
Drug–Drug Interactions: Designing Development Programs and Appropriate Product Labeling

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Abstract

Drug–drug interactions can represent a major public health issue. Drug metabolism science has evolved to the point where interactions with cytochrome P-450 isozymes can be predicted and potentially avoided or managed, but much work remains to allow accurate prediction of non-P-450 mediated interactions. Based on preclinical data, rational clinical plans can be developed to study potential drug–drug interactions in humans and develop labeling that allows optimal usage of new drugs.

2.1 Introduction

Adverse drug reactions are a major public health concern. It has been estimated that approximately 5% of hospital admissions are related to adverse drug reactions (Kongkaew et al. 2008), although other estimates have placed this value between 3% and 28% (McDonnell and Jacobs 2002). Hospital admissions for adverse drug reactions are highest in elderly subjects who are taking multiple medications (Kongkaew et al. 2008), suggesting drug–drug interactions (DDIs) may contribute to this observation. Indeed, in the elderly, 4.8% of hospital admissions were due to DDIs (Becker et al. 2007). These data suggest DDIs contribute to hospital admissions and health care costs.

On an individual basis, DDIs can have catastrophic and life-threatening consequences. Several high profile drugs have been removed from the market due to DDIs. The antihistamine terfenadine caused QT prolongation, torsades de pointes, and sudden cardiac death in patients who were also receiving CYP3A4 inhibitors. Astemizole and cisapride were removed from the market for similar reasons (Smith and Schmid 2006). The calcium channel blocker mibefradil caused significant DDI's with a number of agents and was removed from the market (Krayenbühl et al. 1999). Most notable were rhabdomyolysis when combined with simvastatin or lovastatin and nephrotoxicity in combination with cyclosporine or tacrolimus. In all of these cases, changes in the product labeling were ineffective in avoiding DDIs in clinical use and thus precluded the continued safe prescribing of these agents.

Given the potential societal and individual impact of drug interactions, assessment of drug interaction potential has been an important aspect

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of drug development for decades. Initially- decisions on which drug interactions were studied were based on the likelihood two drugs would be co-administered in the targeted patient population. This philosophy resulted in a large number of drug interaction studies conducted to support marketing and medical practice, many of which were redundant and may not have been truly necessary or scientifically informative. With increased understanding of the mechanisms of drug transport, metabolism, and elimination, in vitro data have been increasingly used to assess potential risk for drug interaction. The Food and Drug Administration's 2006 draft guidance (Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling) helped codify this concept and laid out strategies for how in vitro data can be used to screen for potential metabolic interactions and on how to use in vitro data to decide which clinical interaction studies should be run.

2.2 Why Use Preclinical Data to Guide Clinical Drug Development

Using in vitro methods to guide a drug interaction program for a new chemical entity (NCE) has a number of advantages. First, in vitro studies can be used to identify drug combinations that may result in large changes in exposure, either for one drug or for both. The observation of a substantial in vitro interaction can be a strong signal indicating an in vivo investigation is warranted. In addition, in vitro data allows the results of in vivo studies to be readily generalized based on the mechanism of interaction. An example of this is evident in the labeling for ZYFLO CR[®] tablets, which contain zileuton as the active ingredient. A number of drug interaction studies were performed (theophylline, warfarin, propranolol, prednisone, ethinyl estradiol, digoxin, phenytoin, sulfasalazine, and naproxen), with clinically significant interactions noted for theophylline, propranolol, and warfarin. The increase in theophylline and propranolol AUC was approximately twofold, while the increase

in R-warfarin AUC was about 22%. Subsequent in vitro work has shown zileuton is an inhibitor of CYP1A2, with minimal effects on other CYP isozymes (Lu et al. 2003). These in vitro data could have been generalized to predict interactions with other CYP1A2 substrates, rather than having discussions in the labeling concerning drugs in the same therapeutic class.

Additional benefits can be gained by limiting the conduct of in vivo drug interaction studies to those where interactions are likely to occur. The decreased cost of drug development is accompanied by a reduction in the generation of data that does not provide additional information beyond that obtained in vitro. In the zileuton example, the in vitro data showing lack of effect on CYP isozymes other than CYP1A2 could have been used to avoid conducting drug interaction studies with drugs that are substrates of other isozymes, most notably CYP3A4 and CYP2C9 (terfenadine, prednisone, ethinyl estradiol, phenytoin, and naproxen).

A recent evaluation of the use of in vitro data on p-glycoprotein inhibition to predict drug interactions with digoxin illustrates this point on a broader basis (Fenner et al. 2009). Clinical drug interaction studies with digoxin were routinely conducted in drug development programs, both before and after the main mechanism for this interaction was elucidated (p-glycoprotein inhibition in the gut and kidney). The primary reason for the routine conduct of this study was digoxin's narrow therapeutic window and the potentially serious consequences of digoxin toxicity. As described by Fenner et al. 93% of 123 digoxin DDI studies examined showed changes in digoxin area under the curve <25%. Thus in the vast majority of studies, the magnitude of change was less than the upper limit typically used for bioequivalence trials and therefore the interaction is not considered clinically relevant. Fenner et al. argue that appropriate in vitro studies of p-glycoprotein inhibition by new drugs, with appropriate cutoff criteria, will substantially lower the number of times DDIs with digoxin would need to be studied clinically. They also argue, for NCEs that are p-glycoprotein substrates and have reasonable therapeutic ranges, drug interactions with p-glycoprotein

would rarely, if ever, require substantial dose adjustment and that these interactions may not require detailed *in vivo* investigation. Hence, the use of *in vitro* models makes sense scientifically and financially.

2.3 Preclinical Assessment of DDI Potential in Drug Development

In order to reduce the risks associated with clinical DDIs, and meet the mutual goal of providing safe and effective medicines to the public, health authorities and pharmaceutical companies have focused on the development of *in vitro* strategies for characterizing metabolism early in the drug discovery process. Evaluation of inhibition and induction of cytochrome P450 enzymes by NCEs is now common practice for clinical candidate selection. With the ready availability of recombinant DNA expressed P450 isozymes, commercially available human hepatocytes and microsomes, known substrates and inhibitors for P450 isozymes, and rapid liquid chromatography/mass spectrometry (LC-MS) techniques, early assessment enables an understanding of the structural features leading to inhibition (e.g. structure-activity relationships, SAR). This section of the

chapter will focus on the most recent *in vitro* methods for assessing DDIs, as it pertains to cytochrome P450 inhibition, induction of cytochrome P450, and transporter DDI (e.g. p-glycoprotein). In addition, methods for predicting clinical DDI using *in vitro* data, as well as the future direction of screening strategies for assessing clinical DDI risk, will be discussed.

2.4 Inhibition of Cytochrome P450 and Other Drug-Metabolizing Enzymes

In humans, the biotransformation of xenobiotics is most often catalyzed by the cytochrome P450 family of drug-metabolizing enzymes. It is well understood that the P450 isozymes 3A4, 2D6, 2C9, 2C19, and 1A2 contribute to some degree to the metabolism of >90% of marketed drugs (Fig. 2.1, Wienkers and Heath 2005; Guengerich 2008). Consequently, most labs supporting preclinical pharmacokinetics and metabolism in drug discovery (so-called ADME groups) screen synthesized test compounds against these enzymes in high throughput inhibition assays. The format of these assays has evolved substantially over the years, as the balance between

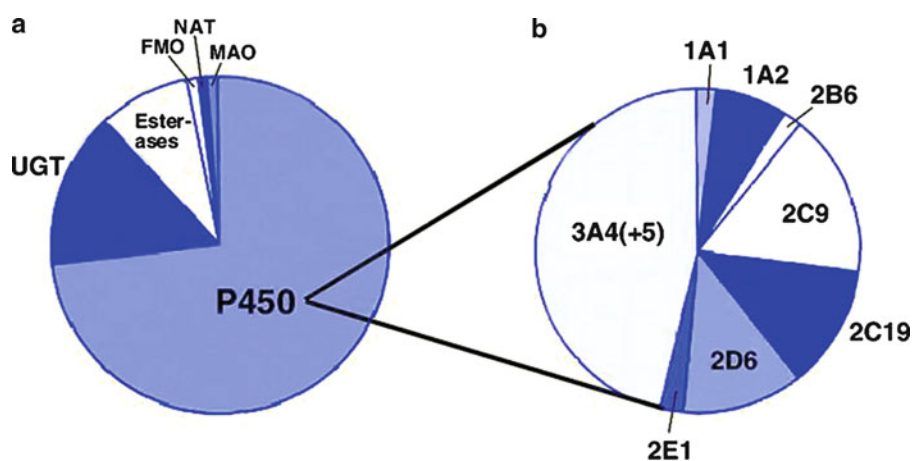


Fig. 2.1 Contributions of enzymes to the metabolism of top 200 prescribed drugs. The results are from a study of Pfizer drugs (Williams et al. 2004). (a) Fraction of metabolic clearance catalyzed by various human drug-metabolizing enzymes. UGT uridine glucuronosyltransferase,

FMO flavin-containing monooxygenase, NAT *N*-acetyltransferase, and MAO monoamine oxidase. (b) Fractions of P450 oxidations on drugs catalyzed by individual P450 enzymes. Reprinted with permission from review in *Chemical Research in Toxicology* (Guengerich 2008)

generation of robust predictive data and high throughput to support rapid cycle times for discovery chemistry has been a continuous challenge. Traditional academic methods for testing inhibition of cytochrome P450 enzymes using HPLC with UV or fluorescence detection were simply unable to accommodate the high-throughput screening (HTS) requirements of drug discovery to evaluate hundreds or even thousands of drug molecules on a weekly basis. Instead, assay technologies that enable improved throughput at a low cost have been developed over the years, including the use of nonselective fluorogenic P450 probe substrates (resorufins, coumarins, quinolines, etc.) and 96 or 384 plate-reading techniques (Crespi and Stresser 2000; Miller et al. 2000). Despite the clear advantage of ultra-high throughput, the fluorogenic probe approach is fraught with issues such as the potential for test compounds to interfere with or quench the fluorescence signal (unpublished observations), and the fact that fluorogenic probes are not “drug-like,” in that they are not used in DDI trials, making it more challenging to extrapolate this type of in vitro data to the clinical setting. In addition, studies comparing the use of fluorogenic

probes with traditional LC/MS methods have found poor correlations, especially for CYP3A4 (Cohen et al. 2003, Fig. 2.2; Bell et al. 2008), raising concerns about the predictability of these methods. Despite the aforementioned issues with using fluorogenic probes, some pharmaceutical/biotechnology companies are still willing to accept this risk in an early screening environment to take advantage of throughput capabilities. Alternatively, another approach, developed to circumvent the potential for interference with fluorescence signal while maintaining high throughput, is the use of luminogenic cytochrome P450 inhibition assays (Cali et al. 2006).

With substantial advances in bioanalytical technology in recent years, ADME groups within the pharmaceutical industry have moved toward the use of clinically relevant and selective probe substrates of the P450 enzymes, according to guidelines generated by the FDA (Table 2.1), using LC-MS. Assay incubations are typically conducted in human liver microsomes (HLMs) pooled from at least 50 donors. This HLM system better represents of the intact liver compared to recombinantly expressed P450 enzymes (e.g. Supersomes[®] from BD-Biosciences or

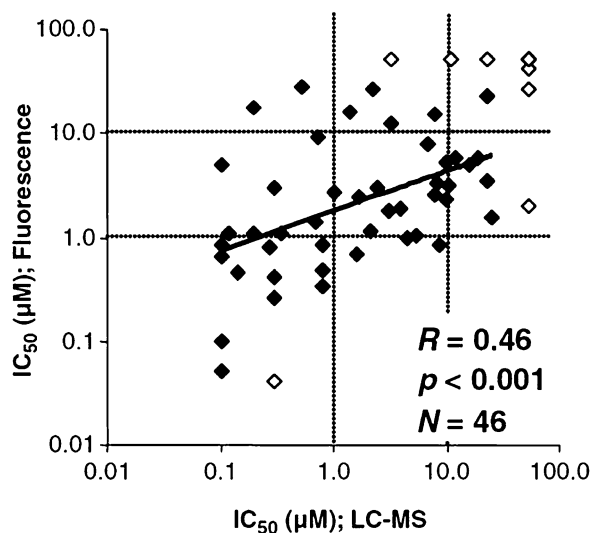


Fig. 2.2 Comparison of IC_{50} s generated using recombinant CYPs with fluorescent probe (dibenzylfluorescein, DBF) to HLM with LC/MS detection with traditional probe substrate (midazolam). Dotted lines define the IC_{50} limits used for DDI risk binning (<1 μ M, high risk;

1–10 μ M, moderate risk; >10 μ M, low risk). Shaded areas represent concordance in binning between the two assays approaches. Reprinted with permission from *Journal of Biomolecular Screening* (Bell et al. 2008)

Table 2.1 Substrates and positive controls for assessing DDI in vitro

Enzyme	Activity	Positive control inhibitor (recommended concentration)	Positive control time-dependent inactivator (TDI)
CYP1A2*	Phenacetin O-deethylase	Furafylline (10 μ M)	Furafylline
CYP2B6	Bupropion hydroxylase	PPP (2-phenyl-2-(1-piperidinyl)propane)	PPP (2-phenyl-2-(1-piperidinyl)propane)
CYP2C8*	Amodiaquine N-deethylase	Montelukast (0.1 μ M)	thioTEPA
CYP2C9	Diclofenac 4'-hydroxylase (S)-warfarin 7-hydroxylase	Sulfaphenazole (10 μ M)	Tienilic acid
CYP2C19	(S)-Mephenytoin 4'-hydroxylase	(+)-N-Benzyl-nirvanol (10 μ M)	Ticlopidine
CYP2D6*	Dextromethorphan O-demethylase	Quinidine (1.0 μ M)	Paroxetine
CYP2E1	Chlorzoxazone 6-hydroxylase	Diethyldithiocarbamate (10 μ M)	Diethyldithiocarbamate
CYP3A4	Midazolam 1'-hydroxylase Testosterone 6 β -hydroxylase Felodipine dehydrogenase	Ketoconazole (1.0 μ M)	Verapamil, erythromycin, TAO

Baculosomes[®] from Invitrogen), which are coexpressed with the electron-shuttling co-proteins oxidoreductase and cytochrome b5 at nonphysiological ratios. To meet the demands of higher throughput in a drug discovery environment, a critical development has been ultraperformance liquid chromatography/tandem mass spectrometry (UPLC-MS-MS), where the use of sub-2 μ m porous particle LC coupled to high flow rates results in sufficient resolution for fast separation methods (Fig. 2.3, Plumb et al. 2008). In addition, the bioanalysis and/or the incubation itself may be conducted in a cocktail assay format (Testino and Patonay 2003; Kim et al. 2005; Dixit et al. 2007), and early “tier 1” ADME screening assays may be performed at one or two concentrations of test compound, with data reported as percent (%) inhibition. Algorithms for estimating an IC_{50} based on a single-point inhibition assessment have also been employed as a screening approach to P450 inhibition (Gao et al. 2002).

