidepressants such as nortriptyline and imiprimine were attributed to genetic factors (56,57). The debrisoquine-CYP2D6 polymorphism is a more recent and famous example of drugs whose exposure and disposition is dramatically affected by genetic polymorphisms at the CYP2D6 locus (30,58,59). Many functional polymorphisms have now been identified in many gene families, with the variant alleles occurring with varying frequencies among different ethnic populations (60–70). Pharmacogenetics/genomics approaches are beginning to be fruitful as evidenced by the approval of the drug BiDil for the treatment of heart failure in African Americans, based on knowledge of differences in the synthesis of nitric oxide in African Americans, compared to other ethnic groups (71,72). As another example, Perlegen Sciences and Mitsubishi Pharma are developing MCC-555 (a peroxisome proliferator activated receptor (PPAR) agonist) for the treatment of diabetes and other metabolic disorders. They are applying whole genome pharmacogenomics technologies to select patients most likely to benefit from the treatment (73). While these efforts and successes show a continuing maturity of the field, data interpretation and application still face many challenges, as the genetic basis of any disease is often multifactorial and also impacted by the interplay between nature and nurture, hence the relevance of any gene, SNP or genotype cannot always be predicted based on genetic analysis alone.

Nuclear Receptors and Transcriptional Regulation

Interest in gene regulation and the potential impact on the efficacy and safety of new drug candidates is steadily increasing. One of the consequences of transcriptional regulation is clinical drug–drug interactions with co-administered drugs and also the observation that some drugs induce the enzymes involved in their metabolism; hence altering their kinetic properties. It is therefore important that the effects of new drug compounds on the expression of drug metabolism genes form an integral part of drug metabolism studies. The discovery and characterization of the cytochrome P450s in the 1950s (1,74–76), their solubilization (14,77) and elucidation of the mechanisms underlying their by polycyclic aromatic hydrocarbons (78,79) are critical milestone in our understanding of the biotransformation of xenobiotics including pharmaceuticals drugs. Although it had been known since their characterization that some P450s were inducible, (79,80) it was knowledge of the interactions between steroid hormones and their targets within the cell that led to the hypothesis that induction of these microsomal enzymes by ligands was via a mechanism similar to that of steroid hormones (81–83). This hypothesis was later supported in subsequent experiments that eventually led to the characterization and identification of the aryl hydrocarbon hydroxylase (Ah) receptor as responsible for CYP1A1 induction (83). Several nuclear receptors have now been cloned and the downstream genes they regulated have been identified. (84–86). These include pregnene X receptor PXR, with CYP3A4, CYP2C and UGT as the prototypic downstream genes, constitutive androstane receptor (CAR), which regulates genes in the CYP2B family and UGTs, liver X receptor (LXR), the master regulator of cholesterol homeostasis, farnesoid X receptor (FXR), peroxisome proliferator-activated receptor (PPAR), vitamin D receptor (VDR), glucocorticoid receptor (GR) and hepatocyte nuclear receptor 4 (HNF4) (87–89). These receptors control a complex network of endogenous pathways and often exhibit cross-talk between them in relation to their ligands and downstream regulated genes (90). It is therefore not surprising that regulation of nuclear receptors by drug candidates has the potential not only to impact targeted pathways, but also affect off target genes leading to unexpected efficacy and/or safety outcomes.

Applications of Genetics/Genomics in Drug Metabolism and Safety

Genotyping Studies. One of the major reasons for inter-individual variations in drug response is genetic polymorphism that result in proteins with variable activities (50,69,91–93).

While polymorphisms have been identified in drug trans porters, receptors and pathway genes, polymorphisms in the *P450* gene family have had the most impact on the fate of pharmaceutical drugs (94). CYP2D6, CYP2C9 and CYP2C19 polymorphisms are the most clinically relevant since these three isozymes contribute to the metabolism of about 40% of drugs currently on the market and exhibit single nucleotide polymorphisms that can be directly correlated with a particular phenotype (95).

The efficacy and or safety of a drug predominantly metabolized by a polymorphic gene, is directly impacted by the genotype of the individual taking the drug (96,97). Information on the genotypes of participants in clinical trials will not only provide data that could be used to explain potential individual differences in response, but also ensure that those with an altered phenotype are properly monitored. This is particularly important with drugs that have a narrow therapeutic window, outside of which severe toxicity or therapeutic failure may occur (98). The impact of genetic variations at the CYP2C and CYP2D6 loci on the metabolism and safety of drugs such warfarin, phenytoin and debrisoquine is well known. While the impact of polymorphisms on these drugs was identified when the drug were already on the market, genetics information is increasingly being used proactively during the discovery and development process. An example is the use of CYP genetic information in designing the clinical program for a CNS program in which the authors are involved. CYP2C9 contributes to approximately 40% of the metabolism of the investigational drug and this information is being incorporated in designing phase 3 clinical studies. Genotyping studies are now possible and practical because of technologies such as Taqman allelic discrimination assays, high throughput sequencing and SNPs. The availability of site directed mutagenesis, recombinant DNA and transgenic technologies are also powerful tools with which to study genetic polymorphisms and to correlate genotypes with phenotypes (99–102). This could potentially lead to a better classification of patients, with prescriptions and doses individualized and optimized based on a patient's genotype.

Different pharmaceutical companies have adopted different genotyping paradigms. A few companies conduct genotyping studies on preclinical samples, for instance, to offer a rationale for differences in efficacy or toxicity, observed in different animals (103–106). However, preclinical genotyping data cannot be applicable in the late stages of drug development since most polymorphisms are species-specific and not likely to be conserved in humans. The predominant practice is to genotype samples from phase 1 clinical trials, for compounds that are metabolized by a polymorphic CYP. Nevertheless, due to redundancies in the drug metabolizing enzymes, not all polymorphisms translate into clinical implications because other enzymes may compensate for the poor phenotype, *in vivo*. As such most genotyping is done retroactively only if clinical observations suggest influence of the polymorphisms. Depending on the purpose of a study, volunteers may be genotyped prior to enrollment, to select the volunteers most likely to respond or not. This approach is particularly useful when comparing pharmacokinetic parameters between extensive and poor metabolizers, to determine the potential for drug–drug interactions with co-administered drugs, in the different phenotypic groups. This approach might also be useful when a drug has a narrow therapeutic window and the consequences of the polymorphism are well characterized and understood (e.g. CYP2D6), to avoid any undue exposing trial participants to any undue serious adverse events. One limitation in using phase 1 and 2 clinical samples is the sample size because low frequency alleles may not be present. Thus genotyping data from phase 1 studies should always be interpreted with allowance given for the limitations associated with a small sample size.

Whole blood is the most practical tissue used for genotyping human subjects and the genes commonly genotyped are CYP2D6, CYP2C9 and CYP2C19. Some MDR1 haplotypes have been associated with reduced protein activity and MDR1 genotyping is also being recommended for drugs that are MDR1 substrates (66). At gene loci where more than two allelic variants exist, the decision on which variant to study should be based on the most common allele existing in the population from which the samples are drawn and also its potential consequences (64).

Enzyme Induction Studies. Potential induction of DMEs by new chemical entities is now routinely evaluated because of their potential to cause clinical drug–drug interactions that may result in loss of efficacy or toxicity. In fact, an FDA draft guidance on *in vitro* and *in vivo* drug interaction studies lends credence to the fact that the agency considers induction data as integral to their ability to appropriately evaluate new chemicals entities for potential drug–drug interactions (107). However, despite this interest, there is no consensus on the acquisition, interpretation and application of induction data. One of the reasons is the fact that the clinical significance of gene regulation cannot be determined in isolation, without knowledge of factors such as the relative contribution of the induced isozyme to the metabolism of the inducer or co-administered drug/s, the clinical exposure of the compound/s at the efficacious dose (Cmax) and whether this is significantly altered by induction, whether induction is observed at doses below or above the efficacious Cmax, the magnitude of induction and the therapeutic window of the drug. In addition, since any *in vitro* system is only a proxy of the *in vivo* system, there has to exist the possibility that *in vitro* observations may not translate to *in vivo* and this could be due to compensatory pathways and feedback/feed forward mechanisms operating *in vivo* that may negate *in vitro* findings. The FDA draft guidance is therefore an effort to harmonize methods, systems and data interpretation for easy comparison of induction data across laboratories and different compounds.

Preclinical enzyme induction studies are performed primarily *ex-vivo*, particularly in tissues such as liver where DMEs are predominantly expressed. At Wyeth, induction studies are carried out on a need to bases, in liver samples taken from routine safety studies (maximum tolerated dose, (MTD). Induction studies are conducted if data from first in man studies or other preclinical parameters (such as increases in liver weights, reductions in exposure following multiple dosing, off-target toxicity) suggest induction may be occurring (108). Historically, these studies are conducted by determining enzyme activities in microsomes prepared from these livers and the rate of metabolism between vehicle and

treated groups compared. Increasingly genome-based approaches using technologies such as, real-time quantitative PCR (103,106) and global gene expression (genechips) are being using to determine enzyme induction at the level of transcription, accurately and specifically capture isozyme specific induction (109–111). Preclinical induction studies can also be done *in vitro* systems such as primary cells, cell lines, transgenic models and cell-based gene reporter assays. However, the *in vivo* relevance of such data has not been established, hence can only be used as diagnostic rather than predictive tools (105,108,112).

The FDA draft guidance on enzyme induction studies is an attempt to harmonize systems, methods, study design, data analysis and interpretation of *in vitro* studies. The method recommended by the FDA, is measurement of enzyme activities in primary or platable cryopreserved human hepatocytes, treated with test compound and vehicle controls, although there are known limitations to this medium such donor variability, detection methods, probe substrates and lack of substrate specificity (113–116). In order to correlate *in vitro* to *in vivo* relevance, the FDA recommends the use of three concentrations flanking the predicted or known exposure in humans (up to 10 times Cmax) and induction by test compound should be compared with that observed with an accepted positive control. The compound is considered a strong inducer, with a potential to cause *in vivo* drug–drug interactions, if it causes induction that is greater than or equal to 40% of that observed with the positive control (107).

Although analysis of mRNA expression is becoming routine, the lack of a direct correlation between mRNA expression and enzyme activities for most genes has meant that the FDA still considers mRNA data only as supplemental to enzyme activity data (117,118). Nevertheless we, and others have found transcriptional regulation to be directly correlated with enzyme activity data for most inducible CYPs and UGTs ((108). In addition, mRNA data is useful in cases where both enzyme induction and mechanism base inhibition are concurrently operative. For instance, ritonavir, an antiretroviral drug, acts as both an inducer and mechanism based inhibitor of CYP3A4 protein (119), and enzyme activity measurements alone do not reveal transcriptional regulation of CYP3A4 because enzyme activity remains the same. Nevertheless, although overall CYP3A4 activity is not affected, other genes that are co-regulated with CYP3A4 but are not inhibited by ritonavir may be affected. mRNA data is also useful in cases where genes in the same subfamily are differentially regulated in an isozyme and gender-related manner (120). Gender specific regulation is not common in humans, but it is a common phenomenon in rodents and since preclinical data is often used to predict human data, knowing the mechanism by which these genes are regulated in different species is useful when determining whether preclinical induction will be relevant in humans (121–123). Since no single method or approach is perfect, we have employed an integrated approach that takes advantage of genome-based methodologies as well enzyme activity assays to provide comprehensive data sets that provide better in sights into potential *in vivo* interactions (108).

