

# Drug–Drug Interactions with an Emphasis on Drug Metabolism and Transport

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20% of the injuries occurring annually to hospitalized patients, doubling the length of stay and cost of treatment of hospitalized patients. Nursing home ADRs occur at a rate of 350,000 per year. Costs of ADRs have been estimated at \$136 billion per year. These estimates might underrepresent the outcomes and costs associated with ADRs likely to occur by the end of the first decade of the twenty-first century. That's because two out of three physician visits already result in a prescription. In 2000 there were 10 prescriptions written for every person in the United States. But the United States is in an exponential phase of growth of the very oldest segment of the population; a segment that tends to be composed of individuals requiring the greatest number of prescription drugs. Consider, too, that fewer than 20% of patients with either hypertension, diabetes, or hyperlipidemia are currently even treated at all. If access to healthcare improves and if diagnostic techniques improve it would follow that even more prescriptions will be issued in the years ahead, leading to more ADRs.

Although the purposeful coadministration of two drugs can be designed in such a way as to improve therapeutic outcomes or even to limit adverse effects, it is most often the case that unintended drug–drug interactions comprise a subset of ADRs. They represent between 3 and 5% of all preventable ADRs in hospitals. For decades the notion of drug–drug interactions (DDIs) was given short shrift in pharmacology, medicine, and pharmacy. Interactions were thought to be largely pharmacodynamic, and consequently largely preventable. The very first mention of drug interactions in *The Pharmacological Basis of Therapeutics* by Louis Goodman and Alfred Gilman didn't appear until the fourth edition was published in 1970. Drugs having opposite effects were said to exhibit functional antagonism. Drugs not sharing the same pharmacodynamic

## 12.1 INTRODUCTION

### 12.1.1 Overview and History

Adverse drug reactions (ADRs) have been recognized as an important cause of morbidity and mortality and as a contributor to the spiraling costs of healthcare. The FDA's Center for Drug Evaluation and Research has reported that there are in excess of two million serious ADRs annually in the United States leading to over 100,000 deaths. They are responsible for about

activity, but capable of altering one another's effects, were said to be heterergic. If two heterergic drugs combined to produce an effect greater than the effects of either one of them, then they were said to act synergistically. If two heterergic drugs elicited an effect less than that of either one of them, then the interaction was characterized as antagonistic. Heterergic drug interactions were then ascribed to the effect of one drug on the dispositional characteristics (absorption, distribution, metabolism, excretion) of the other.

The overall treatment of DDIs was given the space of one page in the fourth edition of the book. The eleventh edition of Goodman & Gilman's *The Pharmacological Basis of Therapeutics* was published more than 35 years later in 2006. The general treatment of DDIs expanded by that time to about two pages of text, however a section on drug interactions was written into many of the individual drug monographs. Today there are dozens of books available, each of which provides hundreds of monographs of DDIs. Searchable DDI databases are available online, permitting clinicians to generate DDI reports on virtually any combination of two drugs. These searchable databases allow the user to explore interactions between drugs and nutrients, and also interactions between drugs and herbal remedies.

There are circumstances that can increase the chance of ADRs developing from DDIs. One of those is the use of drugs with narrow therapeutic ranges. For these drugs it is possible that even relatively small changes in exposure to the drug will be met by either an ADR or therapeutic failure. Example of such drugs are:

- Aminoglycoside antibiotics (gentamicin, tobramycin)
- Anticoagulants (warfarin, heparins)
- Carbamazepine
- Estrogens
- Cyclosporine
- Digoxin
- Hypoglycemic agents
- Levothyroxine sodium
- Lithium
- Phenytoin
- Procainamide
- Quinidine
- Theophylline
- Valproic acid

Some disease states or conditions also predispose patients to experience ADRs, including those that result from DDIs. Examples are:

- Aplastic anemia
- Critical care/intensive care patients
- Patients with liver or renal dysfunction

### 12.1.1 Pharmacodynamic Interactions

DDIs that are rooted in the combined pharmacodynamic actions of the interacting drugs are referred to as pharmacodynamic interactions or pharmacodynamic DDIs. These interactions can be fairly obvious

such as the interaction that might occur between caffeine and say, ramelteon or other sleep-inducing drugs. Ramelteon is a melatonin receptor agonist (both MT1 and MT2) that is used to treat insomnia, since it induces sleep. Caffeine is thought to produce CNS stimulation through adenosine receptor antagonism. Though ramelteon and caffeine interact with different receptors, they nevertheless produce opposite pharmacodynamic effects. Thus, it would not be difficult to understand how caffeine (in a cup of coffee, for example) might diminish the sleep-inducing effect of ramelteon. Though this interaction actually is not cited in DDI monographs, contemporary recommendations for good sleep hygiene, of course, do recommend against the use of CNS stimulants, including caffeine.

The foregoing interaction might be a subtle one that is dependent upon the amount of caffeine exposure (numbers of cups of coffee), and easily overlooked since caffeine is often not even regarded as a drug by anyone other than healthcare practitioners and pharmaceutical scientists. On the other hand, there are countless examples of very serious pharmacodynamic DDIs, some of which are actually quite well known even in popular culture. For example, the drugs for erectile dysfunction such as sildenafil (Viagra®) are vasodilators owing to their ability to inhibit phosphodiesterase 5 (PDE5). Nitroglycerin is a drug that is used to relieve the symptoms of angina by virtue of its vasodilatory effects. It is converted to nitric oxide (NO), which activates the enzyme guanylate cyclase, thereby promoting the synthesis of cyclic guanosine 3',5'-monophosphate (cGMP) leading to increases in intracellular cGMP levels. The net downstream effect subsequent to kinase activation culminates in the dephosphorylation of the myosin light chain of smooth muscle fibers, the release of calcium from smooth muscle cells, smooth muscle relaxation, and vasodilation. The combined use of sildenafil or similar drugs for erectile dysfunction along with nitroglycerin or other nitrates or nitrites for angina ultimately was discovered to cause severe, even fatal, hypotension. Other fatal reactions have been reported for the combination of the opioid analgesic, meperidine, combined with monoamine oxidase inhibitors such as tranylcypromine. Accordingly the combination of meperidine and monoamine oxidase inhibitors (MAOIs) is now contraindicated, and it is recommended that meperidine not even be administered within two weeks of an MAOI. MAOIs are used in depressive illness to increase CNS levels of neurotransmitters such as serotonin (5HT) by inhibiting the enzyme (MAO) that oxidatively deaminates 5HT. Though meperidine is used as a narcotic analgesic on account of its ability to stimulate kappa opiate receptors (OP2), it also interferes with the neuronal reuptake of serotonin. Thus, the combined use of meperidine with MAOIs can cause markedly elevated levels of 5HT leading to severe cardiovascular and/or neurologic adverse reactions, though there may be preexisting conditions such as hyperphenylalanemia that predispose patients to these outcomes.