One concern with conducting P450 inhibition assays with a “cocktail” of substrates, is “crosstalk” or nonselective interactions of the probe substrates with other P450 enzymes, potentially compromising the fidelity of the data. To address this concern, multiple studies have been conducted recently comparing IC_{50} values generated from both singlet and cocktail assays (Zientek et al. 2008; Youdim et al. 2008). In methods reported by Youdim et al. a 384-well cocktail

assay evaluating inhibition of CYP1A2 (tacrine), 2C9 (diclofenac), 2C19 (S-mephenytoin), 2D6 (dextromethorphan), and 3A4 (midazolam) was developed with an LC/MS method run time on the order of 1 min. Interestingly, comparison of IC_{50} values (geometric mean) generated with this miniaturized cocktail method closely resembled those generated in the individual P450 inhibition assays tested, with a slight upward bias in the cocktail results. In further studies by Zientek et al. the enzyme kinetics ($K_{m,app}$ and V_{max}) of the selective probe substrate reactions were compared and while it was found there was minimal shift in $K_{m,app}$ values between singlet and cocktail formats, the velocities measured in the cocktail probe substrate format were consistently lower than those measured in the single substrate format (Fig. 2.4, Zientek et al. 2008). Causes for this observation are unclear at this time, but the authors speculate it may be the result of P450 isoform competition for oxidoreductase, as reported by Cawley et al. (1995). Despite these velocity differences, the accuracy of the IC_{50} values compared to those generated in the singlet assay format did not appear to impact decisions made based on their results. One strategy that seems to be the result of some of the difficulties just described is the elimination of CYP2C19 from early screening. Despite being a selective probe for CYP2C19, (S)-mephenytoin is the most problematic probe substrate within the

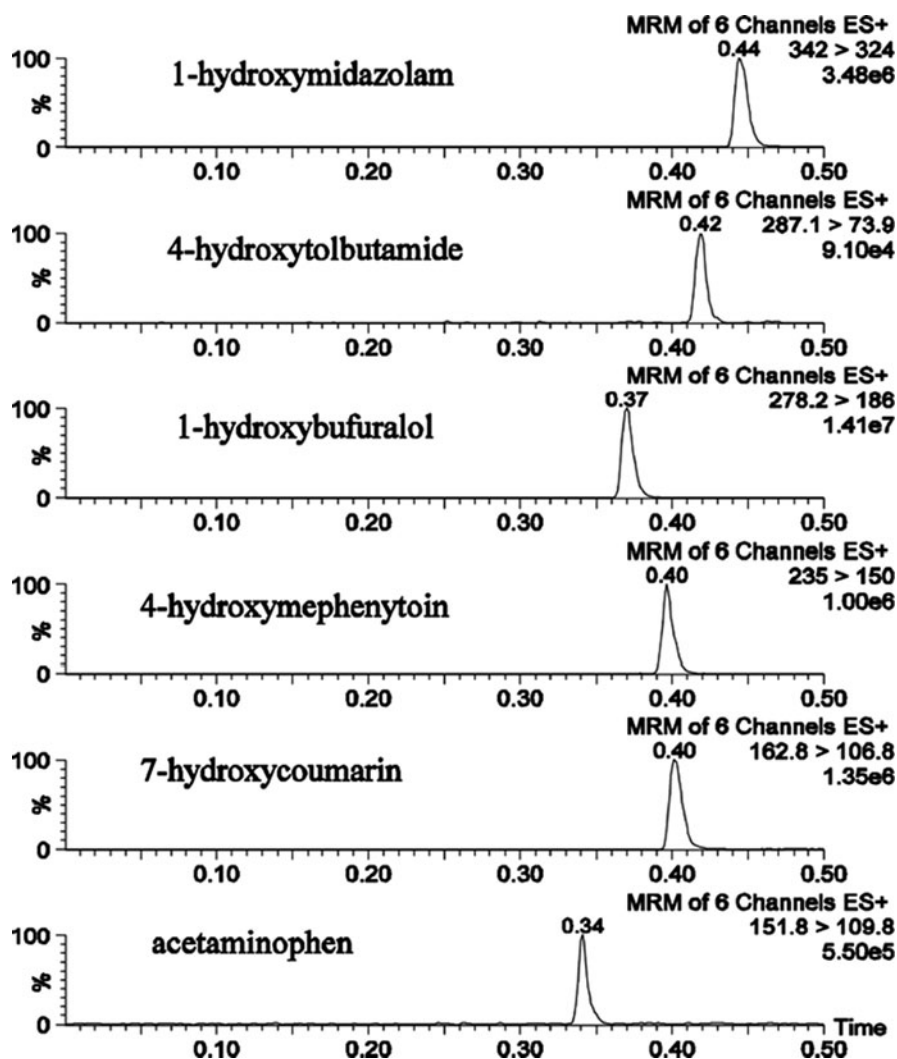


Fig. 2.3 LC/MS/MS analysis of six analytes commonly used in cytochrome P450 inhibition screens in a cocktail format. Use of UPLC/MS/MS methods with a 2.1×50 mm ACQUITY BEH C18 $1.7 \mu\text{m}$ column enables

ultra-high throughput (0.5 min/sample), while maintaining analyte resolution. Reprinted with permission from *Rapid Communications in Mass Spectrometry* (Plumb et al. 2008)

cocktail of substrates, as it is metabolized slowly, and the metabolite monitored (4-hydroxymephenytoin) does not ionize well by mass spectrometry, resulting in an insensitive assay. Identification of a more optimal selective probe substrate for CYP2C19 would be of great benefit. Nonetheless, from these reports, it is clear that cocktail inhibition assays are sufficiently reliable for identifying potent inhibitors of P450 in early stage drug discovery, which is ultimately the

intent of ADME screening. However, at this time, with some of the uncertainties about the cause of the minor differences between cocktail and singlet IC_{50} inhibition assays, it is not recommended to use cocktail IC_{50} data for labeling purposes until additional work is performed to validate the accuracy and use of in vitro cocktail assays beyond screening. Validated singlet IC_{50} assays are recommended for drug labeling, in which the assay conditions have been optimized

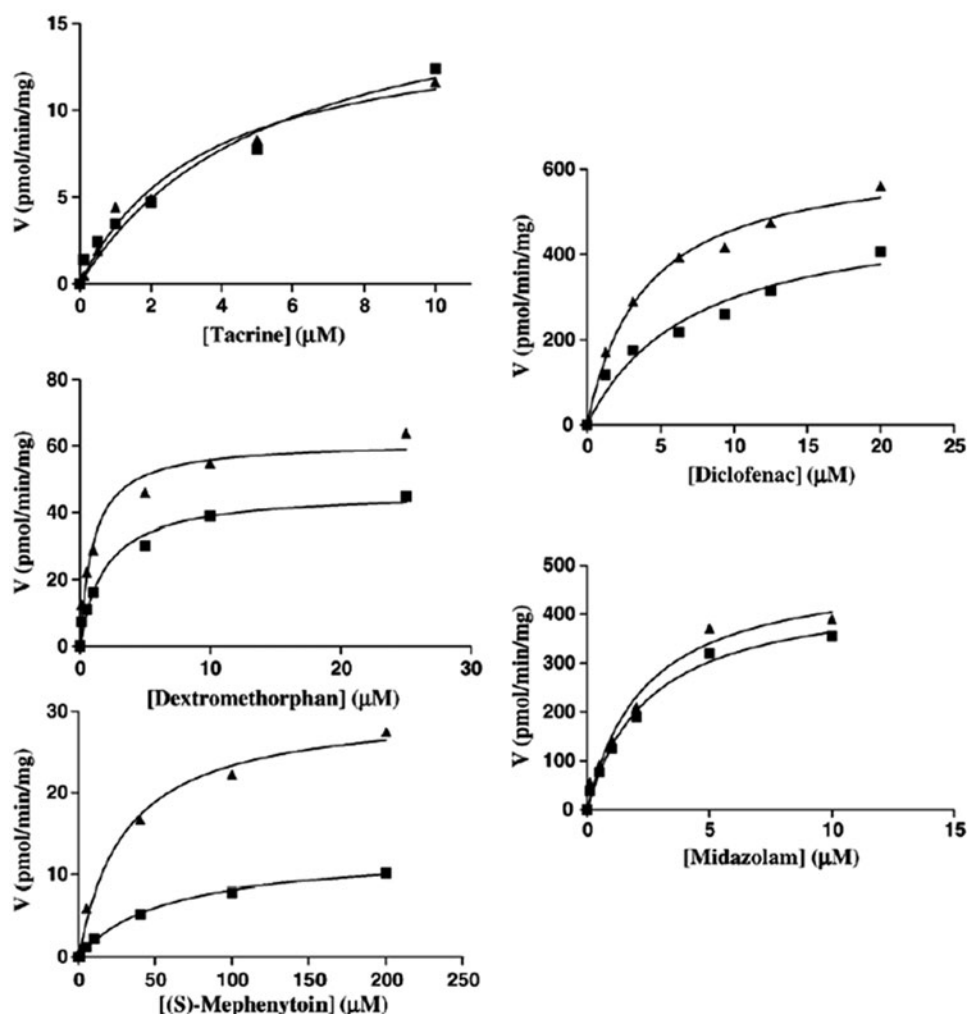


Fig. 2.4 Direct comparison of velocity (pmol/min/mg) versus substrate (μM) enzyme kinetic plots for five traditional P450 probe substrates (tacrine, CYP1A2; dextromethorphan, CYP2D6; (S)-mephenytoin, CYP2C19; diclofenac, CYP2C9; and midazolam, CYP3A4) in a cock-

tail incubation (filled square) and singlet substrate/P450 incubations (filled triangle). Rates are generally lower when incubations are conducted in the cocktail format. Reprinted with permission from *Journal of Pharmacological and Toxicological Methods* (Zientek et al. 2008)

for low protein concentrations to minimize non-specific protein binding (where unbound drug concentration approximates total drug in the incubation) and incubation times are set to maintain linear Michaelis-Menten steady-state kinetics ($<10\%$ consumption of substrate) (Walsky and Obach 2004). It is much more challenging to optimize cocktail assays to maintain these critical conditions, with the selective probe substrates demonstrating such drastic differences in metabolic rates. In addition, multiple probe substrates

for complex P450 enzymes such as CYP3A4 (midazolam, testosterone, and felodipine), where inhibition has been demonstrated to be substrate-dependent in some cases (Wang et al. 2000), must be used for a comprehensive assessment of inhibition of this important drug-metabolizing enzyme.

In addition to screening for inhibition of the major P450 enzymes, Walsky et al. (2005, 2006) have concluded that CYP2C8 and CYP2B6 may also be of potential risk of being inhibited by new

NCEs. Screening efforts discovered a hit rate against CYP2C8 (>50% inhibition at 30 μM) of ~23% when testing >200 compounds in one discovery program. In particular, montelukast, a leukotriene receptor antagonist used in the treatment of asthma, was found to be an especially potent inhibitor of CYP2C8 ($\text{IC}_{50} = 0.02 \mu\text{M}$). In similar efforts, 30 compounds were found to inhibit CYP2B6 by greater than 50% at 30 μM , most notably clopidogrel and ticlopidine, with an IC_{50} of 0.02 and 0.15 μM , respectively (Walsky et al. 2006). While CYP2C8 does not metabolize a large percentage of drugs on the market, testing for inhibition of this enzyme may become of higher importance, especially for discovery programs working in a chemical space outside the Lipinski Rule of 5 guideline for molecular weight (>500 amu), as the size of the CYP2C8 active site rivals that of CYP3A4 (Schoch et al. 2004), metabolizing large molecules such as the chemotherapeutic agent TAXOL[®] (Rahman et al. 1994). These findings suggest compounds should be tested for inhibition of CYP2C8 and CYP2B6 at some time prior to dosing patients who may be taking medications metabolized by these enzymes.