Other *in vitro* systems that are increasingly being applied in enzyme induction studies are reporter gene assays that result from transient or stably transfected cell lines expressing the DME of interest. High throughput AhR and PXR reporter gene assays have been developed and utilized to screen compounds for CYP1A and CYP3A induction (122,124). The CYP3A4 assay entails co-transfecting cells with a CYP3A4 luciferase reporter construct containing the CYP3A4 proximal promoter and distal enhancer, integrated into a modified luciferase vector, and a PXR construct containing the full length open reading frame of human PXR (124). As with many *in vitro* systems, cell-based reporter gene assays also have certain limitations, one of which is the fact that correlation of receptor binding and activation with *in vivo* enzyme induction has not yet been established and standardized.

Drug Transporters

The role of transporter proteins in drug and endogenous substance disposition has increasingly gained recognition. As late as 1995, the role of transporters in biliary excretion was not recognized and as a result, species differences in biliary excretion were not readily explainable (125). It has now become clear that transporters are responsible both for the uptake and efflux of drugs and other chemicals in various tissues and may be key determinants of the pharmacokinetic characteristics of a drug (126–130). Several transporters have been cloned and considerable progress has been made in understanding the molecular characteristics of individual transporters. Table III gives a list of transporter families and the individual genes expressed in humans.

Transporters have been classified as primary, secondary or tertiary active transporters. Primary active transporters require ATP-dependent hydrolysis as the first step in catalysis. Examples are ATP-binding cassette transporters such as multidrug resistance protein (MDR), multidrug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP). Secondary or tertiary active transporters are driven by an exchange or cotransport of intracellular and/or extracellular ions with the substrate. Examples include organic anion transporter (OAT), organic anion transporting polypeptides (OATP), sodium taurocholate co-transporting peptide (NTCP), organic cation transporter (OCT), novel organic cation transporter (OCTN) and oligopeptide transporters (PEPT) (131).

Of the known transporters, P-glycoprotein (P-gp or MDR1) is by far the most characterized and understood efflux transporter. It is predominantly expressed in tissues such as intestines, brain and liver, and plays an important role in intestinal absorption, brain penetration and biliary excretion of drug substrates. Drugs that are P-gp substrates tend to have lower oral absorption and brain penetration, due to P-gp-mediated drug efflux at the intestinal lumen or blood–brain-barrier. Considerable efforts continue to be exerted during the drug discovery phase to identify and select drug candidates that are not (or poor) P-gp substrates in an attempt to improve oral absorption, and for CNS-targeted drugs, to improve brain penetration. The absence of P-gp-mediated efflux, together with high passive permeability, distinguishes between CNS and non-CNS drugs (132). P-gp has also been implicated in clinical drug–drug interactions involving concomitant drugs that are either its substrates or

Table III. Human Drug Transporter Gene Families (125,130)

Gene Family	Gene Product (Gene Symbol)
Multidrug Resistant Protein/P-glycoprotein	MDR1/P-gp *(ABCB1)*
Bile Salt Export Pump	BSEP *(ABCB11)*
Multidrug Resistance-Associated Protein	MRP1 *(ABCC1)*
	MRP2 *(ABCC2)*
	MRP3 *(ABCC3)*
	MRP4 *(ABCC4)*
Breast Cancer Resistant Protein	BCRP *(ABCG2)*
Sodium Taurocholate Co-Transporting Peptide	NTCP *(SLC10A1)*
Oligopeptide Transporters	PEPT 1 *(SLC15A1)*
	PEPT 2 *(SLC15A2)*
Organic Anion Transporting Polypeptides	OATP-A/OATP1A2 (*SLC21A3*)
	OATP-B/OATP2B1 (*SLC21A9*)
	OATP-C/OATP1B1 (*SLC21A6*)
	OATP8/OATP1B3 (*SLC21A8*)
	OATP-D/OATP3A1 (*SLC21A11*)
	OATP-E/OATP4A1 (*SLC21A12*)
	OATP-F/OATP1C1 (*SLC21A14*)
Organic Cation Transporters	OCT1 (*SLC22A1*)
	OCT2 (*SLC22A2*)
	OCT3 (*SLC22A3*)
Novel Organic Cation Transporters	OCTN1 (*SLC22A4*)
	OCTN2 (*SLC22A5*)
Organic Anion Transporters	OAT1 (*SLC22A6*)
	OAT2 (*SLC22A7*)
	OAT3 (*SLC22A8*)
	OAT4 (*SLC22A9*)

inhibitors. Examples of clinical drug–drug interactions involving P-gp are shown in Table IV. For drugs with narrow therapeutic windows such as digoxin, anticancer agents and immunosuppressants, adverse effects resulting from P-gp-related drug interactions are particularly problematic.

Other transporters whose possible role in clinical drug–drug and drug–food interactions continue to emerge are MRP2, OATPs and bile salt export pump (BSEP). OATPs are a superfamily of uptake transporters with wide tissue distribution including the liver, gut, and blood–brain-barrier. They are responsible for the transport of a variety of amphipathic organic anions such as steroid conjugates, bile acids and drugs. OATP has been implicated as a factor in the interactions between fruit juices and fexofenadine (133), in which the systemic exposure of orally administered fexofenadine is reduced by approximately 30% when taken with grapefruit, apple or orange juice. The mechanism appears to be inhibition of OATP-mediated intestinal absorption of fexofenadine by fruit juices and this has been demonstrated using cell lines expressing OATPs (134).

Some drug transporters such as MRP2 and BSEP have been implicated in drug and endogenous substrate-induced toxicity. Identification of ligands that are substrates or inhibitors of MRP2 or BSEP may help to reduce the possibility of drug-induced toxicity. MRP2 is involved in the biliary excretion of anion drugs and their conjugates at the bile canalicular membrane. It is now believed that the toxic effects of methotrexate in the intestines result from the active excretion of methotrexate into the bile by MRP2, with subsequent accumulation in the intestine leading to the observed toxicity (135). The excretion of the reactive glucuronides of diclofenac by MRP2 and its accumulation in bile is also thought to contribute to the toxic effects diclofenac on bile canalicular membranes (136). Inhibition of BSEP has also been implicated in drug-induced cholestasis caused by intracellular accumulation of toxic bile salts (taurocholate). Inhibition of BSEP-mediated transport of bile salt is also suggested as a mechanism responsible for cholestasis caused by cyclosporine and estradiol-17β-glucuronides (137). Other examples of transporter involve-

Table IV. Examples of the Possible Involvement of P-gp in Clinical Drug–Drug Interactions (125)

Inhibitor	Inhibited Drug
Atrovastatin	Digoxin
Clarithromycin	Digoxin
Cyclosporine	Doxorubicin, Taxol, sirolimus
Diltiazem	Tacrolimus
Erythromycin	Atrovastatin, Digoxin, Fexofenadine, Cyclosporine, Saquinavir, Talinolol
Itraconazole	Digoxin, Quinidine
Ketoconazole	Fexofenadine, Saquinavir, Tacrolimus
Propafenone	Digoxin
Quinidine	Digoxin
Ritonavir	Saquinavir
Talinolol	Digoxin
Verapamil	Digoxin
Valspodar	Digoxin, Taxol, dexamethasone
Inducer	Induced Drug
Rifampicin	Fexofenadine, Talinolol, Tacrolimus, Digoxin, Saquinavir
St. John's Wort	Digoxin, Cyclosporine

Table V. Key Milestones in the History of Drug Metabolism Research (197)

Date	Key Participants	Key Event
1828	Friedrich Woehler	Synthesizes urea, a compound he had examined in his studies on urinary waste products
1841	Alexander Ure	Conducts first human metabolism study by observing the conversion of benzoic acid to hippuric acid
1931	R.T Williams	Founder of field of Drug Metabolism, introduces concept of phase II and phase II metabolism
1947	Bernard B. Brodie *et al*	Develop separation and detection techniques that enable measurement of parent compound and metabolites
1955	Axelrod	Determines microsomal oxidizing system as sub-cellular component of metabolism of drugs and other chemicals
1955–1958	Garfinkel, Klingenberg, Hayashi, Mason, Omura, Sato	Discovery and characterization of Cytochrome P450s
1969	Lu and Coon	Isolation of membrane-bound P450s
1970s	Nebert *et al*	Identification of the AhR and Mechanism of P450 induction
1977–1990	Mahgoub, Smith, Eichelbaum, Meyers, Gonzales, Wolf *et al*	Identification and characterization of the CYP2D6 polymorphism, linkage of genotype with phenotype
1990s	Various	Characterization of drug transporters and elucidation of and their role in drug metabolism
1990s	Various	Advances in Molecular Biology and associated technological tools; high throughput sequencing and genotyping, gene-reporter assays, real-time quantitative PCR, genechips siRNA, transgenic models, SNPs
1998	Kliewer, Moore	Cloning and characterization of PXR, the nuclear receptor responsible for the transcriptional regulation of CYP3A4

ment in toxicity include troglitazone, a diabetic agent that was withdrawn from the market due to liver toxicity. It is believed the toxicity was via a cholestatic mechanism, since troglitazone and its sulfate conjugate have been shown to inhibit BSEP-mediated taurocholate transport (138).

Methods in Drug Transporter Studies

A variety of *in vitro* and *in vivo* models are available to assess the role of different transporters in drug disposition. Amongst *in vitro* systems, the colon carcinoma cell line (Caco-2) is one of the most widely used model for predicting intestinal drug absorption (139,140). Caco-2 cells express a variety of drug transporters including MDR1/P-glycoprotein (P-gp) and MRP2 and can be used to assess drug–drug interactions involving multiple transporters. However, the presence of multiple transporters can also be a limitation because it can be difficult to determine the involvement of a particular transporter, unless there are specific inhibitors and/or substrates available. As a result, Caco-2 cells are used mainly to evaluate P-gp interactions in a monolayer-based format, with selective inhibitors or substrates of P-gp. Various factors such as culture time, passage number and culture conditions can affect P-gp expression, hence these conditions need to be optimized and standardized to ensure data reproducibility. Other cell lines include canine kidney cell line (MDCK). Their advantage over Caco-2 cells is shorter culture time and the fact that they can be specifically transfected to over express human P-gp, thus increasing sensitivity and specificity (140). Sandwich-cultured hepatocytes represent a potentially useful *in vitro model* to study drug transporters (141,142). Biliary excretion in long-term sandwich-cultured rat hepatocytes has been shown to correlate well with *in vivo* biliary excretion, though evaluation of *in vitro–in vivo* correlation in humans remains difficult. Sandwich-cultured hepatocytes may also allow the study of the biliary excretion of metabolites, provided the relevant metabolic activities are maintained.