Yet another example of a pharmacodynamic DDI would be the combined use of an adrenergic receptor agonist along with an adrenergic receptor antagonist. For example, men with benign prostatic hypertrophy (BPH) are likely to be treated with alpha 1 adrenergic receptor antagonists such as doxazosin (Cardura®). Doxazosin is a competitive inhibitor at postsynaptic alpha 1 adrenergic receptors in the sympathetic nervous system, and as a consequence it lowers blood pressure and also interferes with norepinephrine-induced stimulation of the prostatic stromal and bladder neck tissues. Thus the sympathetic tone-induced urethral stricture causing BPH symptoms is partially ameliorated by antagonists such as doxazosin. On the other hand, common over-the-counter (OTC) cough and cold remedies frequently contain the drug phenylephrine, which is used as a decongestant. Phenylephrine is an adrenergic agonist with some selectivity for the alpha 1 adrenergic receptor. Thus, patients with hypertension who take doxazosin may experience poorer blood pressure regulation when taking cold medications with phenylephrine, and patients with BPH who benefit from alpha 1 antagonists such as doxazosin, can experience relapses or worsening of symptoms if they should also use a cold product containing phenylephrine.

One final and more general example (of countless possible examples) of a pharmacodynamic DDI might be the combined use of aspirin and warfarin. Warfarin is an anticoagulant drug that can be taken orally for the treatment of thrombotic conditions such as deep vein thrombosis, or to prevent the risk of thrombus formation in patients who have recently had a myocardial infarction (MI) or who have atrial fibrillation (AF). Its anticoagulant activity derives from its ability to inhibit a hepatic enzyme, vitamin K epoxide reductase (VKOR), which plays a key role in the synthesis of four vitamin K-dependent clotting factors, factors II, VII, IX, and X. Aspirin shares indications in the management of post-MI patients to reduce the risk of a recurrent MI, and also for the reduction of the risk of stroke in patients with AF. Its value in these indications resides in its antiplatelet activity by virtue of its ability to inhibit cyclooxygenase enzymes and ultimately the production of thromboxane A2 by platelets, since thromboxane A2, an arachidonic acid metabolite, stimulates platelet aggregation. Thus, warfarin decreases the likelihood of thrombogenesis by slowing clot formation, and aspirin decreases the likelihood of thrombogenesis by limiting platelet aggregation. Another way of looking at it is that each drug independently also increases the risk of spontaneous bleeding, though the mechanisms are different. It follows, then, that the combined use of these two drugs, though sometimes warranted, nevertheless increases the risk of spontaneous bleeding more than the use of either drug alone. This risk is dose-dependent as well. Aspirin, in doses that might be used for treating the pain of arthritis, actually exhibits some anticoagulant activity that is mechanistically similar to that of warfarin. Thus the inadvertent use of high doses of aspirin by a patient who requires warfarin poses a serious risk of bleeding.

### 12.1.1.2 Pharmacokinetic Interactions

Pharmacokinetic (PK) interactions (PK DDIs) sometimes are also referred to as dispositional interactions. These interactions are characterized by the alteration of the PK or disposition (ADME) of one drug by another. Should these alterations be of sufficient magnitude, then significant changes in exposure to the affected drug can occur, ultimately leading to alterations in the effect of the drug. Exposure is quantified in PK terms by the area circumscribed by the plasma concentration versus time curve of a drug. That area is referred to as the area under the curve or AUC (see Chapter 10). If the exposure (i.e., AUC) is significantly increased, the pharmacological effects may become exaggerated and/or unwanted side effects may occur. If the exposure is significantly decreased, the therapeutic effects may fail to develop, and therapeutic failure may ensue. In some instances, changes in the peak plasma concentration ( $C_{max}$ ) of one drug caused by another drug can be sufficient to elicit significant adverse consequences.

This is exemplified by drugs that are HERG channel blockers in cardiac cells. Spikes in the plasma concentrations of those drugs can cause cardiac arrhythmias marked by prolonged QT intervals on the EKG and Torsade de pointes, which is potentially fatal. Some of the earliest second-generation antihistamines (i.e., nonsedating antihistamines) such as astemizole and terfenadine were ultimately withdrawn from the market after it was discovered that inhibition of their metabolism by other drugs such as erythromycin could increase their plasma concentrations and increase their risks for causing fatal cardiac arrhythmias. Greenblatt and colleagues, a group of basic and clinical pharmacologists at Tufts University Medical Center who have characterized the mechanisms and magnitude of DDIs for decades, have often referred to the drug in a DDI whose disposition is altered as the “victim” drug, and the drug that causes the change in the victim’s disposition as the “perpetrator.”

By what mechanisms might a perpetrator alter the AUC of a victim drug? Simply put, a perpetrator might be expected to increase the AUC of a victim drug by:

- Slowing its metabolism in the liver or the intestine
- Blocking the efflux of the victim drug from hepatocytes into the bile
- Increasing its oral absorption; this could occur if the perpetrator slowed intestinal metabolism of the victim or blocked transporter-mediated efflux of the victim back from intestinal epithelial cells into the lumen of the intestine
- Slowing renal secretion of the victim drug into the urine
- Decreasing the protein or tissue binding of the victim drug

Conversely, a perpetrator could be expected to decrease the AUC of a victim drug by interfering with its absorption or bioavailability, accelerating its metabolism in the intestine or liver, or accelerating its efflux from intestinal cells into the lumen of the intestine or

from hepatocytes into the bile. In theory, interference by a perpetrator drug with any facet of ADME of a victim drug could lead to an alteration in the AUC of the victim drug. For many years this broad mechanistic view of DDIs opened the door to predicting innumerable two-drug DDIs. Many of these potential DDIs were subsequently validated by case reports appearing in the medical and pharmaceutical literature that described adverse outcomes of small numbers of patients taking two drugs that had been predicted to exhibit dispositional interactions. Many times these case reports were not further validated (i.e., truly validated) with randomized control clinical trials, but they nevertheless became inscribed in the drug interaction literature, and ultimately included in DDI databases. The problem is that although many dispositional interactions can be predicted qualitatively on the basis of what is known about the ADME characteristics of interacting drugs, the quantitative changes in AUC are often too small to be clinically significant.

Dr. Benet of the University of California, San Francisco, has developed a compelling quantitative assessment of the clinical significance of DDIs arising from a perpetrator interfering with the plasma protein binding of a victim drug. That analysis makes it fairly clear that those types of DDIs will simply not likely be clinically significant. With regard to DDIs arising from the inhibition of hepatic metabolism of a victim drug, strategies that take into account the magnitude of AUC changes in predicting the clinical significance of DDIs weren't really codified in the DDI literature until the beginning of the twenty-first century. Quantitative treatment of perpetrator-induced AUC changes for victim drugs will be presented later in this chapter.