An intense area of research within the pharmaceutical industry in recent years is time-dependent inhibition (TDI) of cytochrome P450 enzymes, where an increase in the extent of

inhibition is observed following pre-incubation with the test inhibitor. This phenomenon may be the result of generation of inhibitory metabolites generated in situ, or mechanism-based inactivation (MBI), where the metabolism of a substrate to a reactive electrophilic species (e.g. bioactivation) leads to either covalent modification of a nucleophilic amino acid residue in the P450 active site or to the heme moiety itself (Ortiz de Montellano 2005). MBI was originally described by Silverman and George (1988) and there are many mechanistic studies that must be performed for a compound to qualify as a MBI. An alternative mechanism of inactivation is formation of what is termed a metabolite-inhibitory complex (MIC), where an intermediate species such as nitroso or carbene noncovalently complexed with the heme iron, also referred to as a pseudoirreversible mechanism because of the tight nature of this complex (Ortiz de Montellano 2005). Whether or not MIC formation is the operable mechanism of TDI can easily be determined by monitoring an increase in absorbance 448–455 nm on a spectrophotometer (Jones et al. 1999; Hutzler et al. 2006). For example, Fig. 2.5 demonstrates a typical MI complex formed when troleandomycin (TAO) is incubated with recombinant CYP3A4 Supersomes[®], with a pronounced time-dependent increase in absorbance at ~455 nm. Regardless of mechanism, the end

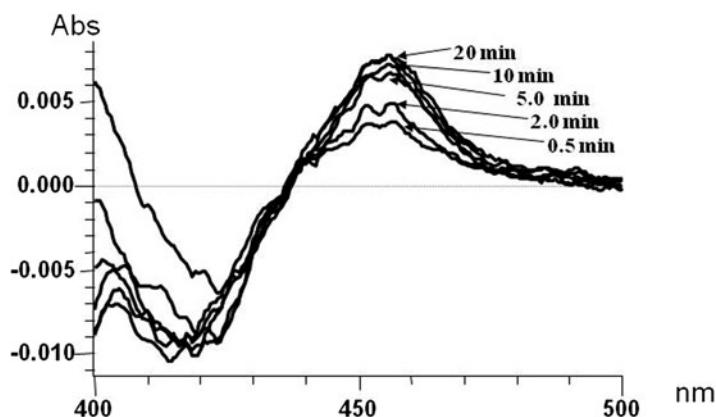


Fig. 2.5 Formation of metabolite-inhibitory complex (MIC) with troleandomycin, a known time-dependent inhibitor of CYP3A4, when incubated with 0.1 pmol/ μL of CYP3A4 Supersomes[®]. There is a clear time-dependent

increase in absorbance at 455 nm, indicative of MIC formation, in this case due to nitroso intermediate complexing with heme iron. Spectra were collected using a Hitachi U3300 dual-beam spectrophotometer

result from a pharmacokinetic perspective is likely similar: potential increase in exposure of a co-administered substrate of the impacted metabolic pathway. Failure to consider this mechanism may lead to drastic underestimation of the magnitude of a clinical DDI.

The mechanism of inactivation may also be relevant as it pertains to drug toxicity. It is known, for example, that inactivation of CYP2C9 by tienilic acid results in covalent modification of the 2C9 apoprotein (Lopez-Garcia et al. 1994), which is proposed to result in an immunogenic response, providing a strong link to observed hepatotoxicity (Lecoeur et al. 1994). There are numerous reviews on the subject of clinical drugs that behave as mechanism-based inactivators of CYP3A4 (Zhou et al. 2004; Hollenberg et al. 2008), as well as comprehensive reviews that summarize the functional groups typically susceptible to bioactivation (Fontana et al. 2005; Kalgutkar et al. 2007). In addition to reviews in the literature, many pharmaceutical companies have also developed their own database of “structural alerts,” summarizing functional groups that may undergo metabolic activation. These alerts can be used by drug metabolism scientists and medicinal chemists as a guide for avoiding or engineering out the risk of TDI of P450 when designing small molecules for therapeutic targets.

The *in vitro* assay design for assessing the kinetics of TDI is quite complex. Briefly, a primary incubation, containing the enzyme system of choice (typically recombinantly expressed P450s or human liver microsomes), test inactivator at various concentrations, and NADPH (or NADPH regeneration system) is initiated, and aliquots from this incubation are subsequently taken at various times (typically up to 30 min) and diluted into a secondary incubation containing a probe substrate at a concentration ≥ 4 -fold K_m to measure residual enzyme activity. It is imperative that if HLMs are used as the enzyme source in the primary assay, a low protein concentration (mg/ml) be used to minimize nonspecific binding of the test inactivator, which may lead to false negative results for highly bound drugs (unpublished observations). From

these kinetic studies, the ability to use *in vitro* time-dependent inhibition (TDI) data to predict the magnitude of a clinical DDI requires the estimation of kinetic parameters k_{inact} (the maximal rate of inactivation) and K_I (the concentration of inactivator resulting in half the maximal rate of inactivation), described in the following equation:

$$k_{\text{obs}} = k_{\text{inact}} \cdot [I]/K_I + [I].$$

As a result of this complex assay design, the pharmaceutical industry has sought alternative screening approaches to enable higher throughput assessment of TDI earlier in the drug discovery process, such as abbreviated inactivation studies with one or two concentrations of test compound and a single pre-incubation time to assess percent loss in activity over a fixed period of time relative to a solvent control (Watanabe et al. 2007). Interpreting data from TDI screening studies is often challenging, but the general consensus is if a NCE causes more than 20–25% loss of activity following a 30 min pre-incubation, then follow-up studies to characterize the kinetics of inactivation may be warranted (Grimm et al. 2009). In addition, IC_{50} -shift studies, where probe substrate is added after a pre-incubation with multiple concentrations of test compound, may be conducted to assess the risk for TDI. While the debate about the predictive ability of IC_{50} -shift data is on-going, reports have shown a good relationship between shifted IC_{50} (measured after pre-incubation) and the k_{inact}/K_I ratio (Obach et al. 2007; Berry and Zhao 2008; Grim et al. 2009), which suggests IC_{50} -shift assays may be useful not only for identifying potential TDI, but also for estimating the kinetics of inactivation.

As previously mentioned, accurate estimation of the kinetics of inactivation (k_{inact} and K_I) is critical to predicting the magnitude of any potential clinical DDI. As demonstrated by Ghanbari et al. (2006), following an exhaustive effort to assemble and compare incubation conditions from literature reports of mechanism-based inactivators, the *in vitro* assay design and subsequent data analysis varies considerably between labs. In particular, protein concentration in the

primary assay, dilution factor into the secondary activity assay, pre-incubation times, correction for decreased enzyme activity in the absence of inhibitor, and probe substrate concentration relative to K_m in the secondary activity assay, all critical to the accurate estimation of k_{inact} and K_I , were identified as being variable. An additional report also focused on assay design for TDI (Van et al. 2006), and should be referred to when questioning the impact on estimation of inactivation kinetics for the purpose of predicting clinical DDIs (discussed in Sect. 2.4). An important point worth mentioning is that on occasion, biphasic inactivation plots may be observed, as was shown by Hutzler et al. with PH-302, an inactivator of CYP3A4 (Hutzler et al. 2006, Fig. 2.6). When biphasicity is observed, it is the initial linear kinetic phase that should be considered and modeled, similar to metabolic stability data from substrate depletion studies for estimating intrinsic clearance (Cl_{int}), so that one is not at risk for underpredicting the magnitude of a DDI. Thus, it is critical to include sufficiently early time points (<5 min) when conducting in vitro TDI studies to enable characterization of the initial inactivation kinetic phase. Additional studies that may be conducted in order to understand the mechanism of time-dependent inhibition include the following (but are not required from a regulatory perspective), which

were discussed in a recent article describing results of an industry-wide PhRMA survey on assessment of time-dependent inhibition of drug metabolizing enzymes (Grimm et al. 2009):

1. Estimation of partition ratio (a measure of biochemical efficiency);
2. Dialysis or microsome washing to establish reversibility;
3. Ferricyanide treatment or spectral studies to diagnose metabolite inhibitory complex (MIC) formation; and
4. Protection of inactivation by addition of competing substrate/inhibitor, reduced glutathione (GSH), and reactive oxygen scavengers such as catalase and superoxide dismutase.

When a compound has advanced to the point of clinical candidate nomination, yet still carries with it the apparent risk of P450 TDI, it is critical to consider all of the clearance pathways of the time-dependent inhibitor, particularly metabolic. Raloxifene serves as a classic example of how this may be critical to a comprehensive risk assessment of time-dependent inactivation of P450. In vitro systems where metabolism is essentially forced to proceed via P450 (e.g. recombinant P450 or human liver microsomes), represent the most sensitive system for evaluating TDI, especially in recombinant systems such as CYP3A4 Supersomes[®], where the catalytic activity is known to be markedly higher than

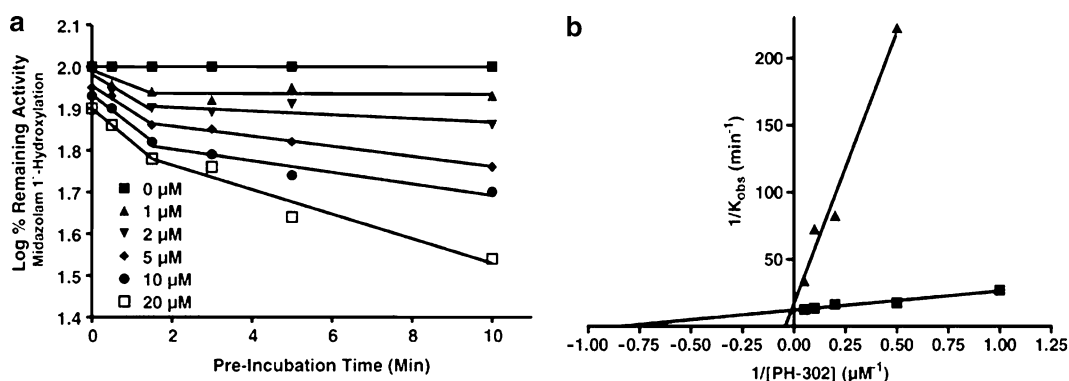


Fig. 2.6 Demonstration of biphasic time-dependent inhibition (TDI) data. (a) Log % remaining activity versus pre-incubation time (0–10 min), illustrating time-dependent inhibition (TDI) of CYP3A4 by PH-302 (0–20 μM). (b) Data from both the initial and terminal phases were subsequently subjected to a

Kitz–Wilson analysis and shown to produce distinct inactivation kinetic parameters (initial phase $k_{\text{inact}} = 0.08 \text{ min}^{-1}$, $K_I = 1.2 \text{ μM}$, terminal phase $k_{\text{inact}} = 0.06 \text{ min}^{-1}$, $K_I = 23.8 \text{ μM}$). Reprinted with permission from *Chemical Research in Toxicology* (Hutzler et al. 2006)

human liver microsomes. Raloxifene is reported to cause time-dependent inhibition of CYP3A4, and is also bioactivated to reactive metabolite species in vitro (Chen et al. 2002; Pearson et al. 2007), yet remains a relatively safe drug. Raloxifene is predominantly cleared by glucuronidation in the gut (Dalvie et al. 2008), and this metabolic clearance is proposed to protect the liver from high exposure to raloxifene. While this may serve as an extreme case, it points to the importance of careful consideration of the complete distributional properties of any drug that shows time-dependent inhibition in early screens. In an effort to account for the complete metabolic pathways of compounds that appear to be time-dependent inhibitors of CYP3A4, inactivation assays in hepatocytes have been conducted and reported (Zhao et al. 2005; McGinnity et al. 2006; Zhao 2008).

2.5 Reaction Phenotyping and Victim DDIs

Reaction phenotyping is the experimental procedure by which one attempts to identify which human enzymes contribute to the metabolism of a NCE, including estimation of relative contributions (e.g. fraction metabolized, f_m) of enzymes to the overall metabolic clearance (Williams et al. 2003). A thorough understanding of the metabolic pathways of a drug molecule is critical to the prediction of pharmacokinetic DDIs, where inhibition of a metabolic pathway by a co-administered inhibitor (called the perpetrator) results in increased exposure of the victim drug (e.g. “victim” drug–drug interaction). Two drugs withdrawn from the market due to DDIs and subsequent unacceptable safety profiles are terfenadine and cerivastatin. Reaction phenotyping information is also useful for the prediction of interindividual variability in drug exposure in the clinic, especially relevant when polymorphic enzymes such as CYP2C9, 2C19, 2D6, and 3A5 contribute significantly to overall clearance, shown to impact the clinical exposure of drugs such as celecoxib (Tang et al. 2001), sertraline (Wang et al. 2001), and metoprolol (Ismail and

Teh 2006) in poor metabolizers. CYP2B6 has also been recently described as the most polymorphic P450 in humans (Zanger et al. 2007), and metabolizes many relevant therapeutics such as efavirenz, nevirapine, and bupropion. Other non-P450 drug metabolizing enzymes shown to be polymorphically expressed include UGT1A1 and N-acetyl transferase 2 (NAT2) (Tomalik-Scharte et al. 2008), however, the science behind non-CYP DDIs generally lags behind the P450 enzymes. As a result of the aforementioned examples, there is a clear need to understand the potential variability in efficacy and toxicity of all new drugs, and thus, it is a requirement from global regulatory agencies that the metabolic pathways of an NCE be characterized prior to submission of any new drug application (NDA).

As indicated in a recent review by Zhang et al. (2007), it is the fraction of total clearance (CL) by metabolism (f_m) and contribution of each individual CYP to total CYP-mediated metabolism ($f_{m,CYP}$) that determines the magnitude of a drug interaction, whether it is by chemical inhibition or compromised metabolism due to polymorphic expression of the drug-metabolizing enzyme.

$$CL_{\text{total}} = CL_{\text{hepatic}} + CL_{\text{renal}} + CL_{\text{nonhepatic}}$$

Typically, the likelihood of a victim DDI is reduced when an NCE is cleared <60% by any one metabolic pathway, especially a polymorphically expressed drug metabolizing enzyme. As shown in Fig. 2.7, the magnitude of DDI (AUC_i/AUC) increases substantially as the fraction metabolized ($f_{m,CYP}$) exceeds 0.6.