Efforts in cloning and transgenic technologies have also been put towards understanding transporter biology and their importance in drug discovery and development. Single-transfected cells that stably or transiently express individual transporters are used to determine whether a compound is a substrate or inhibitor of particular transporters (143,144). Double-transfected cells or monolayers that stably express human or rat uptake and efflux transporters have been developed (144) to better understand the synergistic role of uptake and efflux transporters under *in vivo* conditions. Although these expression systems have the advantages of allowing the qualitative identification of specific drug transporters involved in a drug's disposition, quantitative prediction of *in vitro–in vivo* correlations remain to be established before these systems can gain widespread applications.

In addition to cell based systems, canalicular membrane vesicles (CMVs) are used to assess transport of substances and drugs into the bile (145,146). CMVs have been shown to provide a good prediction of transporter-mediated biliary drug clearance. CMVs express many of the efflux transporters found on the canalicular membrane of hepatocytes, including P-gp, MRP2 and BSEP. CMVs can be prepared from livers of various species using similar procedures and therefore allow for the simultaneous assessment of species differences or similarities. One limitation of CMVs, however, is variability in quality between different livers (human and non-human primates), and strict adherence protocol to ensure reproducible expression of the various transporters. Sinusoidal membrane vesicles (SMVs) are prepared from the sinusoidal (basolateral) membrane of hepatocytes and are used to evaluate transporters expressed on

the basolateral membranes of hepatocytes (125). They are however, not as widely used as more validated systems.

INTEGRATING TRADITIONAL AND EMERGING ASPECTS OF DRUG METABOLISM

A multidisciplinary approach forms the basis of successful drug discovery and development efforts, hence drug metabolism invariably has to adapt to changing science and technology, be more versatile and expand beyond its traditional role. Traditional thinking and approaches in ADME will need to incorporate fields such as pharmacogenomics, pharmacogenetics, drug transporters, metabonomics and systems biology. Even well established and understood areas, such as cytochrome P450 enzymes, enzyme kinetics, metabolic transformations and activations, continue to evolve with increasing understanding of these systems and concepts, thus presenting new challenges for the future. The availability of new *in vitro* and *in silico* tools continues to provide new opportunities to better understand and address questions related to drug metabolism. Examples of areas where integrated approaches are of particular importance are reviewed below.

Identification of Clearance Pathways and Factors that Affect Drug Exposure

Identifying and understanding the pathways involved in the clearance and exposure of a pharmaceutical is one of the most important aspects of drug discovery and development. During the drug discovery and optimization phase, it is often desirable to identify and select compounds that have low clearance and high oral absorption, so that systemic exposure may be maximized and frequency of dosing minimized and still achieve the desired pharmacological activity. Identification of the pathways for clearance and factors that affect exposure is a critical prerequisite for structural modifications when attempting to improve systemic drug exposure of a structural series. During the drug development phase, identifying the clearance pathways allows clinicians to design the proper clinical studies to evaluate the impact of endogenous and exogenous factors on systemic exposure (e.g. drug–drug or drug–food interactions). It is therefore essential that one possess a thorough understanding of possible factors that can affect drug clearance and exposure, to ensure that the proper studies are conducted at the appropriate time. Metabolic clearance is considered the major pathway for drug clearance, followed by renal and biliary clearance (147), and cytochrome-mediated pathways are the most dominant metabolic pathways, with other enzyme systems such as uridine diphosphate glucuronosyltransferases (UGTs), sulfotransferases (SULTs), flavin-containing monooxygenase (FMO), N-acetyltransferases (NATs) and monoamine oxidases (MAOs) playing lesser roles.

For *in vitro* studies, hepatic microsomes and freshly isolated hepatocytes are the *in vitro* systems of choice for the prediction of drug clearance, with freshly isolated hepatocytes regarded as the preferred system since they represent a more realistic physiological entity (148). The fundamental approach is to use intrinsic clearance (V_{max}/K_m) derived from an *in vitro* system to scale-up to *in vivo* hepatic clearance using different physiological models, including parameters such as microsomal yield and liver weights. Due to the need for increased throughout during drug discovery, the use of substrate depletion at a single drug concentration was introduced to estimate *in vitro* intrinsic clearance (149–151) although the inherent limitations of this approach (e.g. drugs with low K_m or limited turnover) need to be considered. In order to improve the accuracy of *in vitro–in vivo* predictions factors such as microsomal binding, hepatic uptake and scaling factors need to be taken into consideration (152,153). More recently, cryopreserved hepatocytes have been demonstrated to provide results comparable to freshly isolated hepatocytes for *in vitro–in vivo* extrapolations, hence have been proposed as the *in vitro* system of choice due to their increased accessibility (153,154). When using *in vitro* metabolism data to predict human pharmacokinetics, interpretations and inferences should always be performed with caution because of inherent assumptions, limitations and exceptions associated with their application. It is also important that the *in vitro* system chosen is relevant to the nature of the compound under investigation. For example, carbamoyl glucuronide conjugation of amine containing drugs may not readily occur under usual *in vitro* incubation conditions since initial reactions required dissolved carbon dioxide followed by glucuronidation of the resulting carboxylic acid (155). Thus the relevance of this pathway may have been overlooked when *in vitro* incubations are performed without enrichment with carbon dioxide. Besides hepatic metabolism, drug-metabolizing enzymes (e.g. CYP3A4 and UGTs) are present in the small intestine and contribute to first-pass metabolic clearance of some drugs (156,157). Currently, there are no reliable and validated models to evaluate the contribution of intestinal drug metabolism to overall metabolic clearance of a drug.

Besides metabolic clearance, direct drug elimination via excretion is not uncommon and should not be overlooked. Drug transporters, aside from passive diffusion processes, have been recognized to play an active role in renal and biliary excretion of many xenobiotics and endogenous substances. Since *in vivo* study of renal or biliary excretion may not be always readily conducted during the drug discovery phase, and realizing that species differences exist in renal or biliary drug clearance (125), *in vitro* approaches in assessing renal or biliary clearance have been proposed, and may provide guidance on the significance of these pathways in drug clearance. These approaches and precautions were previously discussed the Drug Transporters section. Together with physicochemical properties such as molecular weight, solubility and permeability, the identification of transporter involvement (particularly for efflux transporters P-gp and MRP2) greatly enhances our capability to predict compounds that are renally or biliary excreted. Transporter-drug metabolizing enzyme interplay, primarily involving P-gp-CYP3A4 in the intestine, was thought to limit the oral absorption of certain drugs (158). Though *in vitro* systems, such as cell monolayers expressing CYP3A4 and P-gp, to assess the role of transporter-enzyme interplay on drug absorption have been used (159,160), the quantitative extrapolation of *in vitro* data to *in vivo* situations remains to be established.

An example of a drug where transporter-enzyme interplay may play an important role in its metabolic disposition is

sirolimus, a macrolide antibiotic used as an immunosuppressive agent to prevent allograft rejection in kidney transplantation. Sirolimus was metabolized by CYP3A and a majority of the administered dose was biliary excreted (161). Preclinical *in vitro* studies, using models including liver microsomes and Caco-2 cell monolayers expressing CYP3A, indicated that sirolimus was susceptible to macrolide ring-opening via hydrolysis/dehydration, and cytochrome P450 CYP3A-mediated oxidation and P-gp-mediated efflux (160). *In vitro* results therefore suggested that sirolimus would subject to CYP3A- and non-CYP- mediated metabolic clearance, as well as P-gp-mediated biliary excretion; and its oral bioavailability could be limited by intestinal and hepatic CYP3A-catalyzed first-pass effect and intestinal P-gp-mediated efflux. In contrast, failure to recognize the clinical significance of factors that can affect drug exposure can lead to drastic consequences, such as the withdrawal from the market of the non-sedating antihistamine terfenadine, a CYP3A substrate that resulted in life-threatening ventricular arrhythmia when coadministered with CYP3A inhibitors (162). Recognition of the reason for the failure of terfenadine led to the development of fexofenadine, the active metabolite of terfenadine that does not undergo significant biotransformation in humans (163). Wu and Benet (158) recently proposed a revised Biopharmaceutics Classification System (BCS) that may be useful in predicting overall drug disposition, including routes of elimination and effects of efflux and uptake transporters on oral drug absorption, and the clinical significance of transporter-enzyme interplay on oral bioavailability and drug–drug interactions. The widespread application of these proposed usages to facilitate the successful development of future drug candidates remains to be seen.

Prediction of Clinical Drug–Drug Interactions

Due to the central role of P450-catalyzed metabolism in drug clearance and bioavailability, the inhibitory (reversible) effects of a drug on P450 activity is the first step towards assessing the potential for clinical metabolic drug–drug interactions. Michaelis–Menton kinetics is the common model for CYP-mediated reactions but 'atypical kinetics', first described for CYP3A4 and later for CYP2C9, has been identified relevant model for some enzymes. Secondly, non-Michaelis–Menton kinetics, notably autoactivation (cooperativity) and substrate inactivation, can also influence *in vitro–in vivo* extrapolations (164–166). Various *in vitro* systems such as liver microsomes, hepatocytes and recombinant enzymes are employed in inhibition studies, with recombinant enzymes preferred in discovery to provide high throughput data for compound selection (167,168). During preclinical and clinical development stages, liver microsomes are preferred because they are considered to be more physiologically relevant than recombinant enzymes.

Mechanism-based inactivation (MBI) of CYP enzymes and potential *in vivo* consequences are increasingly being recognized (169–175). Understanding the mechanisms leading to MBI allows the development of possible structure-inhibition relationships, and the opportunity to design drugs devoid of structural features that are involved in bioactivation and inhibition. Examples of functional groups involved in MBI are acetylenes, furans, thiophens, secondary or tertiary amines and their associated bioactivation pathways (42–44). Detailed methods and precautions in the use of in vitro MBI data to predict clinical outcome have been described extensively and continue to evolve (164–169, 171–176). Several *in vitro* methodologies are used to identify the possible mechanisms leading to MBI, such as trapping of reactive intermediates using glutathione to determine involvement of covalent binding or measurements of metabolite intermediate complexes for mechanisms involving heme-coordination (42–44). Automated 'cocktail' methods and platforms have been developed to provide high throughput capabilities (170,177). Experimental conditions, such as duration of preincubation and extent of dilution of the preincubation solution for subsequent determination of residual P450 activities, can have a marked effect on inactivation kinetics (K_I and k_{inact}) of MBI and their subsequent extrapolation to *in vivo* situations (175), hence the use of *in vitro* MBI data to predict *in vivo* drug–drug interactions remain to be fully established as methods and data interpretation become validated and standardized. Certain methods, such as the trapping of reactive intermediates, are indirect methods that may not provide unequivocal evidence of the involvement of a particular structural feature in MBI, but provide the basis for further investigations. A recent and practical example of the implication of MBI in clinical drug–drug interactions was the increase in plasma AUCs of CYP2D6 substrate drugs by paroxetine (178,179) The magnitude of the AUC increases appeared inconsistent with the extent of reversible *in vitro* inhibition of CYP2D6, and appeared to be explained, in part, by the mechanism-based inhibition of CYP2D6 by paroxetine presumably via bioactivation of the methylene dioxane moiety (178,180).