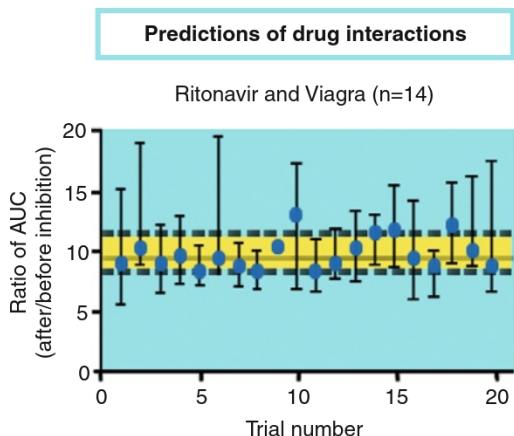
### 12.1.1.3 Sources of Drug Interaction Information

As the knowledge base pertaining to drug metabolism, especially hepatic drug metabolism, grew beginning in the middle of the twentieth century, so, too, did interest in DDIs. Numerous symposia have been organized around this topic. The clinical literature and clinical pharmacological literature became outlets for reviewing DDIs by therapeutic category and by mechanism. The collection of DDI monographs into hard-copy databases was undertaken to provide prescribers and pharmacists alike with handy reference guides to DDIs. A representative group of these DDI books is listed in the References. Some DDI databases have been cast in electronic versions for computer-based queries, or have been integrated into general pharmacy systems software that will flag DDIs for pharmacists prior to dispensing prescriptions that may interact. A problem, however, is that some DDIs enter these databases solely on the strength of small numbers of nonvalidated case reports so that it is possible that DDIs that will not lead to clinical consequences will inadvertently be flagged. DDI databases that are designed to allow user queries are somewhat more sophisticated in that they try to gauge the clinical severity of the interaction and may even characterize the reliability of the source data depending upon whether it arose from isolated case reports, randomly controlled clinical trials, or both.

Examples of DDI database software that permits queries about virtually any two-drug combination include DrugIx, Epocrates Rx, iFacts, Lexi-Interact, mobileMICROMEDEX, MosbyRx, Clinical Pharmacology OnHand, and Tarascon Pocket Pharmacopoeia. The foregoing operate on handhelds as well as on PCs. Comparisons about the accuracy of these databases to predict true positive or true negative interactions (i.e., no DDI) have been published.

With regard to DDIs specifically associated with enzyme inhibition or enzyme induction (refer to Chapter 8) or with transporter inhibition or induction (refer to Chapters 7 and 9), searchable online databases can be accessed that permit the user to explore which enzymes or transporters process a given drug and additionally how a given drug is likely to affect the function of a particular enzyme or transporter. David Flockhart at Indiana University created a database table that lists drugs that are either substrates, inhibitors, or inducers of individual CYP isoforms. The table can be found at <http://medicine.iupui.edu/flockhart/table.htm>. Each drug listed is hyperlinked to literature references and abstracts thereof in the PubMed database. The table and associated links are available *gratis* to users. The Japanese pharmaceutical company Fujitsu maintains an online searchable database that permits users to explore which enzymes and/or transporters process a specific drug, and whether a given drug inhibits or induces enzyme or transporter activity. For drugs that are substrates of enzymes or transporters one can find links to literature citations. Many of those citations are further linked to tables that list enzyme kinetic parameters such as Vmax or Km (see Chapter 8) for a specific drug-enzyme or drug-transporter combination. Likewise, for drugs that are found to be inhibitors of individual enzymes or transporters, there are links that provide the user with the results of *in vitro* inhibition experiments that quantitatively characterize inhibition by either an IC<sub>50</sub> value (see later) or a Ki value (see later). These parameters can be used to quantitatively predict AUC changes (see later). The Fujitsu ADME database is available online only with a paid subscription. Another searchable database providing Ki data is available from the University of Washington.

Much more sophisticated *in silico* predictors of DDIs are available to pharmaceutical scientists who are likely to have different objectives than healthcare practitioners. Here, the goal is to make a reasonable *a priori* prediction about the likelihood of clinically significant DDIs for NCEs even before the substance is placed into clinical trials. Even among these predictors there is a wide range of complexity and sophistication. One such program is Simcyp™, developed by experts in drug metabolism and PK in Great Britain. Simcyp™ combines quantitative *in vitro* data on drug processing, enzyme inhibition, and enzyme induction along with computer-based algorithms to predict quantitative AUC changes in humans for two-drug combinations. An example of the output is presented in Figure 12.1, which is modified from the Simcyp™ web site.



**Figure 12.1** The results of twenty simulations resulting in predicted fold increases in the AUC of sildenafil (Viagra®) after the administration of ritonavir. Note the mean of all twenty trials approaches ten-fold. Redrawn from Simcyp website.

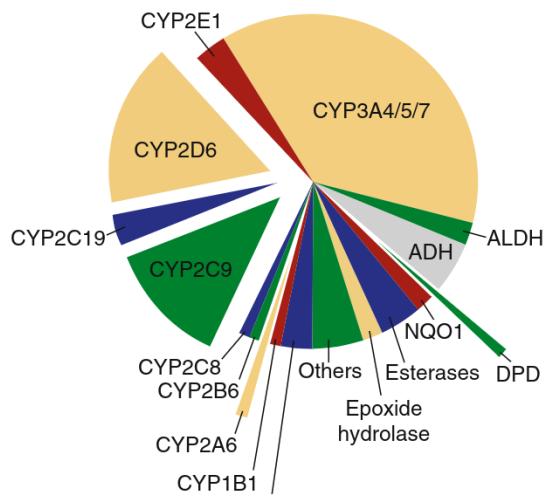
Simcyp™ asserts that its quantitative predictions are accurate about 80% of the time. These quantitative predictions about the PK significance of DDIs enable developers of NCEs to make “go versus no-go” decisions about further drug development even before the DDI is actually studied in a clinical trial. A different approach is taken by Aureus Pharma, a knowledge base company that markets AurSCOPE® ADME/DDI. This is a structured knowledge database that is fully annotated. The knowledge base contains information on 10,360 parent compounds, 2700 metabolites, and 197,560 biological activities extracted from over 7000 published articles. That knowledge base is then used in comparison with novel drug structures to predict a novel drug’s ADME and DDI characteristics. As with the Fujitsu database, both Simcyp and the AuroSCOPE™ ADME/DDI database can only be obtained through purchase or licensing agreements.

## 12.2 DDIs ASSOCIATED WITH ALTERED DRUG METABOLISM

Although, in theory, any inhibitor or any inducer of any drug metabolizing enzyme (see Chapter 8) could function as a perpetrator of DDIs, and any substrate of any drug metabolizing enzyme could become a victim, it is the inhibitors, inducers, and substrates of the cytochrome P450 enzymes (CYPs), for which the great majority of metabolic DDIs occur. To a large extent that is because CYPs are more prominent in the metabolism of drugs than any other type of enzyme, as shown in Figure 12.2.

### 12.2.1 DDIs Associated with Alteration of Cytochrome P450 Activity

As pointed out in Chapter 8, approximately three-fourths of all drugs are processed by CYPs, which are



**Figure 12.2** Relative importance of Phase I enzymes in drug metabolism.

also the most abundant drug metabolizing enzymes in key clearing organs such as the intestines and the liver. Within the intestines the relative contributions of individual CYPs to total CYP content are: CYP3A, 82%; CYP2C9, 14%; CYP2C19, 2%; and CYP2D6, <1%. The relative contributions of individual CYPs to total CYP content in the liver are: CYP3A, 40%; CYP2C9 and 2C19 combined, 25%; CYP1A2, 10%; CYP2E1, 9%; CYP2A6, 6%; CYP2D6, 2%; and CYP2B6, <1%. The importance of CYP enzymes in metabolic DDIs is illustrated by the number of papers appearing in scientific and medical journals in which DDIs have been ascribed to the alteration of CYP activity. These papers numbered fewer than 10 per year since the time of the discovery of the CYPs until the mid-1980s. From the mid-1980s until 2000 the rate of publication of papers investigating the role of CYPs in DDIs increased exponentially with between 150 and 200 such papers published in 2000.