“Definitive” reaction phenotyping can only be performed with a radiolabeled human ADME study, which often is not performed until late in drug development after a drug candidate has demonstrated some measure of safety and effectiveness in patients (e.g. after “proof-of-concept”). As a result, reaction phenotyping studies to support early clinical plans must be done using non-labeled (e.g. cold) test drug, and often without the advantage of having authentic metabolite standards. A traditional approach in discovery would include substrate depletion methods in the in vitro system of choice (e.g. human liver

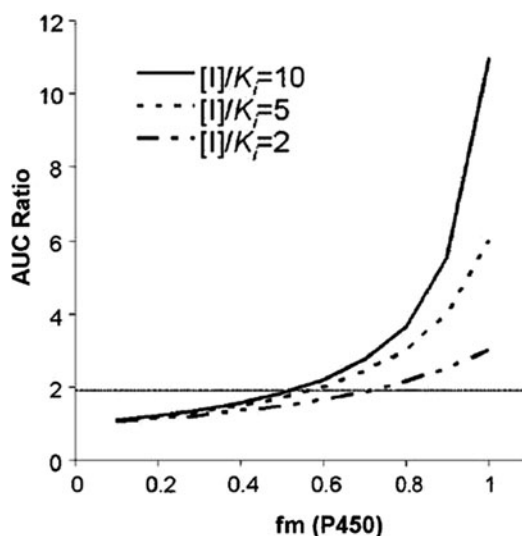


Fig. 2.7 Simulated effect of fraction metabolized (f_m) on the change in AUC ratio (AUC of victim drug in presence of co-administered inhibitor/AUC minus inhibitor). The AUC ratio begins to drastically increase with increase in f_m of victim drug, especially when the $[I]/K_i$ ratio exceeds 2 (e.g. when in vivo inhibitor concentration $> K_i$). Reprinted with permission from *Drug Metabolism and Disposition* (Rock et al. 2008)

microsomes with 1 μM test compound), where selective chemical inhibitors or antibodies of the individual P450s would be coincubated to determine the effect on the in vitro intrinsic clearance (Cl_{int}). Table 2.1 includes recommended P450 inhibitors to use in reaction phenotyping studies, as well as recommended concentrations to optimize selectivity. Complete inhibition by chemical inhibitors while maintaining selectivity has proven to be challenging, which complicates interpretation of reaction phenotyping data. Rock et al. (2008) report that typically, maximal inhibition of CYP3A activity with a chemical inhibitor such as ketoconazole is only ~80%. They showed inhibition $>99\%$ could be achieved by including both CYP3A chemical inhibitor and antibody, leading to improved accuracy in estimating fraction metabolized. Along those same lines, the use of 1-aminobenzotriazole (1-ABT) as a nonselective P450 inhibitor in phenotyping studies to decipher P450 from non-P450 mediated metabolism has recently been called into question. Linder et al. (2009) demonstrated

that while 1-ABT does inhibit all P450 enzymes, it is only somewhat selective and is especially weak against CYP2C9.

In order to effectively use the substrate depletion approach, there should be sufficient depletion of substrate to measure a difference in Cl_{int} in the presence of an inhibitor. Often, drug molecules are slowly metabolized in HLMs (half-life > 60 min), and thus, alternative methods must be employed, such as the use of recombinant P450 enzymes (rCYPs) with the appropriate scaling factors included for predicting $f_{m,CYP}$. For example, use of an intersystem extrapolation factor (ISEF), which accounts for differences in enzymatic activity between the recombinant system and human liver microsomes, as well as the liver abundance of the CYP in question, has been recommended (Proctor et al. 2004).

Monitoring for metabolite formation may also be a valuable approach, as this is often easier to observe than depletion of substrate. Figure 2.8 demonstrates how use of rCYPs and metabolite formation in a single experiment for slow-turn-over compounds may be complementary, as formation of metabolites M1 and M2 were both inhibited by coincubation with 1 μM ketoconazole, and were also shown to be formed with rCYP3A4. If predictions of $f_{m,CYP}$ using data from chemical and/or antibody inhibitors and rCYPs leads to conflicting results, then a third approach such as correlation analysis is recommended. A more detailed description of reaction phenotyping methods has been provided by Wienkers and Stevens (2003).

2.6 Induction of Cytochrome P450 and DDIs

Induction of cytochrome P450 enzymes is also of concern for new molecular entities in drug development, as inducers of P450 enzymes are known to increase drug elimination and cause decreased exposure, resulting in the potential for altered pharmacodynamic profile, and eventual therapeutic failures. For example, acute transplant rejection with cyclosporine and failure of oral contraceptives when co-administered with

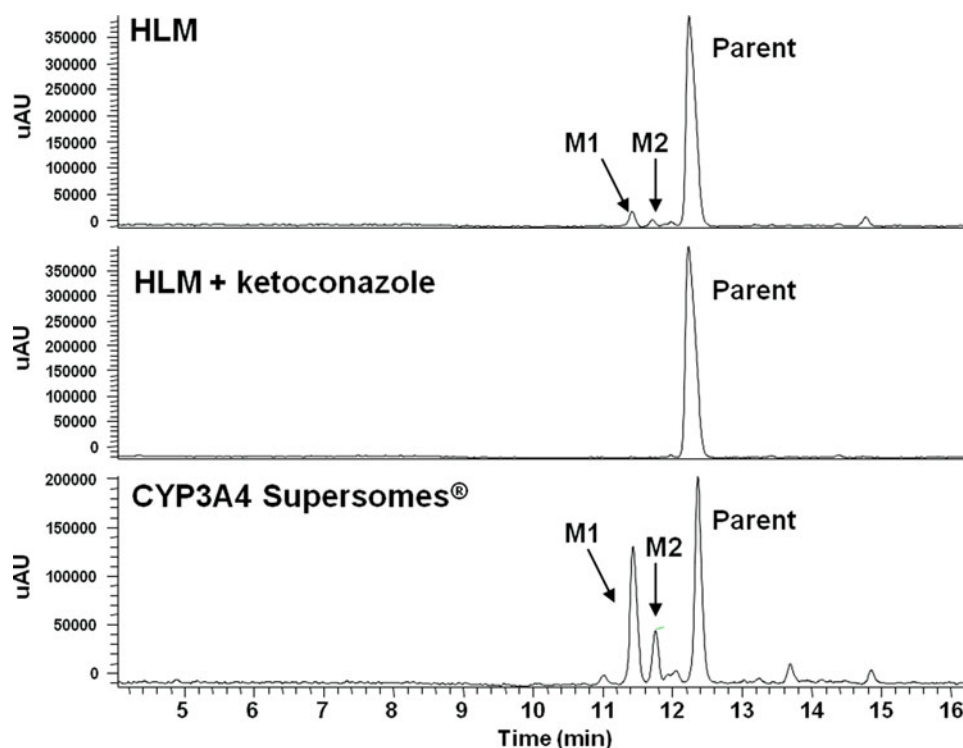


Fig. 2.8 Chromatogram illustrating the value of metabolite formation approach when conducting in vitro reaction phenotyping studies with low-turnover drug molecules, which precludes the use of traditional approaches such as incubation with specific chemical/antibody inhibitors

(unpublished internal data). This figure demonstrates formation of *M1* and *M2* metabolites in both HLMs and recombinant CYP3A4 Supersomes®, and also inhibition of metabolite formation in HLMs by incubation with ketoconazole, a known potent inhibitor of CYP3A4

rifampicin has been reported (Hebert et al. 1992; LeBel et al. 1998). In addition, the potential for toxicity due to increase in reactive metabolite formation exists when induction of an enzyme involved in a bioactivation metabolic pathway occurs (e.g. acetaminophen and hepatotoxicity). Notable inducers of cytochrome P450 include rifampin, phenobarbital, phenytoin, ritonavir, carbamazepine, and St. John's Wort, an herbal treatment for mild to moderate depression (Luo et al. 2004). During the past decade, substantial advances have been made in the technology for investigating induction of cytochrome P450 enzymes. Thus, a broader understanding of the mechanisms regulating expression of drug metabolizing enzymes (and transporters) is now in place. It is now widely accepted that induction of CYP3A4 expression is initiated by binding and transactivation of the nuclear receptor

human pregnane X receptor (hPXR) (Lehmann et al. 1998; Bertilsson et al. 1998). In addition, CYP3A7, CYP2B6, CYP2C8, CYP2C9, UGT1A1, MDR1 (p-glycoprotein), BSEP, and MRP2 appear to be inducible by the transactivation of hPXR (Sinz et al. 2006; Olinga et al. 2008). Constitutive androstane receptor (CAR), glucocorticoid receptor (GR), and aryl hydrocarbon receptor (AhR) have all been shown to play a role in the induction of CYP enzymes (Lin 2006).

Due to the prevailing role of CYP3A4 in elimination of drug molecules, there is increased potential for clinically significant DDIs when expression of this DME is modulated. Thus, the drug industry has devoted much attention to implementation of in vitro assays to evaluate the potential for NCEs to induce CYP3A4, despite the fact that the number of drugs causing metabolic induction in patients appears to be

small (Smith 2000). The gold standard approach for investigating induction potential is the use of primary cultures of human hepatocytes (Lin 2006), while cryopreserved hepatocytes have also been shown to produce similar induction response (Roymans et al. 2005). However, the difficulty in acquiring quality hepatocytes on a routine basis is extremely challenging. To address this, binding and reporter gene assays, which are amenable to higher throughput, have been developed for use in early drug discovery (Jones et al. 2002; El-Sankary et al. 2001). Despite rapid throughput, the disadvantage of the PXR binding assay is that it cannot distinguish between agonists and antagonists, while reporter gene assays only test one mechanism of induction. Nonetheless, comparison of activation of human PXR in a reporter gene assay to induction observed in human hepatocytes resulted in a reasonable correlation (Luo et al. 2002). In a separate study, activation of PXR was shown to be indicative of induction signal in the clinic (Cui et al. 2008), suggesting reporter gene assays may be suitable in an early drug discovery screening paradigm. Lastly, Sinz et al. conducted an extensive study with 170 xenobiotics in a hPXR transactivation assay, and were able to take a hit rate of 54% and conclude only 5% the compounds tested were likely to induce CYP3A4 clinically after consideration of therapeutic C_{\max} , distribution, route of administration, dosing regimen, liver exposure, and potential to inhibit CYP3A4 (Sinz et al. 2006), pointing to the risk of overinterpreting results from reporter gene assays.

With the known complexities of screening for induction using reporter gene assays, and the poor availability of primary human hepatocytes, the search for an intermediate, yet relevant in vitro model for P450 induction is ongoing. In 2004, the use of an immortalized human hepatocyte cell line (Fa2N-4) was introduced (Mills et al. 2004) with the advantage of easier culturing and higher throughput potential relative to primary human hepatocytes. Ripp et al. (2006) went on to test 24 compounds (18 positive, and 6 negative for induction based on previous data from human

hepatocytes) in the Fa2N-4 cells and found all 18 positive controls caused a >2-fold maximal induction, while the six negative controls caused <1.5-fold induction, suggesting these immortalized cells could be used reliably to assess risk of induction. However, reports since have clearly shown limitations to the Fa2N-4 cells, namely low expression of CAR, a mechanism of both CYP3A4 and CYP2B6 induction, as well as hepatic uptake transporters (e.g. OATP1B1), concluding Fa2N-4 cells cannot replace primary human hepatocytes as an in vitro model system for induction (Harparsad et al. 2008; Kenny et al. 2008). Most recently, HepaRG cells have been shown to respond to PXR, CAR, and AhR activators, resulting in induction of CYPs 1A1, 1A2, 2B6, 2C8, 2C9, 2C19, and 3A4 (Kanebratt and Andersson 2008). The apparent success of HepaRG cells in the prediction of induction potential was further supported by a recent study where many of the aforementioned methods for assessing induction were compared (McGinnity et al. 2009). Similar to CYP3A4 time-dependent inhibition, a recent PhRMA survey of current practices for assessment of induction was published (Chu et al. 2009). From this collection of information from 14 PhRMA member companies, some recommendations were made as to how to conduct in vitro and in vivo studies evaluating induction. Worth noting, recommendations for assessment of induction included use of fresh or plateable cryopreserved hepatocytes, treatment with NCE for 2–3 days, measurement of catalytic activity, and at least three donor hepatocytes. For more information and guidance on this subject, see Chu et al. (2009).

Obviously, advantages and disadvantages of each in vitro induction assay (see Table 2.2) need to be weighed against the needs of the particular company. As we continue to explore new chemical space in the drug industry, it would be prudent to assess the risk of enzyme induction in an in vitro system containing the full complement of nuclear receptors, DMEs and transporters prior to clinical development, in order to gain a comprehensive view of induction risk and enable

Table 2.2 Advantages and disadvantages of current in vitro assays used to evaluate induction of drug metabolizing enzymes/transporters

In vitro assay	Advantages	Disadvantages	Reported to be predictive of clinical response?	References
Binding assays	High throughput	Does not differentiate between agonists and antagonists (e.g. potential for false positive)	No	Jones et al. (2002)
	Low cost			
Reporter gene assay (hPXR, AhR, etc.)	Medium-to-high throughput; SAR	Unable to assess other induction mechanisms	Yes	El-Sankary et al. (2001), Luo et al. (2002), Cui et al. (2008)
Primary human hepatocytes	Gold standard for evaluation of induction; full complement of DMEs, nuclear receptors, and transporters	Routine access to high quality cells a challenge; low throughput; variability between donors; cost	Yes	Chu et al. (2009)
Cryopreserved human hepatocytes	Availability	Cost; variable response	No	Roymans et al. (2005)
Human liver slices	Full complement of DMEs, nuclear receptors, and transporters	Availability	No	Olinga et al. (2008)
HepG2 cells	Robust induction response to CYP1A inducers	mRNA levels of most CYPs lower than primary hepatocytes; unresponsive to some CYP3A4 inducers	No	Harmsen et al. (2008)
Immortalized hepatocytes (Fa2N-4)	Ease of culture and handling; good response to CYP3A4 and 1A2 inducers; apparently able to relate data to clinic	Utility limited by very low expression of CAR and several other drug transporters	Yes	Ripp et al. (2006), Hariparsad et al. (2008), Kenny et al. (2008)
HepaRG cells	Functionally resemble primary cultured human hepatocytes	Expression of enzymes and nuclear receptors, and response to enzyme inducers varies depending on media and culture conditions; cost	Yes	Aninat et al. (2006), Kanebratt and Andersson (2008), McGinnity et al. (2009)

identification of novel origins of clinically relevant induction DDIs.