The use of *in vitro* MBI data to extrapolate to *in vivo* situations is further complicated by the fact that certain MBIs, such as HIV protease inhibitors, are also inducers of CYP enzymes, in particular CYP3A4 (119,182). In such cases, the use of liver microsomes or recombinant enzymes to evaluate enzyme inhibition may not provide the correct extrapolation to *in vivo* situations. An approach involving determination of both mRNA changes as well as enzyme activities in an inducible system, such as primary human hepatocytes, should be used (119).

Besides CYP enzymes, the inhibition of drug transporters can result in changes in drug clearance and in drug exposure, leading to either adverse effects or inadequate pharmacological activity (125,126) Since P-gp is the most well understood drug transporter with respect to its role in clinical drug–drug interactions, considerate efforts have been put into identifying and characterizing drugs that P-gp substrates or inhibitors during drug discovery and development, using in vitro models such as bi-directional transport employing monolayers (e.g. Caco-2, MDCK cells), uptake/efflux of fluorescent or radiolabeled probes (e.g. Calcein-AM, rhodamine-123), or simulation of ATPase activity using membrane vesicles derived from tissues or cells expressing P-gp (107,140). Bi-directional transport using monolayers expressing P-gp is considered the most reliable method for identifying P-gp substrates or inhibitors, since the method has the advantages of allowing the direct measurement of efflux across the cell barrier and evaluation of P-gp involvement (107). Similar to studies involving CYPs, criteria can be set

up to identify whether a drug is a substrate (e.g. efflux ratios >2) or an inhibitor (e.g. IC_{50} values in inhibiting digoxin efflux) of P-gp. However, dissimilar to CYPs, the extrapolation of *in vitro* P-gp inhibition data to clinical situation has not been well established, in part due to the wide tissue distribution of P-gp (i.e. intestinal lumen, liver, kidney and blood–brain-barrier) and that relevant inhibitor concentration that needs to be considered (167).

Due to the overlapping substrate and inhibitor selectivity for CYP3A and P-gp, it has become increasingly recognized that drug–drug interactions may also be mediated by this transporter-enzyme interplay. For example, inhibition of P-gp in a CYP3A4 transfected Caco-2 cellular system in the apical to basolateral direction decreased the extraction ratio of sirolimus (a substrate for both P-gp and CYP3A), suggesting that inhibition of intestinal drug efflux can lead to decreased intestinal metabolism of the concomitant drug due to decreased access of the drug to the enzyme by reducing recycling (158,159). In contrast, inhibition of P-gp in the basolateral to apical direction increased the extraction ratio of sirolimus, suggesting that inhibition of hepatic drug efflux can increase the extent of hepatic metabolism of the concomitant drug due to increased access of the drug to the active enzyme. It was suggested (158) that drug–drug interactions as a result of transporter-enzyme interplay might be more significant for drugs with low solubility and extensive metabolism (Class 2 drugs). Continued research is needed in this area to further demonstrate the clinical significance of transporter-enzyme interplay, and to assess approaches to predict likely clinical outcome from *in vitro* methodologies for drugs that are inhibitors of transporters but not of CYP enzymes. An example of a drug where lack of proper integration of drug–drug interaction data can lead to drastic consequence is mibefradil, a calcium channel blocker used for hypertension, which was withdrawn from the market in 1998 due to serious drug interactions with concomitant medications that are CYP3A or P-gp substrates. The serious drug interactions caused by mibefradil might have been anticipated with the proper conduct of preclinical studies, since it is shown to be a potent reversible and mechanism-based inhibitor of CYP3A and a potent inhibitor of P-gp (181,182).

Identification of Metabolism-related Drug Toxicity

Toxicogenomics is a branch of the 'omics' technologies that refers to the study of toxicological changes at the level of transcription, in response to toxic exposure to a chemical (183–185). One of its potential applications is to determine the underling molecular causes of toxicological findings. The application of this technology to characterize toxicity in cells or animals, in order to predict toxicity based on chemical structures, has attracted a lot of attention as its proponents contend that it has the potential to replace and/or shorten the time and cost of traditional toxicology studies conducted during drug development (53,186,187). However, rather than provide an alternative to traditional toxicity studies, toxicogenomics is currently being applied successfully to elucidate molecular-based mechanisms responsible for toxicological observations and also to identify biomarkers for the early detection of toxicity during clinical trials (54).

Adverse drug reactions (ADR) can either be pharmacologic or idiosyncratic. Pharmacologic reactions result from augmentation of the normal/expected pharmacologic response of the drug and are sometimes referred to as an exaggerated response. Idiosyncratic reactions on the other hand, are of unknown etiology and are not manifestations of the expected pharmacologic response (188,189). It is currently difficult to accurately predict which drugs will be associated with a significant incidence of idiosyncratic drug reactions. Formation of reactive metabolites and covalent adduct formation of these intermediates with cellular components such as proteins and DNA has been associated with IDR and is used as a screening tool to predict which molecules can potentially cause IDR (190). However, since not all reactive metabolites covalently bind to cellular components and even when they bind, not all cause IDR, predicting which metabolites will affect endogenous signaling pathways potentially leading to IDR, remains a challenge. In addition, some reactions are species-specific, making it difficult for predictions based on preclinical data to be made (37,190). Recently, it has been suggested that genetic factors play a crucial causative role in IDR and studies have shown that drugs associated with IDR also upregulate protective genes *in vivo* (191). Thus polymorphisms in enzymes that catalyze bioactivation reactions, the proteins to which reactive metabolites covalently bind, receptor proteins and protective genes could potentially affect the formation of IDR and account for the individual differences in susceptibility to IDR. Use of genomics technologies to determine gene expression profiles and genotypes therefore has the potential to develop into a more effective screening tool for IDR, as genetically susceptible subsets of the population could be identified.

Loss-of-function polymorphisms have the potential to affect pathways not directly related to the predicted mechanism of action of a drug. Recent advances in nuclear receptors and signal transduction fields have increased our knowledge and understanding of the complex biological processes of gene regulation and their impact on normal physiology as well as the pathological outcome of variations in this network. We now know for instance that nuclear receptors, initially named 'orphan' receptors because of lack of endogenous ligands, actually control a cascade of biochemical processes and pathways essential for the normal functioning of organisms. Thus the nuclear receptor, LXR, acts as a cholesterol sensor and helps to maintain lipid homeostasis. CAR, AhR and PXR have the ability to modulate thyroid hormone levels by modulating the expression of UGT enzymes, which are involved in the conjugation of thyroid hormones (192). In addition to these critical roles, it is also known that there is cross talk between nuclear receptors such that they share similar ligands and in some cases, regulate the same downstream genes (87). One consequence of this cross talk is that genes in pathways not directly affected by a ligand, may well be modulated and the normal biochemical processes they control, disrupted, leading to unexpected adverse events. Thus understanding nuclear receptor chemistry and their involvement in target independent toxicity provides added value to interpreting toxicological findings. As an example of how a 'toxicogenomics' approach can offer plausible explanations for off-target pre-

clinical safety observations, we used an integrated genome-based approach to provide a molecular mechanism for thyroid hypertrophy and changes in circulating thyroid hormones in a 14-day dose ranging study in rats. A nonsteroidal progestin was shown to cause transcriptional induction of microsomal UGTs, which are predominantly responsible for the conjugation and elimination of thyroid hormones in the rat (193–195).

CONCLUSIONS AND OUTLOOK

Over the last ten years, our understanding of the field of drug metabolism has greatly increased due to advances in areas such as pharmacogenetics, pharmacogenomics and transporters. Some of the key milestones in the history of drug metabolism research are summarized in Table V. Integration of these fields with traditional drug metabolism studies has and continues to have an impact on pharmaceutical research. Their impact of predicting drug–drug interactions, clearance pathways and factors affecting drug exposures is without question and it is anticipated that these emerging sciences will also impact our ability to elucidate the mechanisms involved in idiosyncratic drug reactions and off-target toxicity. Other fields that have the potential to impact drug metabolism research are proteomics, metabonomics, and systems biology. Lack of high throughput capabilities and the associated bioinformatics tools with which to mine the data generated by these technologies currently limit their global application in drug metabolism research. However, as technological advances continue to be made, their application will continue to grow, enabling scientists to benefit from the type of global and high throughput successes that genome-based technologies have achieved. Finally, in order to have the greatest benefits from these advances, drug metabolism scientists need to embrace and incorporate emerging trends in science and technology, ask the right questions, select appropriate tools, conduct studies in a timely manner, and accurately interpret data to facilitate alignment of drug metabolism studies/data with the drug discovery and development process.

ACKNOWLEDGMENTS

The authors would like to thank Dr Julius Enoru-Eta and Dr Joann Scatina for their review of this manuscript.