The strategy for *a priori* prediction of the clinical significance of DDIs evolved from a meeting of academic, regulatory, and pharmaceutical industry scientists who met under the auspices of the European Federation of Pharmaceutical Sciences (EUFEPS), and first published a consensus document in the European Journal of Pharmaceutical Sciences in 2001. Similar reports appeared shortly thereafter in the U.S. literature in key journals in clinical pharmacology and drug metabolism. The main focus of this strategy was to use *in vitro* drug metabolism data, particularly data pertaining to the inhibition of CYP enzymes by a potential perpetrator, in the prediction of the clinical significance of DDIs for this perpetrator. The *in vitro* enzyme inhibition data can be used alone or in combination with limited *in vivo* (clinical) data about the perpetrator as will be discussed shortly. The *in vitro* experiments are designed to quantitate the inhibition of a specific CYP enzyme by the potential perpetrator drug.

### 12.2.1.1 Predicting the Clinical Significance of DDIs Associated with CYP Inhibition from In Vitro Data

Ultimately, these *a priori* predictions rely on accurate *in vitro* estimates of the inhibition of specific CYP enzymes by perpetrators (inhibitors). The removal of a drug (substrate) from the blood by enzymatic metabolism (e.g., a specific CYP enzyme) occurring in a clearing organ such as the liver, is defined by the key quantitative characteristics of the (collective) enzymes that mediate that substrate's metabolism, namely V<sub>max</sub> and K<sub>m</sub> (see Chapter 8). If low enough substrate concentrations are used, then the ability of specific hepatic CYP enzymes to remove or clear drug from the blood can be quantitated as  $\frac{V_{max}}{K_m}$ , which would also be known as the *in vitro* CL<sub>int</sub> or intrinsic clearance. Depending upon the nature of inhibition elicited by an inhibitor (perpetrator, in DDI terms), the addition of a perpetrator to an *in vitro* system might decrease V<sub>max</sub>, increase K<sub>m</sub>, or do both. Accordingly, it is possible to estimate a substrate's (victim, in DDI terms) intrinsic clearance in the absence (CL<sub>int</sub>) and in the presence (CL<sub>int,i</sub>) of a specified concentration of perpetrator (inhibitor) in *in vitro* experiments.

Alternatively we could measure the IC<sub>50</sub> or the K<sub>i</sub> (inhibitory constant) for the perpetrator. The K<sub>i</sub> of a perpetrator that is capable of inhibiting an enzyme (or transporter) is the dissociation constant for the enzyme-inhibitor complex. Accurate estimation of the K<sub>i</sub> requires, among other things, the appropriate definition or specification of the type of enzyme inhibition (e.g., competitive, noncompetitive, or uncompetitive). The appropriate *in vitro* experiments require that multiple concentrations of the inhibitor must be used as well as a range of substrate concentrations that embrace the substrate K<sub>m</sub>, and from these experiments both the type of inhibition elicited by the perpetrator can be deduced and the K<sub>i</sub> value for the perpetrator can be estimated. The K<sub>i</sub> will have units of concentration. Alternatively, K<sub>i</sub> values can be computed from IC<sub>50</sub> values for an inhibitor. The IC<sub>50</sub> is defined simply as the inhibitor concentration that decreases the biotransformation of a substrate at a single, specified concentration by 50%. This parameter obviously also has units of concentration (e.g., μM), and can be related to the K<sub>i</sub> as follows.

For noncompetitive inhibition the IC<sub>50</sub> and K<sub>i</sub> values will be equal. For competitive inhibition  $IC_{50} = K_i \left(1 + \frac{[S]}{K_m}\right)$ , and if [S] is much less than K<sub>m</sub>, then  $IC_{50} \sim K_i$ . For competitive inhibition, if [S]=K<sub>m</sub>, then  $K_i \sim 0.5 * IC_{50}$ . This numerical approximation applies for uncompetitive inhibition as well, when [S] approximates K<sub>m</sub>. Armed with an experimental K<sub>i</sub> for a perpetrator and an *in vitro* measure of CL<sub>int</sub> for the substrate (victim) acquired in the absence of perpetrator, CL<sub>int,i</sub> can be calculated from:

$$CL_{int,i} = \frac{CL_{int}}{1 + \frac{[I]}{K_i}} \quad (12.1)$$

where [I] denotes the concentration of perpetrator (inhibitor) used.

The matrices for conducting these *in vitro* experiments have not been standardized. Some investigators prefer to use human hepatic microsomes, others prefer heterologous systems that express individual CYP enzymes, and others prefer fresh or cryopreserved human hepatocytes or even human liver slices. Quantitative outcomes can be affected by the choice of *in vitro* system and also by the substrate selected as the victim drug. Substrates that have been engineered to be highly selective for specific CYPs and highly fluorescent, making them suitable for use in HTP screens for enzyme inhibition, may not always yield, in the presence of perpetrator (inhibitor) drugs, K<sub>i</sub> values that are comparable to those likely to occur when *bona fide* clinically used drugs are used as victim drugs in the assays. Indeed, The EUFEPS Conference Report recommended that the use of recombinant enzyme systems be limited to qualitative estimates of K<sub>i</sub> owing to the variable expression of enzyme across systems as well as the variable stoichiometries of reductase and cytochrome b5 relative to enzyme levels across systems. These variabilities can at least be partially compensated by the application of relative activity factors (RAF). Alternatively, a normalized rate (NR) for a CYP reaction catalyzed by a recombinant enzyme can be computed by multiplying the reaction rate by the mean specific content of that CYP that is found in native human liver microsomes, and then summed for all CYPs to give a total normalized rate (TNR), and the percent TNR can be directly related to percent inhibition. Unfortunately, for some of the CYP enzymes the specific content within different human livers can vary by more than an order of magnitude.

Another approach that has been used to correct for differences in microsomes isolated from human livers versus individual enzymes expressed in heterologous expression systems involves the application of intersystem extrapolation factors, or ISEFs, that are empirically derived correction factors.

Regardless of the *in vitro* matrix used in the estimation of K<sub>i</sub> and CL<sub>int</sub>, another problem is the failure to account for nonspecific binding of the substrate and inhibitor (i.e., victim and perpetrator, respectively). Failure to account for nonspecific binding within cells, to microsomes, even to plates and other incubation vessels can lead to errant estimates of K<sub>i</sub>.