2.7 Predicting and Simulating Clinical DDIs

2.7.1 Perpetrator DDI (Competitive and Time-Dependent Inhibition)

The early, reliable prediction of DDIs using in vitro and in silico methodologies are important in drug discovery and development for candidate

design and prediction of the human in vivo situation. Understanding the mechanisms of potential DDIs is critical to the successful advancement of drug candidates, as patient safety and labeling restrictions from a commercial standpoint are of the utmost importance in the pharmaceutical industry. The science of using in vitro inhibition data (e.g. K_i or IC_{50}) to predict clinical DDI risk has advanced considerably in recent years, and thus has become more widely accepted, particularly by regulatory agencies such as the FDA, who have issued a regulatory guidance on in vitro drug interaction studies (www.fda.gov).

[gov/cder/guidance/6695dft.htm](http://www.fda.gov/cder/guidance/6695dft.htm)). The underlying mechanisms of DDIs are typically either competitive inhibition of drug clearance, time-dependent inhibition (TDI) or inactivation of drug clearance, and induction, which may result in increased drug clearance and loss of efficacy. Since the cytochrome P450 family of drug-metabolizing enzymes remain the most important enzymes that are involved in the clearance of drug molecules, they will be the focus of this discussion, although the concepts may be applicable to any enzyme involved in drug clearance.

The initial step in conducting a DDI prediction is the generation of the *in vitro* inhibition parameter K_i or IC_{50} . Typically, IC_{50} data is generated early in the discovery continuum due to the ease of conducting this assay, relative to generating a K_i *in vitro*. An approach often utilized is conversion of the IC_{50} to an estimated K_i using the Cheng-Prusoff equation (Equation 1 in Table 2.3), assuming a competitive inhibition mechanism (Cheng and Prusoff 1973). This *in vitro* derived inhibition constant is then used along with the projected *in vivo* inhibitor concentration ($[I]_{in\ vivo}$) in basic equations such as Equation 2. The general relationship of $[I]_{in\ vivo}$ to K_i is shown in Fig. 2.9, and while useful for initial assessment of clinical DDI risk, more sophisticated methodologies with additional input parameters may be used such as the Rowland-Matin equation (Equation 3). Equation 3 incorporates the fraction of the probe substrate (e.g. victim drug) metabolized by CYP ($f_{m(cyp)}$), a key factor to the magnitude of any clinical drug–drug interaction.

A topic of much discussion and debate is what concentration to use for $[I]_{in\ vivo}$, or the *in vivo* inhibitor concentration (e.g. total systemic C_{max} , free systemic C_{max} , $C_{average}$, hepatic portal inlet, etc.). While it is impossible to measure the actual concentration that the enzyme is exposed to within the liver, the best estimates that exist are either the systemic drug level, which is directly measurable, or the hepatic portal inlet, which makes sense conceptually given that for an oral drug absorbed via the portal vein, the liver will be exposed to higher drug concentrations than

the systemic circulation. Additional uncertainty is brought into the discussion as the question of the role of protein binding is on-going (e.g. the “free-drug” hypothesis). The most extensive work to understand and compare the differences associated with using systemic vs. estimated hepatic portal inlet concentrations (and free vs. total) has been reported by Obach et al. (2006). The conclusion from this work was that for competitive (e.g. reversible) inhibitors, the use of estimated free-portal-vein C_{max} for $[I]_{in\ vivo}$ yielded the best predictions of clinical DDI. Equation 4, originally proposed by Kanamitsu et al. (2000), may be used to estimate the free-portal-vein C_{max} for a given dose. Interestingly, work by the same group (Obach et al. 2007; Obach 2009) evaluating mechanism-based inactivation (e.g. time-dependent inhibition) found that the use of free systemic C_{max} for $[I]_{in\ vivo}$ yielded the most accurate overall DDI predictions. The reasons why different surrogate values for $[I]_{in\ vivo}$ appear to work best for competitive and time-dependent inhibition are not clear. Regarding mechanism-based inactivators, it is important to characterize this phenomenon so as to not underpredict the magnitude of the clinical interaction. Accurate estimates of inactivation kinetic parameters k_{inact} and K_I (discussed earlier in this chapter) are key to predicting the clinical DDI. Equation 5, a variation of the Rowland-Matin equation (Equation 4), is reported to yield accurate predictions of clinical DDI (Mayhew et al. 2000; Obach et al. 2007; Foti and Wahlstrom 2008). Yet another important parameter of uncertainty is the k_{deg} , the normal first-order degradation rate of the affected enzyme. This uncertainty is due to the fact that no accurate method for determining k_{deg} in humans exists. The impact of using different k_{deg} values has been reviewed by Yang et al. (2008).

An additional component to accurate DDI predictions is the contribution of the gut to the overall first-pass clearance of drug, particularly for CYP3A4 substrates. For some substrates, the contribution of the gut to first-pass clearance is significant (Galetin et al. 2006, 2007, 2008), despite the fact that there is ~100-fold less

Table 2.3 Summary of equations that may be used for prediction of clinical drug–drug interaction risk using in vitro data

Name	Equation	Descriptors	References
Equation 1 (Cheng-Prusoff)	$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$	K_i = Inhibition constant IC_{50} = Concentration at which 50% inhibition is observed in vitro K_m = Michaelis–Menten constant and the concentration of probe substrate [S] used in in vitro IC_{50} experiment (e.g. [S] = K_m)	Cheng and Prusoff (1973)
Equation 2	$1 + \frac{[I]_{in\ vivo}}{K_i}$	K_i = Inhibition constant $[I]_{in\ vivo}$ = Concentration of inhibitor in vivo	Wienkers and Heath (2005)
Equation 3 (Rowland-Martin)	$\frac{AUC_i}{AUC} = \frac{1}{\frac{f_{m(cyp)}}{1 + \frac{[I]_{in\ vivo}}{K_i}} + (1 - f_{m(cyp)})}$	AUC_i = Exposure of victim drug when co-administered with inhibitor AUC = Exposure of victim drug when given without inhibitor $f_{m(cyp)}$ = Fraction of substrate metabolized by CYP $[I]_{in\ vivo}$ = Concentration of inhibitor in vivo K_i = Inhibition constant	Rowland and Martin (1973)
Equation 4	$[I]_{u,portal} = f_u \cdot \left(C_{max} + \frac{K_a \cdot F_a \cdot Dose}{Q_h} \right)$	f_u = Fraction unbound C_{max} = Maximal systemic concentration K_a = Absorption rate constant F_a = Fraction of dose absorbed Q_h = Hepatic blood flow (21 ml/min/kg)	Kanamitsu et al. (2000), Obach et al. (2006)
Equation 5	$\frac{AUC_i}{AUC} = \frac{1}{1 + \frac{f_{m(cyp)}}{\frac{k_{inact} \cdot [I]_{in\ vivo}}{K_I + k_{deg}}}} + (1 - f_{m(cyp)})$	AUC_i = Exposure of victim drug when co-administered with inhibitor AUC = Exposure of victim drug when given without inhibitor $f_{m(cyp)}$ = Fraction of substrate metabolized by CYP $[I]_{in\ vivo}$ = Concentration of inhibitor in vivo k_{inact} = Maximal rate of inactivation determined from in vitro study (units are min^{-1}) K_I = Concentration of inactivator that yield one-half the maximal rate of inactivation (μM) k_{deg} = Normal rate of enzyme degradation (for CYP3A4, a value of $0.00032\ \text{min}^{-1}$ is typically used)	Obach et al. (2007)
Equation 6	$\frac{F'_g}{F_g} = \frac{1}{F_g + (1 - F_g) \cdot \left(\frac{1}{1 + \left(\frac{k_{inact} \cdot [I]_{gut}}{k_{deg} \cdot (K_I + [I]_{gut})} \right)} \right)}$	F'_g = The fraction of the drug that remains intact following oral dose k_{inact} = Maximal rate of inactivation determined from in vitro study (units are min^{-1}) K_I = Concentration of inactivator that yield one-half the maximal rate of inactivation (μM)	Wang et al. (2004), Obach et al. (2007)

(continued)

Table 2.3 (continued)

Name	Equation	Descriptors	References
		k_{deg} = Normal rate of enzyme degradation (for CYP3A4, a value of 0.00048 min^{-1} is typically used)	
		$[I]_{gut}$ = Concentration of drug in the intestine	
Equation 7	$[I]_{gut} = \frac{k_a \cdot F_a \cdot \text{Dose}}{Q_g}$	k_a = Absorption rate constant F_a = Fraction of dose absorbed Q_g = Villous blood flow (3.5 ml/min/kg)	Obach (2009)
Equation 8	$\text{RIS} = \frac{C_u \cdot E_{max}}{C_u + EC_{50}}$	C_u = Unbound efficacious concentration E_{max} = Maximal observed induction in vitro EC_{50} = concentration of drug that yield 50% induction in vitro	Ripp et al. (2006), Fahmi et al. (2008)

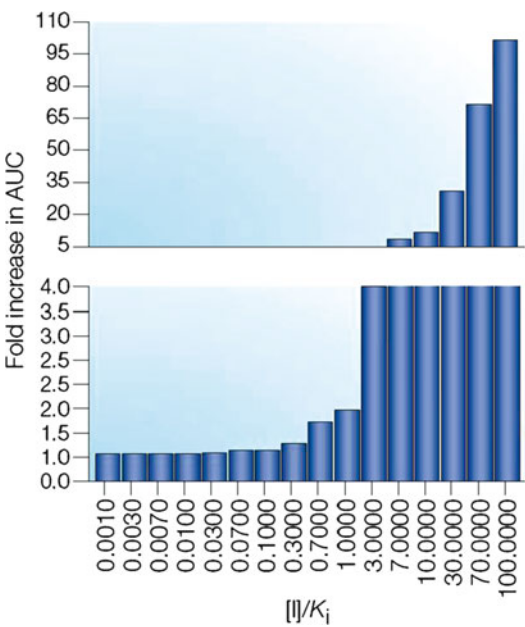


Fig. 2.9 The relationship between the in vivo inhibitor concentration ($[I]_{in\ vivo}$) to the in vitro inhibition constant (K_i) as it relates to fold-increase in AUC of a victim drug.

As the $[I]/K_i$ ratio increases, the fold-increase in AUC increases. Reprinted with permission from *Nature Reviews* (Wienkers and Heath 2005)

CYP3A4 in the gut relative to the liver (Shen et al. 1997). For consideration of the gut in DDI, Equation 6 was proposed by Wang et al. (2004). Within this equation, the component $[I]_{gut}$ is equally important, and may be estimated using Equation 7.

2.7.2 DDI Predictions for Induction

Prediction of DDIs due to induction of cytochrome P450 enzymes is equally important, yet has proven difficult, primarily due to multitude of in vitro models of induction, and extrapolation

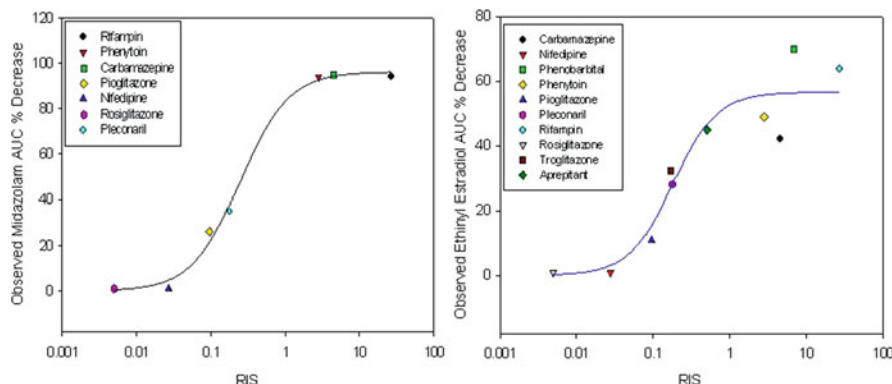


Fig. 2.10 Relationship between the calculated Relative Induction Score (RIS) and clinical observations with co-administered midazolam and ethinyl estradiol with numerous known CYP3A inducers using human cryopre-

served hepatocytes. Observed correlations were $r^2 = 0.96$ and 0.82 , respectively. Reprinted with permission from Drug *Metabolism and Disposition* (Fahmi et al. 2008)

of in vitro induction data to the clinic. Lately, however, several reports have been published which have shown progress in predicting DDI resulting from induction. A novel technique for predicting the magnitude of clinical interaction due to induction of CYP3A was developed using the Fa2N-4 immortalized human hepatocyte line (Ripp et al. 2006). This approach is based on combining in vitro induction parameters (EC_{50} and E_{max}) with the efficacious free plasma concentrations to calculate a relative induction score (RIS) (Equation 8), which was then correlated to the magnitude of clinical DDI for midazolam or ethinyl estradiol, with r^2 values of 0.92 and 0.93 , respectively (Ripp et al. 2006). This RIS method has since been assessed using human cryopreserved hepatocytes with comparable success, as shown in Fig. 2.10 (Fahmi et al. 2008).