REFERENCES

1. J. Axelrod. The enzymatic deamination of amphetamine (benzedrine) *J. Biol. Chem.* **214**:753–763 (1955).
2. R. I. Dorfman, J. W. Cook, and J. B. Hamilton. Conversion by the human of the testis hormone, testosterone, into the urinary androgen, androsterone *J. Biol. Chem.* **130**:285–295 (1939).
3. E. C. Miller, J. A. Miller, R. R. Brown, and J. C. Macdonald. On the protective action of certain polycyclic aromatic hydrocarbons against carcinogenesis by aminoazo dyes and 2-acetylaminofluorene *Cancer Res.* **18**:469–477 (1958).
4. G. C. Mueller and J. A. Miller. The metabolism of 4-dimethylaminoazobenzene by rat liver homogenates *J. Biol. Chem.* **176**:535–544 (1948).
5. G. C. Mueller and J. A. Miller. The metabolism of methylated aminoazo dyes. II. Oxidative demethylation by rat liver homogenates *J. Biol. Chem.* **202**:579–587 (1953).
6. K. J. Ryan. Conversion of androstenedione to estrone by placental microsomes *Biochim. Biophys. Acta* **27**:658–659 (1958).
7. R. T. Williams. *Detoxication Mechanisms: The Metabolism of Drugs and Allied Organic Compounds*, Chapman and Hall, London, 1949.
8. R. T. Williams. *Detoxication Mechanisms: The Metabolism and Detoxication of Drugs, Toxic Substances, and Other Organic Compounds*, Chapman and Hall, London, 1959.
9. P. D. Josephy, F. P. Guengerich, and J. O. Miners. Phase I and II drug metabolism: terminology that we should phase out? *Drug Metab. Rev.***37**:575–580 (2005).
10. H. S. Mason. Mechanisms of oxygen metabolism *Science* **125**:1185–1188 (1957).
11. D. Y. Cooper, S. Levin, S. Narasimhulu, and O. Rosenthal. Photochemical action spectrum of the terminal oxidase of mixed function oxidase systems *Science* **147**:400–402 (1965).
12. T. Omura and R. Sato. A new cytochrome in liver microsomes *J. Biol. Chem.* **237**:1375–1376 (1962).
13. R. W. Estabrook, D. Y. Cooper, and O. Rosenthal. The light reversible carbon monoxide inhibition of the steroid C21-hydroxylase system of the adrenal cortex *Biochem. Z.* **338**:741–755 (1963).
14. A. Y. Lu and M. J. Coon. Role of hemoprotein P-450 in fatty acid omega-hydroxylation in a soluble enzyme system from liver microsomes *J. Biol. Chem.* **243**:1331–1332 (1968).
15. R. W. Estabrook. A passion for P450s (rememberances of the early history of research on cytochrome P450) *Drug Metab. Dispos.* **31**:1461–1473 (2003).
16. F. P. Guengerich. Cytochrome P450: what have we learned and what are the future issues? *Drug Metab. Rev.* **36**:159–197 (2004).
17. D. A. Haugen, T. A. van der Hoeven, and M. J. Coon. Purified liver microsomal cytochrome P-450. Separation and characterization of multiple forms. *J. Biol. Chem.* **250**:3567–3570 (1975).
18. A. Hildebrandt, H. Remmer, and R. W. Estabrook. Cytochrome P-450 of liver microsomes-one pigment or many *Biochem. Biophys. Res. Commun.* **30**:607–612 (1968).
19. D. W. Nebert. Multiple forms of inducible drug-metabolizing enzymes: a reasonable mechanism by which any organism can cope with adversity *Mol. Cell. Biochem.* **27**:27–46 (1979).
20. N. E. Sladek and G. J. Mannering. Induction of drug metabolism. II. Qualitative differences in the microsomal N-demethylating systems stimulated by polycyclic hydrocarbons and by phenobarbital *Mol. Pharmacol.* **5**:186–199 (1969).
21. P. B. Danielson. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans *Curr. Drug Metab.* **3**:561–597 (2002).
22. S. D. Aust, D. L. Roerig, and T. C. Pederson. Evidence for superoxide generation by NADPH-cytochrome c reductase of rat liver microsomes *Biochem. Biophys. Res. Commun.* **47**:1133–1137 (1972).
23. J. T. Groves and G. A. McClusky. Aliphatic hydroxylation by highly purified liver microsomal cytochrome P-450. Evidence for a carbon radical intermediate *Biochem. Biophys. Res. Commun.* **81**:154–160 (1978).
24. F. P. Guengerich. Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity *Chem. Res. Toxicol.* **14**:611–650 (2001).
25. P. R. Ortiz de Montellano. *Oxygen Activation and Reactivity, Cytochrome P450: Structure, Mechanisms, and Biochemistry*. Plenum, New York, 1995, pp. 243–303.
26. P. R. Ortiz de Montellano and J. J. De Voss. Substrate oxidation by cytochrome P450 enzymes. In P. R. O. d. Montellano (ed.), *Cytochrome P450: Structure, Mechanisms, and Biochemistry*, Plenum, New York, 2004, pp. 183–230.
27. R. A. Sheldon and J. K. Kochi. *Activation of Molecular Oxygen by Metal Complexes. Metal-catalyzed Oxidations of Organic Compounds*. Academic, New York, 1981, pp. 108–112.
28. H. W. Strobel and M. J. Coon. Effect of superoxide generation and dismutation on hydroxylation reactions catalyzed by liver microsomal cytochrome P-450 *J. Biol. Chem.* **246**:7826–7829 (1971).
29. S. Chen, J. E. Shively, S. Nakajin, M. Shinoda, and P. F. Hall. Amino terminal sequence analysis of human placenta aromatase *Biochem. Biophys. Res. Commun.* **135**:713–719 (1986).

30. A. Mahgoub, J. R. Idle, L. G. Dring, R. Lancaster, and R. L. Smith. Polymorphic hydroxylation of Debrisoquine in man *Lancet* **2**:584–586 (1977).
31. W. L. Miller. Congenital adrenal hyperplasia *N. Engl. J. Med.* **314**:1321–1322 (1986).
32. D. W. Nebert and D. W. Russell. Clinical importance of the cytochromes P450 *Lancet* **360**:1155–1162 (2002).
33. E. C. Miller and J. A. Miller. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules *Cancer* **47**:2327–2345 (1981).
34. U. Groth and H. G. Neumann. The relevance of chemico-biological interactions for the toxic and carcinogenic effects of aromatic amines. V. The pharmacokinetics of related aromatic amines in blood *Chem. Biol. Interact.* **4**:409–419 (1972).
35. D. J. Jollow, J. R. Mitchell, W. Z. Potter, D. C. Davis, J. R. Gillette, and B. B. Brodie. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo J. Pharmacol. Exp. Ther.* **187**:195–202 (1973).
36. W. Z. Potter, D. C. Davis, J. R. Mitchell, D. J. Jollow, J. R. Gillette, and B. B. Brodie. Acetaminophen-induced hepatic necrosis. 3. Cytochrome P-450-mediated covalent binding in vitro *J. Pharmacol. Exp. Ther.* **187**:203–210 (1973).
37. I. Gardner, M. Popovic, N. Zahid, and J. P. Uetrecht. A comparison of the covalent binding of clozapine, procainamide, and vesnarinone to human neutrophils *in vitro* and rat tissues *in vitro* and *in vivo Chem. Res. Toxicol.* **18**:1384–1394 (2005).
38. A. S. Kalgutkar, I. Gardner, R. S. Obach, C. L. Shaffer, E. Callegari, K. R. Henne, A. E. Mutlib, D. K. Dalvie, J. S. Lee, Y. Nakai, J. P. O'Donnell, J. Boer, and S. P. Harriman. A comprehensive listing of bioactivation pathways of organic functional groups *Curr. Drug Metab.* **6**:161–225 (2005).
39. N. R. Pumford and N. C. Halmes. Protein targets of xenobiotic reactive intermediates *Annu. Rev. Pharmacol. Toxicol.* **37**:91–117 (1997).
40. J. Uetrecht. Prediction of a new drug's potential to cause idiosyncratic reactions *Curr. Opin. Drug. Discov. Dev.* **4**:55–59 (2001).
41. S. Zhou, E. Chan, W. Duan, M. Huang, and Y. Z. Chen. Drug bioactivation, covalent binding to target proteins and toxicity relevance *Drug Metab. Rev.* **37**:41–213 (2005).
42. E. Fontana, P. M. Dansette, and S. M. Poli. Cytochrome p450 enzymes mechanism based inhibitors: common sub-structures and reactivity *Curr. Drug Metab.* **6**:413–454 (2005).
43. S. Zhou, E. Chan, L. Y. Lim, U. A. Boelsterli, S. C. Li, J. Wang, Q. Zhang, M. Huang, and A. Xu. Therapeutic drugs that behave as mechanism-based inhibitors of cytochrome P450 3A4 *Curr. Drug Metab.* **5**:415–442 (2004).
44. S. Zhou, S. Yung Chan, B. Cher Goh, E. Chan, W. Duan, M. Huang, and H. L. McLeod. Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs *Clin. Pharmacokinet.* **44**:279–304 (2005).
45. M. Eichelbaum, M. Ingelman-Sundberg, and W. E. Evans. Pharmacogenomics and individualized drug therapy *Annu. Rev. Med.* **57**:119–137 (2006).
46. G. S. Ginsburg, R. P. Konstance, J. S. Allsbrook, and K. A. Schulman. Implications of pharmacogenomics for drug development and clinical practice *Arch. Intern. Med.* **165**:2331–2336 (2005).
47. D. B. Goldstein, S. K. Tate, and S. M. Sisodiya. Pharmacogenetics goes genomic *Nat. Rev., Genet.* **4**:937–947 (2003).
48. D. W. Nebert. Pharmacogenetics and pharmacogenomics: why is this relevant to the clinical geneticist? *Clin. Genet.* **56**:247–258 (1999).
49. A. Hedgecoe and P. Martin. The drugs don't work: expectations and the shaping of pharmacogenetics *Soc. Stud. Sci.* **33**:327–364 (2003).
50. C. R. Wolf, G. Smith, and R. L. Smith. Science, medicine, and the future: Pharmacogenetics *BMJ* **320**:987–990 (2000).
51. K. A. Frazer, L. Elnitski, D. M. Church, I. Dubchak, and R. C. Hardison. Cross-species sequence comparisons: a review of methods and available resources. *Genome Res.* **13**:1–12 (2003).
52. K. A. Frazer, J. B. Sheehan, R. P. Stokowski, X. Chen, R. Hosseini, J. F. Cheng, S. P. Fodor, D. R. Cox, and N. Patil. Evolutionarily conserved sequences on human chromosome 21 *Genome Res.* **11**:1651–1659 (2001).
53. M. A. Higgins, B. R. Berridge, B. J. Mills, A. E. Schultze, H. Gao, G. H. Searfoss, T. K. Baker, and T. P. Ryan. Gene expression analysis of the acute phase response using a canine microarray *Toxicol. Sci.* **74**:470–484 (2003).
54. N. Kato, M. Shibutani, H. Takagi, C. Uneyama, K. Y. Lee, S. Takigami, K. Mashima, and M. Hirose. Gene expression profile in the livers of rats orally administered ethinylestradiol for 28 days using a microarray technique *Toxicology* **200**:179–192 (2004).
55. G. H. Searfoss, W. H. Jordan, D. O. Calligaro, E. J. Galbreath, L. M. Schirtzinger, B. R. Berridge, H. Gao, M. A. Higgins, P. C. May, and T. P. Ryan. Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional gamma-secretase inhibitor *J. Biol. Chem.* **278**:46107–46116 (2003).
56. B. Alexanderson, D. A. Evans, and F. Sjoqvist. Steady-state plasma levels of nortriptyline in twins: influence of genetic factors and drug therapy *Br. Med. J.* **4**:764–768 (1969).
57. W. Hammer and F. Sjoqvist. Plasma levels of monomethylated tricyclic antidepressants during treatment with imipramine-like compounds *Life Sci.* **6**:1895–1903 (1967).
58. M. Eichelbaum. Polymorphic oxidation of debrisoquine and sparteine *Prog. Clin. Biol. Res.* **214**:157–167 (1986).
59. R. L. Smith. The Paton Prize Award. The discovery of the debrisoquine hydroxylation polymorphism: scientific and clinical impact and consequences *Toxicology* **168**:11–19 (2001).
60. L. Bertilsson. Geographical/interracial differences in polymorphic drug oxidation. Current state of knowledge of cytochromes P450 (CYP) 2D6 and 2C19 *Clin. Pharmacokinet.* **29**:192–209 (1995).
61. C. Bruhn, J. Brockmoller, I. Cascorbi, I. Roots, and H. H. Borchert. Correlation between genotype and phenotype of the human arylamine N-acetyltransferase type 1 (NAT1) *Biochem. Pharmacol.* **58**:1759–1764 (1999).
62. T. K. Chang, L. Yu, J. A. Goldstein, and D. J. Waxman. Identification of the polymorphically expressed CYP2C19 and the wild-type CYP2C9-ILE359 allele as low-Km catalysts of cyclophosphamide and ifosfamide activation *Pharmacogenetics* **7**:211–221 (1997).
63. A. K. Daly, S. Cholerton, W. Gregory, and J. R. Idle. Metabolic polymorphisms *Pharmacol. Ther.* **57**:129–160 (1993).
64. S. Garte, L. Gaspari, A. K. Alexandrie, C. Ambrosone, H. Autrup, J. L. Autrup, H. Baranova, L. Bathum, S. Benhamou, P. Boffetta, C. Bouchardy, K. Breskvar, J. Brockmoller, I. Cascorbi, M. L. Clapper, C. Coutelle, A. Daly, M. Dell'Omo, V. Dolzan, C. M. Dresler, A. Fryer, A. Haugen, D. W. Hein, A. Hildesheim, A. Hirvonen, L. L. Hsieh, M. Ingelman-Sundberg, I. Kalina, D. Kang, M. Kihara, C. Kiyohara, P. Kremers, P. Lazarus, L. Le Marchand, M. C. Lechner, E. M. van Lieshout, S. London, J. J. Manni, C. M. Maugard, S. Morita, V. Nazar-Stewart, K. Noda, Y. Oda, F. F. Parl, R. Pastorelli, I. Persson, W. H. Peters, A. Rannug, T. Rebbeck, A. Risch, L. Roelandt, M. Romkes, D. Ryberg, J. Salagovic, B. Schoket, J. Seidegard, P. G. Shields, E. Sim, D. Sinnet, R. C. Strange, I. Stucker, H. Sugimura, J. To-Figueras, P. Vineis, M. C. Yu, and E. Taioli. Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol. Biomark. Prev.* **10**:1239–1248 (2001).
65. J. A. Goldsteinand and S. M. Moraisde. Biochemistry and molecular biology of the human CYP2C subfamily *Pharmacogenetics* **4**:285–299 (1994).
66. S. Hoffmeyer, O. Burk, O. von Richter, H. P. Arnold, J. Brockmoller, A. Johne, I. Cascorbi, T. Gerloff, I. Roots, M. Eichelbaum, and U. Brinkmann. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc. Natl. Acad. Sci. USA* **97**:3473–3478 (2000).
67. M. Iwasaki, Y. Yoshimura, S. Asahi, K. Saito, S. Sakai, S. Morita, O. Takenaka, T. Inoda, E. Kashiyama, A. Aoyama, T. Nakabayashi, S. Omori, T. Kuwabara, T. Izumi, K. Nakamura, K. Takanaka, Y. Nakayama, M. Takeuchi, H. Nakamura, S. Kametani, Y. Terauchi, T. Hashizume, S. Nagayama, T. Kume, M. Achira, H. Kawai, T. Kawashiro, A. Nakamura, Y. Nakai,