Technical complications notwithstanding, how might the *in vitro* estimation of a victim drug's CL<sub>int</sub> in the presence of a perpetrator (CL<sub>int,i</sub>) be used to predict the clinical significance of a putative inhibitory DDI? If you'll recall, the underlying principle in predicting the clinical significance of a DDI is related to the prediction in the change in exposure (AUC) to the victim drug in the presence of the perpetrator drug (inhibitor). When there is no perpetrator involved, the AUC of the victim drug is inversely related to its intrinsic clearance (CL<sub>int</sub>). In the presence of an inhibiting perpetrator drug the AUC of the victim drug (AUC<sub>i</sub>) is inversely related to its intrinsic clearance (CL<sub>int,i</sub>). Thus, the exposure or AUC to the victim in the presence of an

inhibitor ( $AUC_i$ ) relative to the exposure to the victim in the absence of an inhibitor ( $AUC$ ) is given by:

$$\frac{AUC_i}{AUC} = \frac{CL_{int}}{CL_{int,i}} \quad (12.2)$$

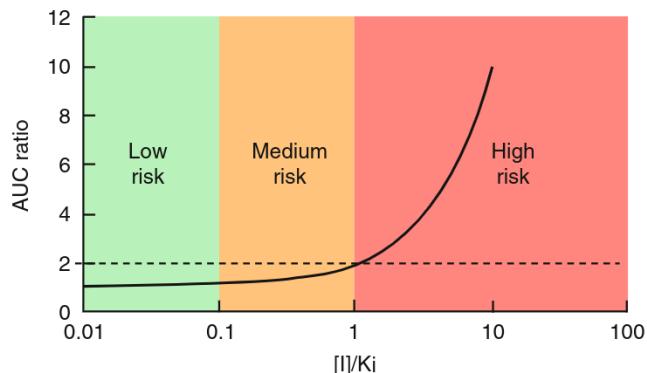
Since  $CL_{int,i}$  is determined by  $K_i$  and by the concentration of the inhibitor ( $[I]$ ) as shown in [Equation \(12.1\)](#), if [Equation \(12.1\)](#) is substituted for  $CL_{int,i}$  in [Equation \(12.2\)](#), then:

$$\frac{AUC_i}{AUC} = 1 + \frac{[I]}{K_i} \quad (12.3)$$

The ratio of  $\frac{AUC_i}{AUC}$  is referred to as the *AUC* ratio.

Once again, it denotes the increase in exposure to the victim in the presence of perpetrator relative to exposure in the absence of perpetrator. At this point there has *not* been a measurement of either  $AUC$  or  $AUC_i$ . These are *in vivo* measurements that could be measured only if the victim drug were administered to humans absent the perpetrator drug ( $AUC$ ) and in the presence of the perpetrator drug ( $AUC_i$ ). Nevertheless, the *AUC* ratio depends only on  $[I]$  and  $K_i$ , and the  $K_i$  value, in fact, can be acquired from *in vitro* experiments. The strategy developed at the EUFEPS conference was to estimate the *AUC* ratio from  $K_i$  values obtained from *in vitro* experiments in accordance with [Equation \(12.3\)](#). The relationship between the *AUC* ratio and the  $K_i$  value for an inhibitory perpetrator is shown in [Figure 12.3](#).

Actually, the predicted *AUC* ratio is plotted as a function of  $[I]/K_i$ , where  $[I]$  denotes the concentration of the inhibitory perpetrator drug. But selecting an appropriate concentration or value for  $[I]$  turns out to be a thorny issue. The *AUC* ratio is supposed to be predicted for the *in vivo* use of the victim drug in the presence of an *in vivo* concentration of the perpetrator. But, for predictive purposes, what *in vivo* concentration of perpetrator ( $[I]$ ) ought to be selected? Should it be the average steady-state concentration? Should it be the peak steady-state concentration? Should it be measured in plasma? Should it represent the concentration in the liver? And, by the way, just how high should the predicted *AUC* ratio be predicted to be, before a DDI is thought to be clinically significant?



**Figure 12.3** Relationship between *AUC* ratio and the ratio of  $[I]/K_i$ .

This last question has been addressed with a consensus opinion that a threshold *AUC* ratio of  $\geq 2$  is required before a DDI is considered a high risk DDI ([Figure 12.3](#)). Likewise an *AUC* ratio of  $\leq 1.25$  (for inhibitory DDIs) signifies low risk or no risk for a clinically significant DDI.

It would appear that by default *AUC* ratios that fall between 1.25 and 2 are viewed as posing moderate risk of a clinically significant DDI. Now, based on [Equation \(12.3\)](#) and [Figure 12.3](#) it should also be apparent that the same risk (for a clinically significant DDI) analysis can be cast in terms of the  $[I]/K_i$  ratio as well, only here the qualitative risk boundaries are as follows:

- $[I]/K_i \leq 0.1$ , small or no risk
- $[I]/K_i$  between 0.1 and 1.0; moderate risk
- $[I]/K_i \geq 1$ ; high risk of clinically significant DDI

Remember, the  $K_i$  is a parameter that can be derived strictly from *in vitro* experiments, and if the  $K_i$  is for a specific CYP enzyme, the experiments can be conducted using hepatic microsomes, cells, liver slices, or heterologous expression systems. The value for  $[I]$ , according to consensus, was supposed to be the steady-state peak plasma concentration ( $C_{max}$ ) of the perpetrator in humans. However, in spite of the consensus opinion, some have found that  $C_{max}$  (i.e.  $[I]_{max}$ ) does not always give the best prediction of the actual *AUC* ratio in humans. Various other fluid concentrations of the perpetrator have been proposed to be used in the computation of  $[I]/K_i$ , and some of them are listed in [Table 12.1](#).

Indeed, Houston and colleagues at the University of Manchester in the U.K., who have been instrumental in establishing and refining the prediction of DDIs in humans from *in vitro*  $K_i$  data, have shown that not only will the predictive accuracy, which they refer to as qualitative zoning (e.g., low risk, moderate risk, and high risk), vary depending upon which value is selected for  $[I]$ , but the predictive accuracy will also vary depending on which value of  $[I]$  is used even for a given CYP enzyme. In evaluating nearly 200 DDIs for which actual human *AUC* ratio data had been

**Table 12.1** Representative Methods for the Selection or Computation of  $[I]$

Choice of $[I]$	Computation of $[I]$
Average steady-state plasma concentration	$[I]_{av} = \frac{FD}{CL \times \tau}$
Peak steady-state plasma concentration	$[I]_{max} = \frac{[I]_{av} k\tau}{(1 - \exp^{-k\tau})}$
Maximum hepatic input concentration	$[I]_{in} = [I]_{av} + \left( \frac{kaFaD}{Q} \right)$
Concentration in the hepatocytes	$[I]_{hep} = \frac{[I]_{in \text{ vitro}}}{[I]_{medium}} \times [I]_{blood}$
Unbound concentration	$[I]_i \times f_u$

**Table 12.2** The Percentage of True Positives, True Negatives, False Positives, and False Negatives as a Function of the Value for  $[I]$ <sup>a</sup>

Result	CYP3A4				CYP2D6				CYP2C9			
	$[I]_{av}$	$[I]_{av_u}^b$	$[I]_{max}$	$[I]_{fin}$	$[I]_{av}$	$[I]_{av_u}^b$	$[I]_{max}$	$[I]_{fin}$	$[I]_{av}$	$[I]_{av_u}^b$	$[I]_{max}$	$[I]_{fin}$
True positives (%)	22	7	24	50	20	0	20	29	23	16	23	31
True negatives (%)	43	43	42	33	65	71	65	25	46	44	35	23
% correct <sup>c</sup>	65	50	66	83	85	71	85	54	69	60	58	54

<sup>a</sup>Values denote the percentage of data points in each category.

<sup>b</sup>This is  $[I]_{av\ unbound}$  or  $[I]_{av*fw}$ .