2.7.3 SimCYP

SimCYP© (SimCYP© Limited, Sheffield, UK) is a computer simulation program developed for the prediction of metabolic DDIs that applies fundamental scaling concepts (Houston 1994) for the prediction of in vivo clearance from in vitro metabolism data (Rostami-Hodjegan and Tucker 2007; Jamei et al. 2009). The SimCYP model requires a number of inputs including molecular

weight and physicochemical properties of the molecule (LogP , pK_a and acid/base/neutral character together with measured $f_{u(\text{plasma})}$ and blood:plasma ratio), as well as predicted clearance pathways and pharmacokinetic properties in humans, if available. SimCYP uses a physiologically based pharmacokinetic model that enables modeling of the dynamic nature of in vivo inhibitor and substrate concentrations, as well as the interindividual variability among the population.

The effects of perpetrator DDIs are predicted using the relationship between the inhibitor concentration in vivo and the K_i determined in vitro. From the IC_{50} values generated in the relevant in vitro system, K_i values are determined assuming competitive inhibition (e.g. Cheng-Prusoff Equation), unless a K_i value has been estimated experimentally. The values determined for the appropriate CYPs are used together with an estimate of its in vivo clearance and absorption characteristics. SimCYP© incorporates the population variability in each parameter (e.g. CYP abundance) by applying a Monte Carlo approach. Using appropriate clinical probes (Table 2.4), the potential for NCE compounds to be involved in a DDI are then predicted. Physiological variability is calculated automatically using databases within SimCYP©, while pharmacokinetic variability can be incorporated by the user in the form of a coefficient of variation. Clinical trials of

Table 2.4 Recommended clinically used probes and inhibitors for perpetrator and victim SimCYP® simulation studies

CYP	Perpetrator studies (clinical probe studied)	Victim studies (clinical inhibitor studied)
1A2	Theophylline	Fluvoxamine
2C9	S-warfarin	Fluconazole
2C19	Omeprazole	Omeprazole
2D6	Desipramine	Paroxetine
	Dextromethorphan	
3A4	Midazolam	Ketoconazole (potent)
		Erythromycin (moderate)

various sizes can be simulated to determine the effects of trial size on variability.

For victim drugs (substrates), CL_{int} values for the individual CYP enzymes are used as inputs for DDI prediction using numbers from HLM incubations or from recombinant human CYPs with appropriate Intersystem Extrapolation Factors (ISEF) correction (Proctor et al. 2004). These data can also be entered using V_{max} and K_m information for the individual CYPs if these are available, allowing saturation of metabolic clearance to be simulated. Since data for clearance and inhibition (inhibition constants for reversible (K_i) or time dependent (K_I , k_{inact}) inhibitors or induction (Ind_{50} , Ind_{max} , Ind slope) are also incorporated in the model, cumulative effects may be modeled simultaneously for complex drugs like ritonavir. Activities for enzymes catalyzing non-CYP mediated metabolism are also inputs in the SimCYP© model so that $f_{m(CYP)}$ (the fraction of metabolism mediated by a particular CYP), is calculated appropriately. In addition, undefined HLM CL_{int} (e.g. for FMO mediated metabolism) and other enzymes can be used and incorporated as a component of the overall clearance using appropriate scaling factors. In this way, all known pathways of metabolic clearance can be incorporated in a SimCYP© model.

2.8 Transporters and DDIs

While alteration of drug metabolizing enzymes is most often the cause of DDIs, there is increasing evidence this may not entirely explain the pharmacokinetic variability observed with some drug

molecules (Zhang et al. 2008). For example, the mechanism of the digoxin–quinidine interaction (Hager et al. 1979) was not truly understood until relatively recently (Fromm et al. 1999). Thus, as the science behind drug transporters continues to evolve, their role in absorption, distribution, and elimination of drugs is becoming one of the topics of highest interest in the ADME discipline. Figure 2.11 demonstrates the numerous uptake and efflux transporters expressed in the hepatocyte, which can complicate the distribution of drug molecules. The FDA has issued a guidance (September 2006) that includes a decision tree to determine if clinical in vivo interaction studies are warranted based on in vitro data. P-glycoprotein (P-gp) is able to transport a diverse range of compound structures, similar to CYP3A4, and is thus the most studied and understood efflux transporter. The expression of P-gp in the body is high in tissues such as the gut, blood–brain barrier and in organs of drug clearance such as the liver and kidney, making it of significance in the distribution and elimination of drugs.

Interestingly, a recent publication has suggested DDIs solely related to P-gp are generally not clinically relevant, as changes in pharmacokinetics were modest, with only moderate changes in drug exposure (Fenner et al. 2009). For example, valsopodar (PSC 833) is one of the most potent inhibitors of P-gp known ($IC_{50} = 0.02 \mu M$) (Kawahara et al. 2000), yet in a clinical DDI study, only a 76–211% increase in digoxin AUC was observed (Kovarik et al. 1999), typically considered insignificant. However, if digoxin or any P-gp substrate with a narrow therapeutic index (TI) is the potential victim drug, then a even a 25% change in drug exposure

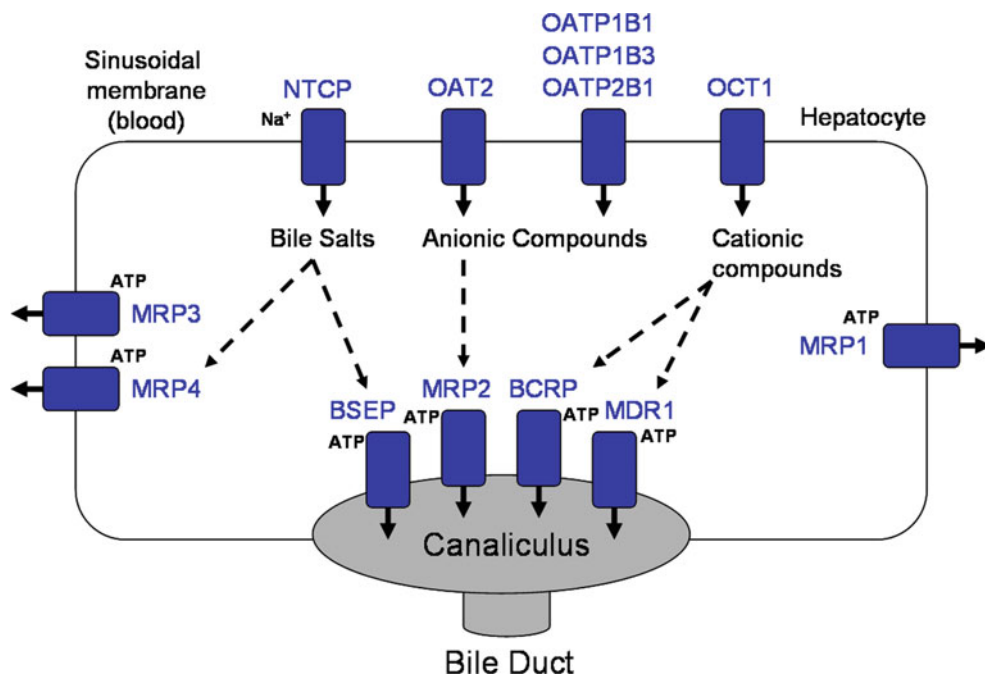


Fig. 2.11 Schematic showing the numerous uptake and efflux transporters that contribute to the distribution of drug molecules within the hepatocyte. Currently, P-gp (MDR1) is the most studied and understood efflux

transporter with respect to DDIs. However, the science of transporters is rapidly evolving, evidenced by the recent reports of DDI with statin drugs, reportedly due to inhibition of uptake transporter OATP1B1

due to inhibition of P-gp may lead to unwanted toxicities (e.g. digitalis for digoxin). As digoxin is a commonly prescribed heart medication, in vitro assessment of the potential for a drug interaction specifically with digoxin is warranted for compounds progressing toward first-in-patient (FIP) or proof-of-concept (POC) in clinical development.

In vitro evaluation of whether an NCE is a substrate or inhibitor of P-gp is relatively straightforward, yet there are numerous assay formats that may be utilized. It is recommended in the FDA guidance document to use bidirectional transport studies with either Caco-2 cells or recombinant epithelial cell lines (MDCK_MDR1 or LLC-PK1_MDR1). In general, if the efflux ratio ($B \rightarrow A / A \rightarrow B$) is ≥ 2 and addition of a specific P-gp inhibitor decreases this efflux ratio then the NCE is categorized as a potential P-gp substrate. Use of a specific P-gp inhibitor is especially critical when Caco-2 cells are used, since it is known other efflux transporters (e.g. BCRP

and MRP2) are expressed in this human cell line (Taipalensuu et al. 2001). Follow-up studies may then need to be performed to evaluate the actual risk for a drug–drug interaction. A simple decision tree adopted from Zhang et al. (2008) is shown in Fig. 2.12. For early evaluation of an NCE as a potential inhibitor of P-gp, the calcein-AM assay, where inhibition of the P-gp-mediated efflux of the fluorescent substrate (calcein) is determined, has been useful in the discovery setting (Tiberghien and Loor 1996; Feng et al. 2008). This assay should be used on an “as needed” basis since, as previously mentioned, most P-gp-mediated DDIs are not significant. The gold-standard assay for assessing P-gp inhibition includes the use of MDCK_MDR1 cells and ^3H -digoxin, and should be conducted where that results would be available prior to the first study where digoxin may be as a possible concomitant medication. To aid in use of in vitro derived inhibition data with transporters, a decision tree for evaluation of NCEs as potential

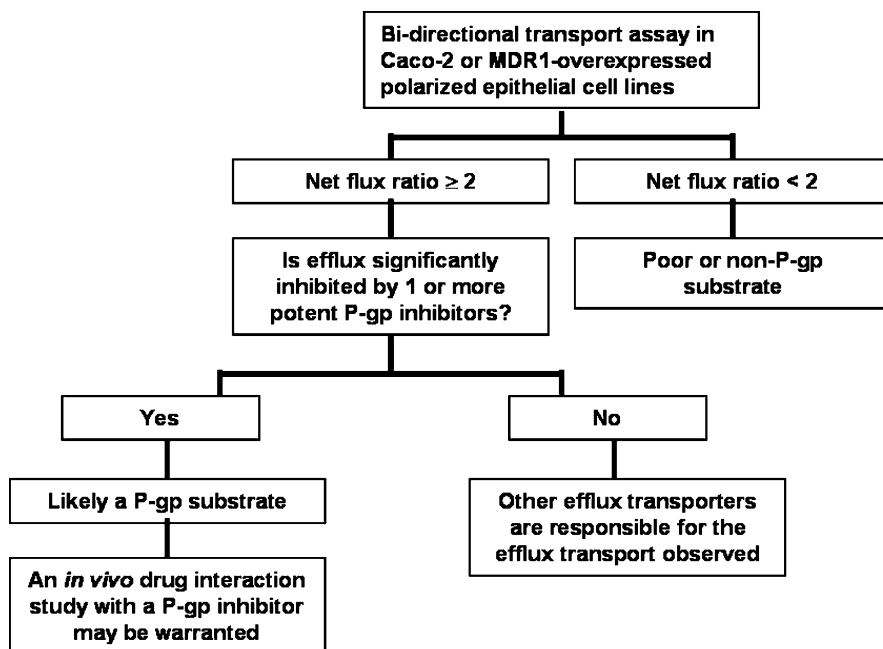


Fig. 2.12 Decision tree for use as a guide in determining if an NCE is a substrate for P-glycoprotein (P-gp). Net flux ratio is calculated as F_{B-A}/F_{A-B} . A net flux ratio of ≥ 2 is considered to be a positive signal for P-gp substrate interaction. Use of a P-gp inhibitor is also recommended in diagnosing P-gp substrate, and reduction of net flux

$>50\%$ or to unity is further evidence for P-gp interactions. Additional data may be needed to decipher the clinical relevance of *in vitro* data, including the potential contribution from other efflux transporters (e.g. BCRP and MRP2)

inhibitors of P-gp has also been proposed by the FDA in a recent report (Zhang et al. 2008), and is shown in Fig. 2.13.

While P-gp clearly remains the most studied transporter, several reports of transporter-mediated DDI with transporters other than P-gp have been published (Simonson et al. 2004; Treiber et al. 2007; Seithel et al. 2007). Regarding uptake transporters, OATP1B1 is of most interest at this time due to the observed DDIs with HMG-CoA-reductase inhibitors (e.g. statin drugs). For an extensive review of OATP and OCT-mediated DDIs, the reader is referred to a recent review on this subject (Kindla et al. 2009). Despite these clinical observations, at this point there has been no consensus established for *in vitro* methods, probe substrates or tool inhibitors to use for transporters such as OATP1B1, BCRP, OATs and OCTs, although an international working group consisting of members from academia, industry,

and the FDA has been formed to establish some guidelines in this area. It is likely that the knowledge and understanding of transporter-mediated DDI will progress significantly in the next few years.

Lastly, it is worth mentioning, even though not considered DDIs, inhibition of efflux transporters multidrug resistance protein 2 (MRP2) and bile salt export pump (BSEP) have been shown to lead to certain toxicities. Specifically, inhibition of MRP2 can lead to hyperbilirubinaemia, while evidence exists that inhibition of BSEP leads to increases in bile salts, and subsequent cholestasis and hepatotoxicity, as observed with nefazodone (Kostrubsky et al. 2006), and more recently with CP-724,714 (Feng et al. 2009). As a result, drug discovery DMPK and toxicology programs have begun to test compounds for their potential to inhibit these hepatobiliary transporters.