A. Kagayama, T. Shiraga, T. Niwa, T. Yoshimura, J. Morita, F. Ohsawa, M. Tani, N. Osawa, K. Ida, and K. Noguchi. Functional characterization of single nucleotide polymorphisms with amino acid substitution in CYP1A2, CYP2A6, and CYP2B6 found in the Japanese population *Drug Metab. Pharmacokinet.* **19**:444–452 (2004).
68. I. Johansson, Q. Y. Yue, M. L. Dahl, M. Heim, J. Sawe, L. Bertilsson, U. A. Meyer, F. Sjoqvist, and M. Ingelman-Sundberg. Genetic analysis of the interethnic difference between Chinese and Caucasians in the polymorphic metabolism of debrisoquine and codeine *Eur. J. Clin. Pharmacol.* **40**:553–556 (1991).
69. U. A. Meyer and U. M. Zanger. Molecular mechanisms of genetic polymorphisms of drug metabolism *Annu. Rev. Pharmacol. Toxicol.* **37**:269–296 (1997).
70. S. Ohgiya, M. Komori, H. Ohi, K. Shiramatsu, N. Shinriki, and T. Kamataki. Six-base deletion occurring in messages of human cytochrome P-450 in the CYP2C subfamily results in reduction of tolbutamide hydroxylase activity *Biochem. Int.* **27**:1073–1081 (1992).
71. K. C. Ferdinand. Isosorbide dinitrate and hydralazine hydrochloride: a review of efficacy and safety *Expert Rev. Cardiovasc. Ther.* **3**:993–1001 (2005).
72. S. B. Hagaand and G. S. Ginsburg. Prescribing BiDil: is it black and white? *J. Am. Coll. Cardiol.* **48**:12–14 (2006).
73. D. M. Maraganore, M. Andradede, T. G. Lesnick, K. J. Strain, M. J. Farrer, W. A. Rocca, P. V. Pant, K. A. Frazer, D. R. Cox, and D. G. Ballinger. High-resolution whole-genome association study of Parkinson disease *Am. J. Hum. Genet.* **77**:685–693 (2005).
74. B. Brodie, J. Axelrod, J. R. Cooper, L. Gaudette, B. N. DuLa, C. Mitoma, and S. Udenfriend. Detoxication of drugs and other foreign compounds by liver microsomes *Science* **121**:603–604 (1955).
75. D. Garfinkel. Studies on pig liver microsomes. I. Enzymic and pigment composition of different microsomal fractions *Arch. Biochem. Biophys.* **77**:493–509 (1958).
76. M. Klingenberg. Pigments of rat liver microsomes *Arch. Biochem. Biophys.* **75**:376–386 (1958).
77. A. Y. Lu, K. W. Junk, and M. J. Coon. Resolution of the cytochrome P-450-containing omega-hydroxylation system of liver microsomes into three components *J. Biol. Chem.* **244**:3714–3721 (1969).
78. D. W. Nebert and L. L. Bausserman. Genetic differences in the extent of aryl hydrocarbon hydroxylase induction in mouse fetal cell cultures *J. Biol. Chem.* **245**:6373–6382 (1970).
79. A. B. Okey, G. P. Bondy, M. E. Mason, D. W. Nebert, C. J. Forster-Gibson, J. Muncan, and M. J. Dufresne. Temperature-dependent cytosol-to-nucleus translocation of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in continuous cell culture lines *J. Biol. Chem.* **255**:11415–11422 (1980).
80. J. E. Gielen and D. W. Nebert. Aryl hydrocarbon hydroxylase induction in mammalian liver cell culture. I. Stimulation of enzyme activity in nonhepatic cells and in hepatic cells by phenobarbital, polycyclic hydrocarbons, and 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane *J. Biol. Chem.* **246**:5189–5198 (1971).
81. J. Gorski and F. Gannon. Current models of steroid hormone action: a critique *Annu. Rev. Physiol.* **38**:425–450 (1976).
82. T. M. Guenthner and D. W. Nebert. Cytosolic receptor for aryl hydrocarbon hydroxylase induction by polycyclic aromatic compounds. Evidence for structural and regulatory variants among established cell cultured lines *J. Biol. Chem.* **252**: 8981–8989 (1977).
83. A. 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 receptor for induction of aryl hydrocarbon hydroxylase *J. Biol. Chem.* **251**:4936–4946 (1976).
84. 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).
85. B. Blumberg, W. Sabbagh, Jr., H. Juguilon, J. Bolado, Jr., 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).
86. 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. Willson, R. H. Zetterstrom, T. Perlmann, and J. M. Lehmann. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway *Cell* **92**:73–82 (1998).
87. S. A. Kliewer, J. M. Lehmann, and T. M. Willson. Orphan nuclear receptors: shifting endocrinology into reverse *Science* **284**:757–760 (1999).
88. J. J. Repa and D. J. Mangelsdorf. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis *Annu. Rev. Cell Dev. Biol.* **16**:459–481 (2000).
89. T. H. Rushmore and A. N. Kong. Pharmacogenomics, regulation and signaling pathways of phase I and II drug metabolizing enzymes *Curr. Drug Metab.* **3**:481–490 (2002).
90. J. H. Lin. Tissue distribution and pharmacodynamics: a complicated relationship *Curr. Drug Metab.* **7**:39–65 (2006).
91. J. Brockmoller, J. Kirchheiner, C. Meisel, and I. Roots. Pharmacogenetic diagnostics of cytochrome P450 polymorphisms in clinical drug development and in drug treatment *Pharmacogenomics* **1**:125–151 (2000).
92. M. Eichelbaum. Pharmacokinetic drug interactions *J. Clin. Pharmacol.* **26**:469–473 (1986).
93. M. T. Zuhlsdorf. Relevance of pheno- and genotyping in clinical drug development *Int. J. Clin. Pharmacol. Ther.* **36**:607–612 (1998).
94. R. Weinshilboum. Inheritance and drug response *N. Engl. J. Med.* **348**:529–537 (2003).
95. M. Ingelman-Sundberg. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms *Naunyn-Schmiedebergs Arch. Pharmacol* **369**:89–104 (2004).
96. W. E. Evans and M. V. Relling. Pharmacogenomics: translating functional genomics into rational therapeutics *Science* **286**: 487–491 (1999).
97. M. M. Shi, M. R. Bleavins, and F. A. Iglesiade la. Pharmacogenetic application in drug development and clinical trials *Drug Metab. Dispos.* **29**:591–595 (2001).
98. M. W. Linder, R. A. Prough, and R. Valdes, Jr. Pharmacogenetics: a laboratory tool for optimizing therapeutic efficiency. *Clin. Chem.***43**:254–266 (1997).
99. T. Friedberg, M. P. Pritchard, M. Bandera, S. P. Hanlon, D. Yao, L. A. McLaughlin, S. Ding, B. Burchell, and C. R. Wolf. Merits and limitations of recombinant models for the study of human P450-mediated drug metabolism and toxicity: an intralaboratory comparison *Drug Metab. Rev.* **31**:523–544 (1999).
100. C. J. Henderson and C. R. Wolf. Transgenic analysis of human drug-metabolizing enzymes: preclinical drug development and toxicology *Mol. Interv.* **3**:331–343 (2003).
101. I. S. Vizirianakis. Challenges in current drug delivery from the potential application of pharmacogenomics and personalized medicine in clinical practice *Curr. Drug Deliv.* **1**:73–80 (2004).
102. C. R. Wolf. The Gerhard Zbinden memorial lecture: application of biochemical and genetic approaches to understanding pathways of chemical toxicity *Toxicol. Lett.* **127**:3–17 (2002).
103. P. M. Holland, R. D. Abramson, R. Watson, and D. H. Gelfand. Detection of specific polymerase chain reaction product by utilizing the 5′–3′ exonuclease activity of Thermus aquaticus DNA polymerase *Proc. Natl. Acad. Sci. USA* **88**:7276–7280 (1991).
104. P. W. Kleyn and E. S. Vesell. Genetic variation as a guide to drug development *Science* **281**:1820–1821 (1998).
105. 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. Graham, K. Carroll, J. Xie, A. Madan, A. Parkinson, D. Christ, B. Selling, E. LeCluyse, and L. S. 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).