<sup>c</sup>Denotes the sum of true positives plus true negatives.

determined in clinical trials and for which they had *in vitro*  $K_i$  data, they were able to actually pair true AUC ratios with  $[I]/K_i$  data. One purpose for matching  $[I]/K_i$  data with actual AUC ratios was to assess the accuracy of the entire predictive strategy discussed earlier. They tabulated the true positive predictions that were defined as all the data points for which the AUC ratio was  $\geq 2$  AND the  $[I]/K_i$  ratio was  $\geq 1$  (Figure 12.3). They also tabulated the true negative predictions that were represented by AUC ratios  $\leq 1.1$  AND  $[I]/K_i$  ratios  $\leq 0.1$ . They did this for inhibitory DDIs involving three distinct CYPs, namely CYP3A4, CYP2D6, and CYP2C9. Their abridged findings are shown in Table 12.2.

The bottom line (literally) in Table 12.2 is merely the sum of the percentages of true positives and true negatives. Note, for example, for CYP3A4 the highest total percent correct AUC ratios that were predicted from  $[I]/K_i$  ratios (83%) occurred when  $[I]_{av}$  was used; that is, the computed concentration of perpetrator drug in the hepatic portal vein (Table 12.1). In contrast, for CYP2D6 the highest percent correct (sum of true positive plus true negative) predictions occurred when either  $[I]_{max}$  or  $[I]_{av}$  was used (85% for both). Interestingly, for inhibitory DDIs ascribed to CYP2C9 inhibition the predictive accuracy of an AUC ratio  $\geq 2$  or  $\leq 1$  was highest when  $[I]_{av}$  was used in the  $[I]/K_i$  ratio, however it only reached 69%.

What about the universality of the  $K_i$  value of a perpetrator for a particular CYP enzyme? Unfortunately the *in vitro*  $K_i$  value may vary depending on which victim drug is used as the substrate. There is evidence that some of the substrates engineered to be used (as victim drugs) with perpetrators (inhibitors) in HTP screens for assessing  $K_i$  values, yield  $K_i$  values for specific CYP enzymes that don't correlate very well with the  $K_i$  values computed when standard (clinically used) drugs are used as victim drugs. And, as mentioned earlier,  $K_i$  values can vary depending upon which *in vitro* matrix (isolated microsomes vs. heterologously expressed enzymes vs. hepatocytes) is used to study the DDI.

Yet another problem may arise from the increasingly apparent phenomenon of atypical enzyme kinetics of the substrate drug. One of the problems that has plagued the drug metabolism community until

recently is the apparent lack of correlation between the clearance rates of multiple probes for the same CYPs. In part, at least, these poor correlations can now be ascribed to the allosteric kinetics of the CYP enzymes. For example, a single substrate for CYP3A4 may be capable of binding at either of two different locations in the broader binding site (see Chapter 8). Two molecules of substrate might bind simultaneously at two different locations within the broader binding site, which could lead to homotropic activation of the enzyme. Two different substrates might bind simultaneously at two different locations within the broader binding site leading to heterotropic activation of the enzyme. Allosteric kinetics occur among CYPs other than CYP3A, and they give rise to atypical enzyme kinetics that are not described by the simple Michaelis-Menten equation. Multiple binding arrangements for different CYP3A4 substrates may contribute to  $K_i$  values for a single inhibitor that vary by more than 10-fold, depending upon the substrate used. Carefully structured *in vitro* studies with more than one substrate and a range of substrate concentrations that enabled multisite kinetic analysis have demonstrated partial inhibition, cooperative inhibition, and concentration-dependent inhibition, but no mutual inhibition of CYP3A4 when combinations of nifedipine, midazolam, felodipine, and testosterone were used simultaneously. Clearly, the failure to account for atypical kinetics in *in vitro* experiments could give rise to errant values for  $K_m$  and  $K_i$ . The EUFEPS conference report recommended that the issue of enzyme cooperativity for CYP3A4 and CYP2C9 be addressed, at least partially, by the determination of IC<sub>50</sub> values using at least two low (therapeutic) concentrations of at least two substrates, one of which is known to exhibit homotropic cooperativity, and by full characterization of activation kinetics when defining *in vitro* CL<sub>int</sub> values.

It could be observed that in light of the vexing and variable issues such as the selection of *in vivo* perpetrator concentration ( $[I]$ ), the experimental system for estimating  $K_i$ , the issue of nonspecific victim and perpetrator binding, and the sometimes atypical kinetics (see Chapter 8) of CYP enzymes associated with multiple substrate binding sites, heterotropic, and homotropic effects, that it is surprising that  $[I]/K_i$  predictions of AUC ratios are as good as they are. After all, for DDIs involving inhibition

of CYP2C9, CYP3A4, and CYP2D6 the accuracy of the clinical significance of DDIs from  $[I]/K_i$  ratios computed from *in vitro*  $K_i$  values paired with *in vivo* perpetrator concentrations ( $[I]$ ) was about 70%, 83%, and 85%, respectively (Table 12.2).

### Refinements to the Prediction of the Significance of Inhibitory DDIs Based on *In Vivo* Values for $[I]$ and *In Vitro* Values for $K_i$

**Accounting for Mechanism-based Inhibition** If a perpetrator elicits either competitive or noncompetitive inhibition of a CYP enzyme, then the *in vivo* AUC ratio can be predicted from an appropriately determined value for  $[I]$  and for  $K_i$  in accordance with Equations (12.1), (12.2), and (12.3). On the other hand, if a perpetrator elicits mechanism-based inhibition (see Chapter 8) of a CYP, then the AUC ratio becomes:

$$AUC_{ratio} = \frac{k_{deg} + \frac{[I] \times k_{inact}}{[I] + K_{iapp}}}{k_{deg}} \quad (12.4)$$

where, as defined in Chapter 8,  $k_{inact}$  is the apparent inactivation rate constant for the enzyme;  $K_{iapp}$  is the concentration of inhibitor (perpetrator) that elicits a  $k_{inact}/2$ ; and  $k_{deg}$  is the degradation rate constant for the enzyme.

There is some evidence that it may be possible to apply Equation (12.3) even in the case of mechanism-based inhibition so long as the inhibiting drug is preincubated for 30 minutes prior to the addition of the substrate.

Mechanism-based enzyme inhibition may be one of the more important types of DDIs, and examples of drugs that inhibit CYP enzymes in this fashion include paroxetine inhibition of CYP2D6, verapamil inhibition of CYP3A4, and the inhibition of CYP3A4 by protease inhibitors such as ritonavir. Some of the other drugs that cause mechanism-based inhibition of CYP enzymes include erythromycin, fluvoxamine, and ethinyl estradiol.