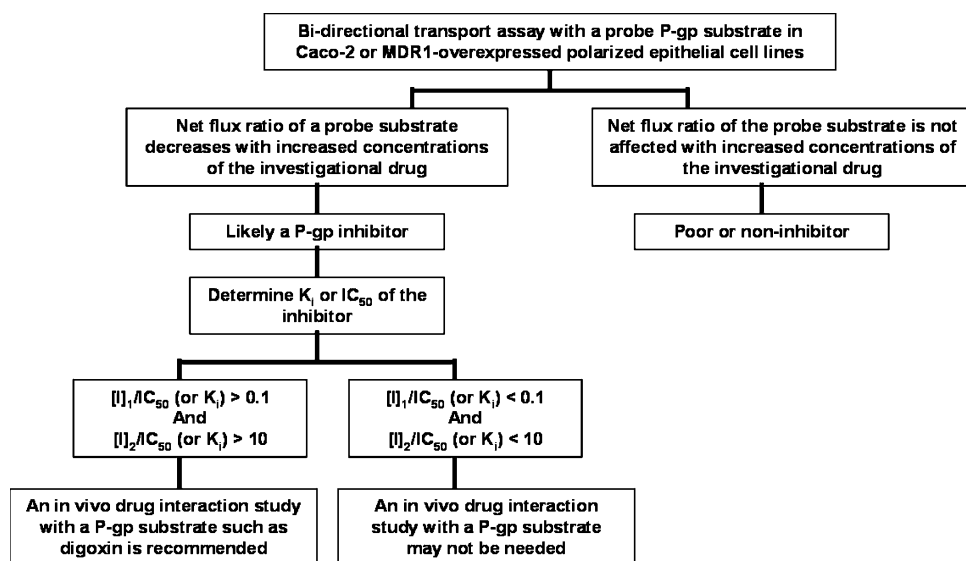


Fig. 2.13 Decision tree for use as a guide in determining if an NCE is an inhibitor of P-glycoprotein (P-gp), and whether a clinical DDI study with digoxin needs to be conducted. Net flux ratio is calculated as $F_{B-A}/F_{A-B} \cdot [I]_1$

represents mean steady-state C_{\max} values for total drug following administration at the highest proposed clinical dose; $[I]_2$ = dose of inhibitor (mol)/250 ml

2.9 The Future of DDI Investigations

2.9.1 Polymorphic Metabolism and DDI

Several of the human cytochrome P450 enzymes (e.g. CYP2B6, 2C9, 2C19, 2D6, and 3A5) are known to be polymorphically expressed to different degrees in the human population. The primary impact of these polymorphisms includes reduced oral clearance for certain medications, and subsequent increase in exposure, resulting in potential for pharmacokinetic variability and increased incidence of adverse events. A relevant question clinically is whether individuals with polymorphic expression are more or less susceptible to a clinical DDI when an inhibitor is co-administered. Understanding this relationship is clearly important as dose adjustments in individuals with particular genotypes (e.g. poor metabolizer or PM) may be different from a normal “extensive” metabolizer (EM). It has been

reported that individuals possessing polymorphisms resulting in expression of inactive protein (e.g. CYP2C19 and 2D6) are less susceptible to DDI (Hamelin et al. 2000; Lessard et al. 2001; Uno et al. 2006). In vitro experiments evaluating this effect are not possible due to the lack of enzymatic activity. However, for polymorphically expressed P450 enzymes or allelic variants (e.g. CYP2C9*2 and *3) that still possess some activity, the same question of susceptibility to DDI is applicable. Kumar et al. (2006) reported that inhibition of CYP2C9 was genotype-dependent, which triggered a clinical study using flurbiprofen to show that individuals homozygous for the *3 allele of CYP2C9 did not have a significant decrease in oral clearance when co-administered with fluconazole (Kumar et al. 2008). This finding is reasonable, as the baseline pharmacokinetics in the *3/*3 individuals were different, suggesting that fraction metabolized by CYP2C9, which contributes to the magnitude of the interaction, is clearly different in PM compared to EM individuals. This finding warrants further investigation, and argues in vitro assays

evaluating inhibition of CYP2C9 allelic variant enzymes should be considered for clinical candidates with a risk of CYP2C9-mediated DDI.

2.9.2 The Role of CYP3A5

While CYP3A4 has been estimated to play a significant role in the metabolism of >50% of all drugs on the market (Guengerich 2002), the contribution of CYP3A5 to the overall metabolism of NCE's has received increased attention due to its polymorphic expression (3A5*3, *6, *7). Interestingly, only ~15% of Caucasians express high levels of CYP3A5, compared to 75% of African-Americans (Xie et al. 2004). Despite 84% amino acid sequence similarity between CYP3A4 and 3A5, several drugs, including midazolam and lidocaine have been reported to be more efficiently metabolized by CYP3A5 compared to 3A4 (Wrighton and Stevens 1992), which suggests CYP3A5 may play a significant role in the overall clearance of some drugs. However, this assessment is complicated by conflicting reports comparing CYP3A4 to 3A5 activity. Some of the differences can be attributed to the different expression systems used, where some enzyme preps were coexpressed with coenzymes (e.g. P450 oxidoreductase and cytochrome b5), and others only supplemented with these coenzymes (Huang et al. 2004). Nonetheless, several examples of pharmacokinetic variability in the clinic for CYP3A5 substrates have been reported. Most notably, reduced oral clearance of tacrolimus was reported in subjects homozygous for the CYP3A5*3 genotype compared to those with 3A5*1 genotype (Hesselink et al. 2003, 2008). Other drugs reported to have substantial clinical pharmacokinetic variability thought to be attributed to CYP3A5 genotype, include cyclosporine (Anglicheau et al. 2007), vincristine, where exposure varied up to 19-fold (Van den Berg et al. 1982), and the PDE5 inhibitor vardenafil, with 14-fold variability observed across subjects given a 20 mg dose (Klotz et al. 2001; Rajagopalan et al. 2003; Ku et al. 2008). Not only has the potential impact of CYP3A5 ex-

pression on pharmacokinetic variability been of concern, it has also been observed that CYP3A5 may in fact produce a distinct metabolite profile compared to 3A4, as observed with quetiapine (Bakken et al. 2009). This may have ramifications not only for pharmacological, but also the toxicological profile of impacted drugs.

Currently, estimating the contribution of CYP3A5 in overall metabolism is challenging, as there are no completely specific inhibitors or substrates used as in vitro tools to differentiate 3A5 from 3A4 activity. A reasonable approach has been a direct comparison of CYP3A4 and 3A5 in vitro intrinsic activity using recombinantly expressed enzymes with the same ratio of reductase and cytochrome b5 (e.g. Supersomes®). However, scaling of this in vitro intrinsic clearance is challenging, as abundances of CYP3A5 in liver are variable. There are now genotyped human liver microsomes available where both CYP3A4 and 3A5 expression levels are known (www.bdbiosciences.com), which may be useful in comparing intrinsic metabolic capacity in vitro in CYP3A5 expressers vs. non-expressers. This approach was taken by Huang et al. with numerous substrates of CYP3A4 and 3A5 (Huang et al. 2004). Another approach with potential for estimating of the contribution of CYP3A5 to overall metabolism of NCEs may be use of raloxifene, which has been reported to be a selective mechanism-based inhibitor (MBI) of CYP3A4 (Pearson et al. 2007), even though it is a competitive inhibitor of both CYP3A4 and 3A5.

2.9.3 Non-P450 Enzymes and DDI

While the cytochrome P450 family of drug-metabolizing enzymes are the clear origin of most clinically relevant DDIs, other enzymes such as UDP-glucuronosyltransferases (UGT), sulfotransferases (ST), monoamine oxidases (MAO-A and MAO-B), aldehyde oxidase (AO), and flavin monooxygenase (FMO) are of interest from a DDI perspective due to their potential role in metabolic clearance. Of these non-P450 enzymes, the UGTs have received the most attention of late, due in part to a report in 2004

by Williams et al. who demonstrated that glucuronidation is a clearance mechanism for 1 in 10 of the top 200 prescribed drugs in 2002 (Williams et al. 2004). While a primary conclusion from this report was that inhibition of UGT enzymes rarely lead to any clinically significant DDI (AUC Ratio >2), recent advances in the understanding of UGT enzymes and in vitro characterization of activity have triggered a re-evaluation of this subject. For example, it has generally been thought UGT enzymes are low-affinity (e.g. high K_m) enzymes. However, recently it has been shown that addition of albumin to HLM and recombinant UGT incubations significantly reduces the K_m for AZT glucuronidation by UGT2B7 (Rowland et al. 2007), a result of albumin sequestering inhibitory fatty acids liberated during microsomal incubations. This realization may lead to more attention being paid to UGT-mediated metabolism, particularly to UGT1A1 since this represents the predominant clearance pathway for bilirubin, and polymorphic expression leads to hyperbilirubinemia, a condition known as Gilbert's Syndrome (Burchell et al. 2000). The other non-P450 drug metabolizing enzymes are currently less understood as it relates to DDIs, probably because they rarely contribute 100% to the clearance of drug molecules. This may change in the future as NCEs continue to be profiled, especially as chemistry and drug metabolism scientists work to reduce P450-mediated metabolism in optimization efforts.

2.10 Design and Execution of a Clinical Assessment of DDI Potential in a Drug Development Program

The major determinant of drug interaction studies is no longer the list of approved drugs most likely to be co-administered, rather than determination is based on likely mechanisms for a drug interaction. Thus, the first step in determining which drug interactions studies should be performed in a clinical program is to utilize in vitro and preclinical data to judge the likely risk of interactions. Typical interactions to be

considered are those where one drug might alter the pharmacokinetics of another, such as inhibiting clearance. Additional consideration must also be given to pharmacodynamic interactions, which can alter the drug's pharmacology, as has been observed with the sedative effects of compounds with inhibitory central nervous system activity.

For pharmacokinetic interactions, equal consideration should be given to circumstances where a drug is an object, or victim, of the interaction and where the drug is the perpetrator (precipitant, inhibitor, or inducer). When the drug is the victim, the projected metabolic pathways are potential sites of interactions and the more predominate a pathway, the higher the risk of a clinically relevant interaction. Thus for drugs predominately cleared by a single pathway, there will almost always be interactions of clinical relevance. Conversely, if a molecule is cleared by multiple mechanisms, then the risk of a clinically relevant interaction is lower. Additionally clinically relevant interactions due to transporters have been observed. For instance inhibitors of liver uptake transporters resulted in 2 to 25-fold increases in systemic statin concentrations (Neuvonen et al. 2006).

Other distribution phenomena are generally less important. For example, alterations in plasma protein binding typically do not necessitate a dose adjustment, as they do not affect the clearance of free drug, the presumed active form. Thus, while plasma total drug concentrations may be altered, free drug concentrations will return to the same steady-state levels as observed prior to administration of the perpetrator. The exceptions to the above generalization are except in two instances. The first is when a drug has the attributes of a high extraction ratio, a narrow therapeutic index, and is parentally given (e.g. lidocaine). The second case is for a drug that has a narrow therapeutic index, is orally administered and has a very rapid pharmacokinetic–pharmacodynamic equilibration time. In these rare cases, DDIs causing protein binding changes can have clinical consequences (Benet and Hoener 2002).

Studies typically involve sensitive probe substrates or potent specific inhibitors to elicit the

maximum magnitude of an interaction. Thus if no interaction is observed, one can be reasonably assured there will not be a relevant interaction with the investigational drug and other drugs acting through the same pathway. Further, if there is an interaction, other interactions with the investigation drug involving the same pathway will be of a similar or lesser magnitude. Thus, if an enzyme or transporter is identified as potentially important, a single probe substrate and/or inhibitor study is conducted and the results extrapolated. Less potent inhibitors may be considered for study, however, where an interaction has been shown and the results require contraindication or dose adjustment. In these cases, the resulting smaller magnitude of the interaction could result in different dosing recommendations based on inhibitor potency. A list of probe substrates and inhibitors can be found in the FDA draft drug interaction guidance (Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling, September 11, 2006, <http://www.fda.gov/downloads/Drugs/Guidance/ComplianceRegulatoryInformation/Guidances/ucm072101.pdf>).

2.10.1 Metabolism Based Drug Interactions

The FDA has published guidance on drug interaction strategies for cytochrome P450 (CYP) based interaction studies (previously cited). The guidance suggests interactions with CYP1A2, 2C8, 2C9, 2C19, 2D6, and 3A be considered. Clinical studies should be performed when the drug is a substrate of an isozyme and when the isozyme is a major elimination pathway or it is unclear as to if it is a major elimination pathway. The guidance does not provide explicitly state to what constitutes a major pathway. However, the guidance does show that human in vivo data indicate CYP enzymes contribute more than 25% of a drug's overall clearance, investigations to identify responsible CYP isozymes should be

conducted. A theoretical maximal change in systemic drug concentrations can be estimated as:

$$\% \text{ Increase in systemic exposure} = \frac{100\%}{(1 - \text{fraction of overall clearance due to pathway})} - 100\%$$

Thus if a particular isozyme is responsible for 25% of a drug's clearance, maximal inhibition of the pathway would cause a 33% increase in systemic drug concentrations.