106. L. G. Yengi, Q. Xiang, J. Pan, J. Scatina, J. Kao, S. E. Ball, R. Fruncillo, G. Ferron, and C. Roland Wolf. Quantitation of cytochrome P450 mRNA levels in human skin *Anal. Biochem.* **316**:103–110 (2003).
107. FDA. *Guidance for Industry. Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling*, 2006.
108. L. G. Yengi. Systems biology in drug safety and metabolism: integration of microarray, real-time PCR and enzyme approaches *Pharmacogenomics* **6**:185–192 (2005).
109. C. Debouck and P. N. Goodfellow. DNA microarrays in drug discovery and development *Nat. Genet.* **21**:48–50 (1999).
110. C. A. Harrington, C. Rosenow, and J. Retief. Monitoring gene expression using DNA microarrays *Curr. Opin. Microbiol.* **3**:285–291 (2000).
111. R. J. Lipshutz, S. P. Fodor, T. R. Gingeras, and D. J. Lockhart. High density synthetic oligonucleotide arrays *Nat. Genet.* **21**:20–24 (1999).
112. C. J. Henderson, D. M. Otto, D. Carrie, M. A. Magnuson, A. W. McLaren, I. Rosewell, and C. R. Wolf. Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. *J. Biol. Chem.* **278**: 13480–13486 (2003).
113. E. 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. Mol. Toxicol.* **14**:177–188 (2000).
114. A. P. Li, S. M. Colburn, and D. J. Beck. A simplified method for the culturing of primary adult rat and human hepatocytes as multicellular spheroids *In Vitro Cell Dev. Biol.* **28A**:673–677 (1992).
115. R. E. Pearce, C. J. McIntyre, A. Madan, U. Sanzgiri, A. J. Draper, P. L. Bullock, D. C. Cook, L. A. Burton, J. Latham, C. Nevins, and A. Parkinson. Effects of freezing, thawing, and storing human liver microsomes on cytochrome P450 activity *Arch. Biochem. Biophys.* **331**:145–169 (1996).
116. A. P. Li, D. L. Kaminski, and A. Rasmussen. Substrates of human hepatic cytochrome P450 3A4 *Toxicology* **104**:1–8 (1995).
117. J. D. Cleary, L. A. Walker, and R. L. Hawke. Antimycotic drug discovery in the age of genomics *Am. J. Pharmacogenomics* **5**:365–386 (2005).
118. D. Gerhold, M. Lu, J. Xu, C. Austin, C. T. Caskey, and T. Rushmore. Monitoring expression of genes involved in drug metabolism and toxicology using DNA microarrays *Physiol. Genomics.* **5**:161–170 (2001).
119. D. J. McConn and Z. Zhao. Integrating *in vitro* kinetic data from compounds exhibiting induction, reversible inhibition and mechanism-based inactivation: *in vitro* study design *Curr. Drug Metab.* **5**:141–146 (2004).
120. G. Luo, J. Lin, W. D. Fiske, R. Dai, T. J. Yang, S. Kim, M. Sinz, E. LeCluyse, E. Solon, J. M. Brennan, I. H. Benedek, S. Jolley, D. Gilbert, L. Wang, F. W. Lee, and L. S. Gan. Concurrent induction and mechanism-based inactivation of CYP3A4 by an L-valinamide derivative *Drug Metab. Dispos.* **31**:1170–1175 (2003).
121. Y. Konno, M. Sekimoto, K. Nemoto, and M. Degawa. Sex difference in induction of hepatic CYP2B and CYP3A subfamily enzymes by nicardipine and nifedipine in rats *Toxicol. Appl. Pharmacol.* **196**:20–28 (2004).
122. D. Projean, S. Dautrey, H. K. Vu, T. Groblewski, J. L. Brazier, and J. Ducharme. Selective downregulation of hepatic cytochrome P450 expression and activity in a rat model of inflammatory pain *Pharm. Res.* **22**:62–70 (2005).
123. M. P. Murphy. Current pharmacogenomic approaches to clinical drug development *Pharmacogenomics* **1**:115–123 (2000).
124. B. Goodwin, E. Hodgson, and C. Liddle. The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module *Mol. Pharmacol.* **56**:1329–1339 (1999).
125. N. Mizuno, T. Niwa, Y. Yotsumoto, and Y. Sugiyama. Impact of drug transporter studies on drug discovery and development *Pharmacol. Rev.* **55**:425–461 (2003).
126. P. M. Beringer and R. L. Slaughter. Transporters and their impact on drug disposition *Ann. Pharmacother.* **39**:1097–1108 (2005).
127. K. Ito, H. Suzuki, T. Horie, and Y. Sugiyama. Apical/basolateral surface expression of drug transporters and its role in vectorial drug transport *Pharm. Res.* **22**:1559–1577 (2005).
128. J. H. Lin. Species similarities and differences in pharmacokinetics *Drug Metab. Dispos.* **23**:1008–1021 (1995).
129. Y. Sai. Biochemical and molecular pharmacological aspects of transporters as determinants of drug disposition *Drug Metab. Pharmacokinet.* **20**:91–99 (2005).
130. J. E. van Montfoort, B. Hagenbuch, G. M. Groothuis, H. Koepsell, P. J. Meier, and D. K. Meijer. Drug uptake systems in liver and kidney. *Curr. Drug Metab.* **4**:185–211 (2003).
131. A. Bodo, E. Bakos, F. Szeri, A. Varadi, and B. Sarkadi. The role of multidrug transporters in drug availability, metabolism and toxicity *Toxicol. Lett.* **140–141**:133–143 (2003).
132. K. M. Mahar Doan, J. E. Humphreys, L. O. Webster, S. A. Wring, L. J. Shampine, C. J. Serabjit-Singh, K. K. Adkison, and J. W. Polli. Passive permeability and P-glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs *J. Pharmacol. Exp. Ther.* **303**:1029–1037 (2002).
133. G. K. Dresser, D. G. Bailey, B. F. Leake, U. I. Schwarz, P. A. Dawson, D. J. Freeman, and R. B. Kim. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine *Clin. Pharmacol. Ther.* **71**:11–20 (2002).
134. M. Cvetkovic, B. Leake, M. F. Fromm, G. R. Wilkinson, and R. B. Kim. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine *Drug Metab. Dispos.* **27**:866–871 (1999).
135. M. Masuda, Y. I'Izuka, M. Yamazaki, R. Nishigaki, Y. Kato, K. Ni'inuma, H. Suzuki, and Y. Sugiyama. Methotrexate is excreted into the bile by canalicular multispecific organic anion transporter in rats *Cancer Res.* **57**:3506–3510 (1997).
136. S. Seitz and U. A. Boelsterli. Diclofenac acyl glucuronide, a major biliary metabolite, is directly involved in small intestinal injury in rats *Gastroenterology* **115**:1476–1482 (1998).
137. B. Stieger, K. Fattinger, J. Madon, G. A. Kullak-Ublick, and P. J. Meier. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver *Gastroenterology* **118**:422–430 (2000).
138. C. Funk, M. Pantze, L. Jehle, C. Ponelle, G. Scheuermann, M. Lazendic, and R. Gasser. Troglitazone-induced intrahepatic cholestasis by an interference with the hepatobiliary export of bile acids in male and female rats. Correlation with the gender difference in troglitazone sulfate formation and the inhibition of the canalicular bile salt export pump (Bsep) by troglitazone and troglitazone sulfate *Toxicology* **167**:83–98 (2001).
139. T. Korjamo, P. Honkakoski, M. R. Toppinen, S. Niva, M. Reinisalo, J. J. Palmgren, and J. Monkkonen. Absorption properties and P-glycoprotein activity of modified Caco-2 cell lines *Eur. J. Pharm. Sci.* **26**:266–279 (2005).
140. J. W. Polli, S. A. Wring, J. E. Humphreys, L. Huang, J. B. Morgan, L. O. Webster, and C. S. Serabjit-Singh. Rational use of *in vitro* P-glycoprotein assays in drug discovery *J. Pharmacol. Exp. Ther.* **299**:620–628 (2001).
141. P. P. Annaert and K. L. Brouwer. Assessment of drug interactions in hepatobiliary transport using rhodamine 123 in sandwich-cultured rat hepatocytes *Drug Metab. Dispos.* **33**:388–394 (2005).
142. P. P. Annaert, R. Z. Turncliff, C. L. Booth, D. R. Thakker, and K. L. Brouwer. P-glycoprotein-mediated *in vitro* biliary excretion in sandwich-cultured rat hepatocytes *Drug Metab. Dispos.* **29**:1277–1283 (2001).
143. W. Lee, H. Glaeser, L. H. Smith, R. L. Roberts, G. W. Moeckel, G. Gervasini, B. F. Leake, and R. B. Kim. Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry. *J. Biol. Chem.* **280**:9610–9617 (2005).
144. M. Sasaki, H. Suzuki, J. Aoki, K. Ito, P. J. Meier, and Y. Sugiyama. Prediction of *in vivo* biliary clearance from the *in vitro* transcellular transport of organic anions across a double-transfected Madin-Darby canine kidney II monolayer