**Accounting for the Fraction of Drug Metabolized by a Given CYP Enzyme** How much of a victim drug must be metabolized by an enzyme, before enzyme inhibition by a perpetrator is likely to be clinically significant? Benchmarks have been set as low as 30%, but a good case can be made for 50%. For a drug that is cleared in part by metabolism via a CYP enzyme, its clearance in the presence of an inhibitor (perpetrator) of that enzyme can be defined as follows:

$$CL_{int,i} = CL_{int}(1 - fm_{cyp}) \quad (12.5)$$

where  $CL_{int}$  represents all clearance routes. Accordingly, the maximum impact on the AUC ratio that could occur if the metabolic contribution to drug clearance were completely stopped by a perpetrator (inhibitor) would be given by:

$$AUC_{ratio} = \frac{1}{1 - fm_{cyp}} \quad (12.6)$$

Thus, only if  $fm_{cyp}$  were 0.5 would the AUC ratio double by CYP inhibition. However, as described earlier, the extent of clearance inhibition associated with enzyme inhibition actually depends upon the  $K_i$  value for the perpetrator. If a victim drug is metabolized by a single CYP enzyme, and if metabolism accounts for  $\geq 50\%$  of the victim drug's total clearance, then the AUC ratio arising from inhibition of that enzyme by a perpetrator is given by the Rowland-Matin equation:

$$AUC_{ratio} = \frac{1}{\frac{fm_{cyp}}{1 + \frac{[I]}{K_i}} + (1 - fm_{cyp})} \quad (12.7)$$

If a victim drug (i.e., substrate) is subject to multiple metabolic pathways accounting for more than 50% of the total clearance of the victim drug, and if a perpetrator (inhibitor) affects those enzymes in quantitatively different ways, another layer of complexity is added to the estimation of the AUC ratio from  $[I]/K_i$  data. If, say, two pathways are subject to inhibition by a particular inhibitor, the fraction of victim drug metabolized by each of the two pathways is known, and the  $K_i$  values for the perpetrator on each of the two pathways is known, then the AUC ratio can be described by:

$$AUC_{ratio} = \frac{1}{\left( \frac{fm_{cyp1}}{1 + \frac{[I]}{K_i, 1}} \right) + \left( \frac{1 - fm_{cyp2}}{1 + \frac{[I]}{K_i, 2}} \right)} \quad (12.8)$$

**Accounting for Cooperativity** In Chapter 8 it was noted that the kinetics of drug processing do not always occur in a manner that can be described by the Michaelis-Menten model, and that non-Michaelis-Menten kinetics can occur when an enzyme binding site accommodates more than one molecule at a time, opening the door to the phenomenon of cooperativity. Two identical molecules binding at the same time (homotropic) can be either positively or negatively cooperative. Two different molecules binding at the same time (heterotropic) can be positively or negatively cooperative. For an enzyme that can accommodate two molecules at the same time, the AUC ratio elicited by an inhibitor (perpetrator) can be described as follows:

$$AUC_{ratio} = \frac{\left(1 + \frac{[I]}{K_i}\right)^2}{1 + \frac{\gamma [I]}{\delta K_i}} \quad (12.9)$$

where  $\gamma$  is an interaction factor that denotes the change in catalytic efficacy in the presence of inhibitor, and  $\delta$  is an interaction factor that denotes the change in binding affinity in the presence of inhibitor. When these two interaction factors are equivalent, Equation (12.9) simplifies to Equation (12.3).

**Uncompetitive Inhibition** As with mechanism-based inhibition, the failure to acknowledge the occurrence of uncompetitive inhibition could lead to errors in the prediction of the AUC ratio and risk of a DDI. Although there are, as yet, no prominent examples of uncompetitive inhibition of CYPs, the AUC ratio for the phenomenon would be given as:

$$AUC_{ratio} = 1 + \left( \frac{[I]}{K_i} \right) \left( \frac{[S]}{[S] + K_m} \right) \quad (12.10)$$

where  $[S]$  and  $K_m$  are the concentration and Michaelis constant for the victim drug, and  $[I]$  and  $K_i$  have their usual meanings for the perpetrator drug.

### 12.2.1.2 Interactions Resulting from Increased Enzyme Activity

In Chapter 8, the major role played by nuclear receptors as transcription factors in the increased expression of drug metabolizing enzymes was described. The key nuclear receptors in this regard are the AhR causing increased expression of CYP1A; PXR causing increased expression chiefly of CYP3A but also CYP2C9; and CAR causing increased expression of CYP2B6 and CYP2C. Actually, activation of PXR or CAR can lead to increased expression of some of the same enzymes due to their overlapping activities. Just as CYP3A plays a greater role in the oxidative metabolism of drugs compared to other CYP enzymes, PXR agonism is primarily responsible for the increased expression of enzymes, namely CYP3A4, and also for increased expression of P-glycoprotein (PgP), an efflux transporter. To be sure, CYP activities can be increased by mechanisms other than transactivation of CYP genes. For example, ethanol induces its own metabolism by CYP2E1 via stabilization of the protein thereby slowing its rate of degradation. And, as mentioned earlier and described in Chapter 8, some drugs can increase rates of drug metabolism by heterotropic or homotropic activation. However, it would appear that the majority of DDIs associated with perpetrator-elicited increases in the rate of metabolism of victim drugs is associated with perpetrator agonism of orphan receptors such as the PXR.

Long before the roles of AhR, PXR, or CAR in enzyme induction were known, it was recognized that numerous xenobiotics, including some drugs, were capable of increasing the activity of drug metabolizing enzymes; most notably the activity of CYPs. One of the earliest comprehensive compilations of information about the experimental induction of drug metabolizing enzymes by xenobiotics was compiled by Dr. A. H. Conney. Dozens of monographs and review papers about enzyme induction and DDIs have been published in both the clinical and scientific literature since Conney's review of enzyme induction and its pharmacological consequences in 1967. Some focus on therapeutic categories of drugs that are affected by enzyme induction. Others focus on the types of drugs that elicit enzyme induction such as the rifamycins. Still others focus on phytochemicals found within

herbal remedies or nutraceuticals that can cause enzyme induction. Both the Flockhart database on DDIs and the Fujitsu database (*vide supra*) can both be searched for drugs that are CYP inducers.

If a perpetrator can, through transactivation of one or more CYP genes, increase the expression of CYP enzymes in, say, the liver, then might it be possible, as with inhibitory DDIs, to predict the clinical significance of that transactivation based on *in vitro* measures of transactivation? Possibly, but just how analogous to *in vitro* enzyme inhibition is the phenomenon of *in vitro* enzyme induction? For *in vitro* inhibitory experiments the key quantitative parameter denoting a perpetrator's ability to inhibit a specific enzyme is  $K_i$ . On the other hand, transactivation experiments typically measure the ability of, for example, a PXR agonist, to increase PXR activation in a reporter gene assay using luminescence intensity as the signal for PXR activation. The magnitude of transactivation is referenced to a positive control such as rifampin and also to a negative control such as DMSO. If transactivation is referenced only to a negative control such as DMSO, then induction could be quantitated as the luminescence response of the perpetrator relative to the luminescence response to DMSO, or  $\frac{signal_{agonist}}{signal_{control}}$ .

However, in the absence of a positive control, this data would be difficult to interpret. For example would a signal ratio of 1.4 indicate a low, medium, or high risk of a clinically significant *in vivo* interaction? If the signal were referenced to a positive control such as rifampin, the extrapolation of the *in vitro* findings to *in vivo* significance may also be difficult. For example, the positive control (e.g., rifampin) is typically used in a single concentration in the *in vitro* experiments (e.g., 10 μM). Suppose the positive control caused the luminescence signal to be 10 times higher than background (i.e., 10 × transactivation). The test perpetrator, however, causes the luminescence signal to be five times higher than background. This response is 50% of the response caused by rifampin. How should that be interpreted? Of course it's possible to combine both the negative and positive controls into the quantitation of the response of a perpetrator *in vitro*. One way of doing so is as follows:

$$\% \text{ activation} = \frac{(signal_{agonist} - signal_{control})}{(signal_{rifampin} - signal_{control})} \times 100 \quad (12.11)$$

While taking account of the signal produced by a vehicle control such as DMSO, this method for quantitating transactivation is fundamentally still done in the context of the positive control (rifampin). Again, if the response were to be 50%, how should that be interpreted in a clinical sense? What if the response had been 20%? Such a response still might denote a two-fold increase (compared to control) in PXR activation if, for example, the rifampin signal were 10 times higher than the control signal.