The same CYP isozymes are to be evaluated when considering a drug's potential to be an inhibitor or inducer. Only those instances where preclinical data suggest a drug is an inhibitor or inducer of that isozyme need to be considered for clinical study. To evaluate the potential for reversible inhibition, the likelihood of an interaction is determined by the $[I]/K_i$ ratio where $[I]$ represents the mean steady-state C_{\max} value following administration of the highest proposed clinical dose. As the ratio increases, the likelihood of an interaction increases. An estimated $[I]/K_i$ ratio of greater than 0.1 is considered positive and the guidance recommends a clinical study. Further, it is suggested that the most sensitive isozymes (i.e. where the drug has the lowest inhibitor 50 or induction 50 concentration) should be studied first. If no clinical interaction is observed for these most sensitive pathways, other less sensitive pathways do not need to be studied in clinical studies. For mechanism-based inhibition, the guidance recommends clinical evaluation if any time dependent inhibition is detected. Finally for induction potential, no guidance is given as to what the threshold for clinical evaluations is, however the guidance does note that if induction studies with a compound confirm that it is not an inducer of CYP3A4 then it can be concluded the drug is not an inducer of CYP2C8, CYP2C9, or CYP2C19.

Strategies for non-CYP metabolic pathways are less well established. However, a similar philosophy of using a sensitive probe substrate or potent inhibitor can be applied.

2.10.2 Transporter Based Drug Interactions

Strategies for transporter based interaction studies are less well defined as transporters are less well understood. The FDA does offer a guidance for P-glycoprotein based drug interactions (cited above). A drug is considered a substrate if the net flux ratio is greater than 2, where net flux ratio is the ratio of basal-apical permeability to apical-basal permeability in preclinical models. Evaluation in clinical studies is recommended for substrates where pre-clinical models show inhibition of transport by 1 or more known P-glycoprotein inhibitors. To assess the potential for P-glycoprotein inhibition, the $[I]/IC_{50}$ value is considered, where IC_{50} is the concentration causing half the maximal drug related inhibition. The guidance suggests $[I]/IC_{50}$ values greater than 0.1 warrant clinical investigations. Recent work by Cook et al. (submitted) suggest this threshold may result in an unacceptable false-negative rate (i.e. indication a lack of potential to inhibit P-glycoprotein when clinically relevant interaction does occur) and suggest cut-off values for $[I]/IC_{50} < 0.1$ and $[I_2]/IC_{50} < 5$ (I_2 is the dose/250 ml) were identified to minimize the error rate which resulted in a reduction of false negatives to 9%. Finally for induction potential, the guidance notes a lack of predictable preclinical models but does state if a drug is not an inducer of CYP3A4, then it can be concluded the drug does not induce P-glycoprotein.

Specific criteria for transporters other than P-glycoprotein have not been established. However, Wu and Benet (2005) have utilized the Biopharmaceutics Classification System to assess the potential importance of transporters. For Class 1 compounds (highly soluble, highly permeable), transporters are expected to have minimal impact on drug disposition and thus drug interactions are not expected. For Class 2 compounds (low solubility, highly permeable), efflux transporter effects will predominate. For Class 3 compounds (highly soluble, low permeability), absorptive transporter effects will predominate. For Class 4 compounds (low solubility, low permeability) absorptive or efflux transporters could be important.

Once transporters important to a drugs disposition have been identified, a preclinical assessment as to their importance can be estimated using a model proposed by Shirasaka et al. (2008):

$$\begin{aligned} V_{app} &= V_{passive} \pm V_{transporter} \\ &= P_{passive} \cdot S \cdot C \pm \frac{V_{max} \cdot C}{K_m + C} \end{aligned}$$

where V is the flux and app, passive and transporter subscripts denote whether the flux is apparent, passive or due to the transporter respectively; S is the surface area of the membrane, P is the permeability, V_{max} and K_m are the maximum velocity and concentration producing half-maximal velocity, and C is the concentration at the membrane. The sign in front of the transporter term depends on whether the transporter is an absorptive transporter (+) or efflux transporter (−) with respect to the driving concentration, C . $V_{passive}$ can be determined in preclinical experiments through the use of inhibitor. The difference between the apparent flux without and with an inhibitor will yield the flux due to the transporter. If passive permeability predominates ($V_{passive} \gg V_{transporter}$) then drug interactions are not expected. Similarly the impact of a drug's inhibition potential may be considered using the following model (Tamai 2009):

$$P_{app} = P_{passive} \pm \frac{P_{max, transporter}}{(1 + [I]/K_i)}$$

where, P_{max} is the maximum change in permeability of the substrate due to the transporter, $[I]$ is the concentration of the inhibitor and K_i is the inhibitory constant. Again if passive permeability predominates, no clinically relevant interaction is expected.

2.10.3 Pharmacodynamically Based Drug Interactions

In general there are only two typical cases where pharmacodynamic interactions are considered. The first is for drugs with activity in the central

nervous system. Generally if a drug is centrally acting and sedation a frequent side-effect, evaluation of co-administration of other CNS active agents is warranted using a pharmacodynamic endpoint. The other typical case is drug interaction studies involving warfarin. S-Warfarin is a substrate probe for CYP 2C9 based interaction studies. Because of the relatively low therapeutic window, warfarin pharmacodynamics are often evaluated using prothrombin time and the international normalized ratio (INR). There are certain instances where drugs are co-administered because they have beneficial additive or synergistic pharmacodynamic effects. These are not considered further in this chapter as the interaction is typically evaluated in clinical efficacy trials.

2.10.4 Other Interaction Studies

There may be instances where it is beneficial to conduct clinical drug interaction studies even though there is no basis for an interaction. One instance may be to facilitate recruitment into Phase II or III trials. There are some instances where a background therapy may be given while patients are given the investigational drug. In some instances the drug given as background therapy may have a narrow therapeutic index. Another example might be interactions with oral contraceptives (OCs). Patients in clinical trials may be required to use barrier forms of contraception while enrolled and for a period thereafter. Showing the absence of an interaction with OCs may allow this restriction to be lifted. In these cases, these studies are conducted to show the absence of a clinically significant interaction, and thus assure investigators that the compounds can be given concomitantly. A second reason for conducting a drug interaction when there is no basis for an interaction is to demonstrate a competitive treatment advantage. Both patient and prescriber benefit from knowledge that a new medicine does not carry the same drug interaction liabilities as other available treatments. For example clinical evaluations for pregabalin were undertaken to show a lack of interaction

with a number of antiepileptic drugs (carbamazepine, valproic acid, lamotrigine, phenytoin, phenobarbital, and topiramate), when most of other antiepileptic drugs have clinically relevant drug interactions. Thus, pregabalin could be added to a patient's regimen for seizure control without fear of altering the pharmacokinetics, and potentially the efficacy and safety of the therapy.

2.10.5 Determination of When to Do Drug Interaction Studies

Once it is determined what clinical drug interaction studies need to be done in a drug development program, the timing of these studies should be determined. Considering only one in nine compounds entering human drug development are eventually marketed and clinical safety (of which drug interactions contribute some fraction) accounts for approximately 12% of the failures, it would seem drug interactions are not typically go/no-go studies once a drug development program starts with clinical studies. (Presumably preclinical assessments have caused the attrition of most compounds with major drug interaction liabilities). Consequently it is economically prudent not to spend too many resources early in clinical development to assess drug interactions. It is therefore recommended that prior to initial studies in patients, two types of drug interaction studies are performed. The first are those where definitive "go/no-go" development decisions can be made based on the interaction: where there is an unacceptably high risk of a major drug interaction that would preclude further development. If the interaction study does not need to be conducted (e.g. the efficacy trial can be run without the need for concomitant medication), the level of risk of a relevant interaction [P(DDI)] and cost of the drug interaction study (DDI\$) should be considered against the risk of lack of efficacy [P(LOE)] and the cost of the initial efficacy trial (EFF\$). The drug interaction study should be done first if:

$$(1 - P(\text{DDI})) \cdot P(\text{LOE}) \cdot \text{Eff\$} + \text{DDI\$} \\ < (1 - P(\text{LOE})) \cdot P(\text{DDI}) \cdot \text{DDI\$} + \text{Eff\$}$$

And the estimates of probability can be based on historical rates or efficacy trials and past performance of preclinical models used to estimate the magnitude of clinical interactions.

The second type of drug interaction study that should be conducted are those where concomitant medications need to be given in the initial efficacy trials, and the pharmacokinetics or pharmacodynamics involve pathways identified as likely to be affected by or likely to alter the pharmacokinetics of the investigational drug. For example, if the investigational drug is predominately metabolized by CYP 3A4 and a typical concomitant medication is likely to be a CYP 3A4 inhibitor, a drug interaction study investigating the effect of CYP 3A4 inhibition of the investigational drug is likely needed prior to the study so that dosing recommendations can be made. After the initial efficacy trial, it may be necessary to perform similar enabling studies (those necessary to allow dosing recommendations for other possible concomitant medications as well as those required by investigators to facilitate recruitment) prior to larger clinical trials in order to expand enrollment criteria to encompass a more diverse patient population.

During Phase II and III, sparse pharmacokinetic sampling is now routinely included to evaluate exposure and/or effect relationships in the population pharmacokinetics of patients. These same data can be used to evaluate the influence of concomitant medications on the pharmacokinetics of the investigational drug. Commonly, these data are used to confirm the lack of an interaction when preclinical data suggest that no interaction is expected or to confirm the clinical importance of an interaction previously observed in healthy volunteers. Regulatory agencies have label statements describing the findings (Duan 2007). They are typically not used to confirm the lack of an interaction when preclinical data suggest an interaction is likely, for safety reasons. These analyses may also be useful in detecting unsuspected DDIs. Before such ana-

lyses are conducted, simulations are recommended to optimize study design elements (e.g. sample collection) and to assess the study's ability to characterize an interaction if one were to occur.

Frequently, drug interaction studies are conducted near the end of the development plan to provide labeling information. Many of these studies are conducted to confirm a lack of interaction with commonly co-administered medications in the target treatment population. For instance the FDA guidance on drug interactions (previously cited) suggests conducting at least one drug interaction study with a concomitant inducing or inhibiting drug. The concomitant drug is chosen based on its interaction with the CYP isozyme most sensitive to the investigational drug (e.g. the one for which the investigational drug has the lowest K_i). If the clinical study results in no important interaction, no further studies involving CYP isozymes are warranted. If an interaction does occur, another drug interaction study should be considered with the probe substrate selected based on the next most sensitive CYP isozyme.

2.11 Labeling Considerations

As previously mentioned, the results of drug interaction studies with probe substrates or potent inhibitors are generally extrapolated to other drugs where the interaction would occur through the same pathway. For example if a clinical study indicated no interaction, the drug interaction section of the label might look like the following example suggested by the FDA draft guidance on In Vivo Drug Metabolism/Drug Interaction Studies – Study Design, Data Analysis, and Recommendations for Dosing and Labeling 11/24/1999. (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072119.pdf> last accessed 7/14/2009.):

Data from a drug–drug interaction study involving (drug) and (probe drug) in _____ patients/healthy individuals indicate that the PK disposition of (probe drug) is not altered when the drugs are co-administered. This indicates

that (drug) does not inhibit CYP3A4 and will not alter the metabolism of drugs metabolized by this enzyme.

Similarly, if a significant interaction did occur, the following is an example of a possible portion of the drug interaction section of the label.

The effect of (drug) on the pharmacokinetics of (probe drug) has been studied in _____ patients/healthy subjects. The C_{max} , AUC, half-life and clearances of (probe drug) increased/decreased by _____% (90% Confidence Interval: _____ to _____%) in the presence of (drug). This indicates that (drug) can inhibit the metabolism of drugs metabolized by CYP3A4 and can increase blood concentrations of such drugs. (See PRECAUTIONS, WARNINGS, DOSAGE AND ADMINISTRATION, or CONTRAINDICATIONS sections.)

Declaration of a clinically relevant effect or lack thereof is based on a statistical comparison of pharmacokinetic (or pharmacodynamic) parameters, typically C_{max} and AUC for metabolic based interactions. Proof of a lack of interaction is based on the 90% confidence interval limits for the ratio of pharmacokinetic parameter values (substrate + inhibitor as numerator, substrate only as denominator) and whether or not the 90% confidence intervals lies between no effect boundaries. Two approaches can be used to set no effect boundaries. The first is to use boundary limits that are used for bioequivalence: 80% and 125%. This is the most conservative approach in that lack of interaction is accepted without question. A second approach is to consider exposure-response relationships for both safety and efficacy and to set boundary limits where changes in exposure will not lead to clinical consequences. Thus a large therapeutic window and/or a shallow exposure-efficacy relationship can lead to no effect boundaries that are wider than the 80% and 125% limits used in the first approach. Choice of the approach, the expected magnitude of the interaction and the variability of the substrate can all be used to size the clinical study.

In cases where a clinically relevant interaction is expected, the size of the study should be based on the expected magnitude of the interaction, the variability of the substrate and the precision

required to provide appropriate dosing recommendations. For example, if a very large interaction is expected that is likely to result in a contraindication, few subjects are typically needed than for a study that might result in a dosage adjustment or declaration of no interaction. In general the study design should reflect the expected labeling by either demonstrating that there is no clinically relevant interaction (e.g. powered to rule out a interaction) or resulting in recommendation for dosage adjustment or contraindication (e.g. sized to yield a sufficiently precise estimate of effect).

Conclusions

DDIs can represent a major public health issue. The science has evolved to the point where the risk interactions occurring at various cytochrome-P450 isozymes in humans can be reliably predicted from in vitro data. The science in the area of drug interactions with other enzymes will continue to evolve, hopefully to the point where the likelihood of clinically significant interactions may be more accurately predicted. Clinical drug development scientists and regulators will use these data to assure that the most critical information on drug interactions are obtained in the clinic and to provide appropriate information to physicians and patients to guide the safe and effective use of new and existing medications.

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