expressing both rat organic anion transporting polypeptide 4 and multidrug resistance associated protein 2 *Mol. Pharmacol.* **66**:450–459 (2004).
145. H. Ishizuka, K. Konno, T. Shiina, H. Naganuma, K. Nishimura, K. Ito, H. Suzuki, and Y. Sugiyama. Species differences in the transport activity for organic anions across the bile canalicular membrane *J. Pharmacol. Exp. Ther.* **290**:1324–1330 (1999).
146. A. D. Shilling, F. Azam, J. Kao, and L. Leung. Use of canalicular membrane vesicles (CMVs) from rats, dogs, monkeys and humans to assess drug transport across the canalicular membrane *J. Pharmacol. Toxicol. Methods* **53**: 186–197 (2006).
147. J. A. Williams, R. Hyland, B. C. Jones, D. A. Smith, S. Hurst, T. C. Goosen, V. Peterkin, J. R. Koup, and S. E. Ball. Drug–drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios *Drug Metab. Dispos.* **32**:1201–1208 (2004).
148. J. B. Houston and D. J. Carlile. Prediction of hepatic clearance from microsomes, hepatocytes, and liver slices *Drug Metab. Rev.* **29**:891–922 (1997).
149. H. M. Jones and J. B. Houston. Substrate depletion approach for determining *in vitro* metabolic clearance: time dependencies in hepatocyte and microsomal incubations *Drug Metab. Dispos.* **32**:973–982 (2004).
150. R. S. Obach. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of *in vitro* half-life approach and nonspecific binding to microsomes *Drug Metab. Dispos.* **27**:1350–1359 (1999).
151. S. X. Zhao, D. Forman, N. Wallace, B. J. Smith, D. Meyer, D. Kazolias, F. Gao, J. Soglia, M. Cole, and D. Nettleton. Simple strategies for reducing sample loads in in *in vitro* metabolic stability high-throughput screening experiments: a comparison between traditional, two-time-point and pooled sample analyses *J. Pharm. Sci.* **94**:38–45 (2005).
152. Y. Naritomi, S. Terashita, A. Kagayama, and Y. Sugiyama. Utility of hepatocytes in predicting drug metabolism: comparison of hepatic intrinsic clearance in rats and humans *in vivo* and *in vitro Drug Metab. Dispos.* **31**:580–588 (2003).
153. R. J. Riley, D. F. McGinnity, and R. P. Austin. A unified model for predicting human hepatic, metabolic clearance from *in vitro* intrinsic clearance data in hepatocytes and microsomes *Drug Metab. Dispos.* **33**:1304–1311 (2005).
154. Y. Shibata, H. Takahashi, M. Chiba, and Y. Ishii. Prediction of hepatic clearance and availability by cryopreserved human hepatocytes: an application of serum incubation method *Drug Metab. Dispos.* **30**:892–896 (2002).
155. L. P. Delbressine, C. W. Funke, M. Tilborgvan, and F. M. Kaspersen. On the formation of carbamate glucuronides *Xenobiotica* **20**:133–134 (1990).
156. S. D. Hall, K. E. Thummel, P. B. Watkins, K. S. Lown, L. Z. Benet, M. F. Paine, R. R. Mayo, D. K. Turgeon, D. G. Bailey, R. J. Fontana, and S. A. Wrighton. Molecular and physical mechanisms of first-pass extraction *Drug Metab. Dispos.* **27**:161–166 (1999).
157. M. F. Paine, H. L. Hart, S. S. Ludington, R. L. Haining, A. E. Rettie, and D. C. Zeldin. The human intestinal cytochrome P450 "pie" *Drug Metab. Dispos.* **34**:880–886 (2006).
158. C. Y. Wu and L. Z. Benet. Predicting drug disposition via application of BCS: transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system *Pharm. Res.* **22**:11–23 (2005).
159. C. L. Cummins, W. Jacobsen, U. Christians, and L. Z. Benet. CYP3A4-transfected Caco-2 cells as a tool for understanding biochemical absorption barriers: studies with sirolimus and midazolam *J. Pharmacol. Exp. Ther.* **308**:143–155 (2004).
160. M. F. Paine, L. Y. Leung, H. K. Lim, K. Liao, A. Oganesian, M. Y. Zhang, K. E. Thummel, and P. B. Watkins. Identification of a novel route of extraction of sirolimus in human small intestine: roles of metabolism and secretion *J. Pharmacol. Exp. Ther.* **301**:174–186 (2002).
161. L. Y. Leung, H. K. Lim, M. W. Abell, and J. J. Zimmerman. Pharmacokinetics and metabolic disposition of sirolimus in healthy male volunteers after a single oral dose *Ther. Drug Monit.* **28**:51–61 (2006).
162. R. L. Woosley, Y. Chen, J. P. Freiman, and R. A. Gillis. Mechanism of the cardiotoxic actions of terfenadine *JAMA* **269**:1532–1536 (1993).
163. A. Markham and A. J. Wagstaff. Fexofenadine *Drugs* **55**:269–274 (1998).
164. P. Lu, M. L. Schrag, D. E. Slaughter, C. E. Raab, M. Shou, and A. D. Rodrigues. Mechanism-based inhibition of human liver microsomal cytochrome P450 1A2 by zileuton, a 5-lipoxygenase inhibitor *Drug Metab. Dispos.* **31**:1352–1360 (2003).
165. T. M. Polasek, D. J. Elliot, B. C. Lewis, and J. O. Miners. Mechanism-based inactivation of human cytochrome P4502C8 by drugs *in vitro*. *J. Pharmacol. Exp. Ther.***311**:996–1007 (2004).
166. Z. Y. Zhang and Y. N. Wong. Enzyme kinetics for clinically relevant CYP inhibition *Curr. Drug Metab.* **6**:241–257 (2005).
167. T. D. Bjornsson, J. T. Callaghan, H. J. Einolf, V. Fischer, L. Gan, S. Grimm, J. Kao, S. P. King, G. Miwa, L. Ni, G. Kumar, J. McLeod, R. S. Obach, S. Roberts, A. Roe, A. Shah, F. Snikeris, J. T. Sullivan, D. Tweedie, J. M. Vega, J. Walsh, and S. A. Wrighton. The conduct of *in vitro* and *in vivo* drug–drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective *Drug Metab. Dispos.* **31**:815–832 (2003).
168. W. Tang, R. W. Wang, and A. Y. Lu. Utility of recombinant cytochrome p450 enzymes: a drug metabolism perspective *Curr. Drug Metab.* **6**:503–517 (2005).
169. A. L. Blobaum. Mechanism-based inactivation and reversibility: is there a new trend in the inactivation of cytochrome p450 enzymes? *Drug Metab. Dispos.* **34**:1–7 (2006).
170. E. A. Dierks, K. R. Stams, H. K. Lim, G. Cornelius, H. Zhang, and S. E. Ball. A method for the simultaneous evaluation of the activities of seven major human drug-metabolizing cytochrome P450s using an *in vitro* cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectrometry *Drug Metab. Dispos.* **29**:23–29 (2001).
171. K. Ito, D. Hallifax, R. S. Obach, and J. B. Houston. Impact of parallel pathways of drug elimination and multiple cytochrome P450 involvement on drug–drug interactions: CYP2D6 paradigm *Drug Metab. Dispos.* **33**:837–844 (2005).
172. B. S. Mayhew, D. R. Jones, and S. D. Hall. An *in vitro* model for predicting in vivo inhibition of cytochrome P450 3A4 by metabolic intermediate complex formation *Drug Metab. Dispos.* **28**:1031–1037 (2000).
173. J. P. O'Donnell, D. K. Dalvie, A. S. Kalgutkar, and R. S. Obach. Mechanism-based inactivation of human recombinant P450 2C9 by the nonsteroidal anti-inflammatory drug suprofen *Drug Metab. Dispos.* **31**:1369–1377 (2003).
174. Y. H. Wang, D. R. Jones, and S. D. Hall. Prediction of cytochrome P450 3A inhibition by verapamil enantiomers and their metabolites *Drug Metab. Dispos.* **32**:259–266 (2004).
175. J. Yang, M. Jamei, K. R. Yeo, G. T. Tucker, and A. Rostami-Hodjegan. Kinetic values for mechanism-based enzyme inhibition: assessing the bias introduced by the conventional experimental protocol *Eur. J. Pharm. Sci.* **26**:334–340 (2005).
176. N. Greene, P. N. Judson, J. J. Langowski, and C. A. Marchant. Knowledge-based expert systems for toxicity and metabolism prediction: DEREK, StAR and METEOR *SAR QSAR Environ. Res.* **10**:299–314 (1999).
177. H. K. Lim, N. Duczak Jr., L. Brougham, M. Elliot, K. Patel, and K. Chan. Automated screening with confirmation of mechanism-based inactivation of CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP1A2 in pooled human liver microsomes *Drug Metab. Dispos.* **33**:1211–1219 (2005).
178. K. M. Bertelsen, K. Venkatakrishnan, L. L. Von Moltke, R. S. Obach, and D. J. Greenblatt. Apparent mechanism-based inhibition of human CYP2D6 *in vitro* by paroxetine: comparison with fluoxetine and quinidine. *Drug Metab. Dispos.* **31**:289–293 (2003).
179. A. Hemeryck and F. M. Belpaire. Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update *Curr. Drug Metab.* **3**:13–37 (2002).
180. K. Venkatakrishnan and R. S. Obach. *In vitro–in vivo* extrapolation of CYP2D6 inactivation by paroxetine: prediction of nonstationary pharmacokinetics and drug interaction magnitude *Drug Metab. Dispos.* **33**:845–852 (2005).

181. T. Prueksaritanont, B. Ma, C. Tang, Y. Meng, C. Assang, P. Lu, P. J. Reider, J. H. Lin, and T. A. Baillie. Metabolic interactions between mibefradil and HMG-CoA reductase inhibitors: an *in vitro* investigation with human liver preparations *Br. J. Clin. Pharmacol.* **47**:291–298 (1999).
182. C. Wandel, R. B. Kim, F. P. Guengerich, and A. J. Wood. Mibefradil is a P-glycoprotein substrate and a potent inhibitor of both P-glycoprotein and CYP3A *in vitro Drug Metab. Dispos.* **28**:895–898 (2000).
183. F. Longuevillede, F. A. Atienzar, L. Marcq, S. Dufrane, S. Evrard, L. Wouters, F. Leroux, V. Bertholet, B. Gerin, R. Whomsley, T. Arnould, J. Remacle, and M. Canning. Use of a low-density microarray for studying gene expression patterns induced by hepatotoxicants on primary cultures of rat hepatocytes *Toxicol. Sci.* **75**:378–392 (2003).
184. M. R. Fielden and T. R. Zacharewski. Challenges and limitations of gene expression profiling in mechanistic and predictive toxicology *Toxicol. Sci.* **60**:6–10 (2001).
185. T. Storck, M. C. von Brevern, C. K. Behrens, J. Scheel, and A. Bach. Transcriptomics in predictive toxicology. *Curr. Opin. Drug Discov. Dev.* **5**:90–97 (2002).
186. F. Boess, M. Kamber, S. Romer, R. Gasser, D. Muller, S. Albertini, and L. Suter. Gene expression in two hepatic cell lines, cultured primary hepatocytes, and liver slices compared to the *in vivo* liver gene expression in rats: possible implications for toxicogenomics use of *in vitro* systems. *Toxicol. Sci.***73**:386–402 (2003).
187. J. F. Waring, G. Cavet, R. A. Jolly, J. McDowell, H. Dai, R. Ciurlionis, C. Zhang, R. Stoughton, P. Lum, A. Ferguson, C. J. Roberts, and R. G. Ulrich. Development of a DNA microarray for toxicology based on hepatotoxin-regulated sequences *EHP Toxicogenomics* **111**:53–60 (2003).
188. R. A. Roth, J. P. Luyendyk, J. F. Maddox, and P. E. Ganey. Inflammation and drug idiosyncrasy-is there a connection? *J. Pharmacol. Exp. Ther.***307**:1–8 (2003).
189. D. P. Williams, N. R. Kitteringham, D. J. Naisbitt, M. Pirmohamed, D. A. Smith, and B. K. Park. Are chemically reactive metabolites responsible for adverse reactions to drugs? *Curr. Drug Metab.* **3**:351–366 (2002).
190. D. C. Liebler and F. P. Guengerich. Elucidating mechanisms of drug-induced toxicity. *Nat. Rev. Drug Discov.* **4**:410–420 (2005).
191. J. Uetrecht. Screening for the potential of a drug candidate to cause idiosyncratic drug reactions *Drug Discov. Today* **8**:832–837 (2003).
192. C. Chen, J. L. Staudinger, and C. D. Klaassen. Nuclear receptor, pregname X receptor, is required for induction of UDP-glucuronosyltranferases in mouse liver by pregnenolone-16 alpha-carbonitrile *Drug Metab. Dispos.* **31**:908–915 (2003).
193. J. P. Nagpal, K. L. Khanduja, R. R. Sharma, S. Majumdar, R. Singh, M. P. Gupta, and S. C. Dogra. The effect of medroxyprogesterone acetate on the hepatic drug-metabolizing enzymes in normal and protein-deficient female rats *Biochem. Med.* **34**:11–16 (1985).
194. H. U. Saarni. Time course of hepatic changes produced by medroxyprogesterone acetate in the rat *Gen. Pharmacol.* **17**:25–29 (1986).
195. L. G. Yengi, Q. Xiang, L. Shen, C. Appavu, J. Kao, and J. Scatina. Application of pharmacogenomics in drug discovery and development: correlations between transcriptional modulation and preclinical safety observation. *Drug metab. Lett* **1**:(2007).
196. I. Kola and J. Landis. Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* **3**:711–715 (2004).
197. M. P. Murphy, M. Bickel, D. Birkett, B. Clement, R. W. Estabrook, J. Gorrod, J. Hinson, P. Kissinger, T. Kunze, P. Maurel, P. Van Bladeren, and C. R. Wolf. History of Xenobiotic Metabolism. http://www.issx.org.