As in the *in vitro* inhibition experiments, the outcomes must be referenced to some *in vivo*

concentration of the perpetrator. Michael Sinz and colleagues at Bristol-Myers Squibb Co. have evaluated the *in vitro* transactivation of the human PXR by 170 xenobiotics using [Equation \(12.11\)](#). They constructed dose-response curves for an entire range of concentrations for each perpetrator. Then they then asked the question, “What would be the expected *in vivo* percent transactivation for an *in vivo* concentration if perpetrator was the same as its expected peak plasma concentration,  $C_{max}$ ? They delineated perpetrators with a high potential of inducing CYP3A4 *in vivo* as those that caused 40% transactivation *in vitro* at concentrations equal to the known *in vivo*  $C_{max}$ . Those that caused less than 15% transactivation *in vitro* at concentrations equal to the known *in vivo*  $C_{max}$  were said to have a low potential to cause enzyme induction *in vivo*. Compounds that caused between 15 and 40% transactivation *in vitro* at concentrations equal to the known *in vivo*  $C_{max}$  were deemed to have a moderate potential to cause enzyme induction *in vivo*. This is broadly similar to the qualitative zoning that evolved for the prediction of the clinical significance of inhibitor DDIs based on  $[I]/K_i$  ratios. Using the qualitative zoning boundaries set forth by the group at Bristol-Myers Squibb, their analysis of 170 putative enzyme inducers revealed only nine that they predicted to have a high potential for clinically significant enzyme induction, and another four that they predicted to have a moderate potential. The rest had low or no potential.

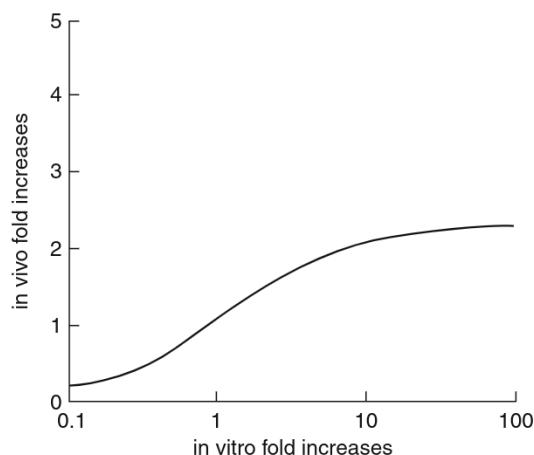
Again, whether [Equation \(12.11\)](#) constitutes the best way to characterize *in vitro* transactivation, or whether activation referenced exclusively to a negative control would be more meaningful still remains to be determined. For example an alternative strategy might be to look at the ratio of  $\frac{signal_{agonist}}{signal_{control}}$ . Suppose this were

3.0 at an *in vitro* concentration equal to the known *in vivo*  $C_{max}$ ? How could we rule out a significant *in vivo* enzyme induction effect? Moreover, any prediction that includes a value for an *in vivo* concentration of perpetrator raises the same issues that were discussed in [Section 12.2.1.1](#). That is, should total concentration in plasma be used or should the unbound concentration be used? Should the concentration be a  $C_{max}$  as proposed by the group at Bristol-Myers Squibb, or should it be a concentration at the inlet of the liver or the concentration within hepatocytes? A group of Pfizer scientists suggest using the plasma  $C_{max}$  adjusted for binding (i.e., unbound  $C_{max}$ ).

Just as these issues are still under investigation for the use of the  $[I]/K_i$  paradigm in the prediction of the clinical significance of inhibitory DDIs, so, too, must they be evaluated in any predictive method aimed at inferring the clinical significance of enzyme induction-related DDIs that might be drawn from a mix of clinical drug concentrations and *in vitro* measures of enzyme induction. For inhibitory DDIs you may recall that a consensus opinion that perpetrator  $K_i$  values  $\leq 1\mu M$  tend to be predictive of a high probability of a clinically significant DDI. There is insufficient analysis of induction-type experiments to permit a similar generalization about putative inducing agents.

Among the “high probability” inducers that produced *in vitro* transactivation greater than 40% of that produced by rifampin, all but one of the EC50 values for induction was less than 2  $\mu M$ . However, only nine substances were high probability inducers, and even some low and medium probability inducers exhibited EC50 values less than 2  $\mu M$ . On the other hand, the Pfizer group used both *in vitro* Emax and EC50 values to try and characterize the extent of induction. They first computed relative induction scores (RIS) for each of about two dozen inducers. The RIS was computed as  $\frac{C_{max,unb} * E_{max}}{C_{max,unb} + EC50}$ . Next they correlated the *in vitro* RIS with the percent reduction in AUC measured *in vivo*. This was done for two separate CYP3A4 substrates, namely midazolam and ethinyl estradiol. Though the curves were not identical for both substrates, the data points for both curves (one curve for midazolam and another for ethinyl estradiol) fit a three-parameter Hill function extremely well. Maximum *in vivo* induction plateaued once the RIS reached a value of about 0.5. From this analysis, we might infer that *in vitro* RIS values very much greater than 0.5 (e.g., 9 or 10) will produce no greater *in vivo* decreases in AUC than RIS values at approximately 0.5. Unfortunately, the slopes of the *in vivo* decrease in AUC versus RIS curves for midazolam and ethinyl estradiol were quite different from one another, making it hard to delineate the lowest RIS score that might define a clinically significant inductive DDI. If, for example, we were to assume that a 25% decrease in AUC would represent the least likely occurrence of a clinically significant DDI, then the RIS for inducers of midazolam metabolism would have to exhibit an RIS of approximately 0.02, whereas inducers of ethinyl estradiol metabolism would have to exhibit only an RIS of about 0.07.

It must also be recognized that there is not a linear correlation between the extent of *in vitro* induction or transactivation and in the magnitude of *in vivo* increases in clearance or decreases in AUC in humans. Though it represents a fairly crude attempt to match *in vivo* changes in drug metabolism (e.g., decreases in AUC, increases in clearance, or increases in metabolic ratios) to *in vitro* increases in transactivation or metabolic activity (expressed as fold increases), the nonlinearity between the two is exemplified in [Figure 12.4](#). *In vitro* data was paired with *in vivo* data when both could be found for common perpetrator/victim pairs. For example, *in vivo* data for rifampin-induced changes in warfarin clearance was paired with *in vitro* data for induction of CYP2C9 by rifampin. The inducers for which both *in vivo* and *in vitro* data could be matched—typically not from the same study—were ritonavir, rifampin, sulfispirazine, phenobarbital, troglitazone, and either hyperforin or St John’s wort. The victim drugs (or endobiotics) were warfarin, midazolam, erythromycin, alfentanil, and cortisol. As can be seen from the plot in [Figure 12.4](#), even for perpetrators that could elicit more than 15-fold increases in *in vitro* metabolism or transactivation, those same perpetrators appeared to cause *in vivo* increases in clearance or metabolic ratios or decreases in AUC of not much more than two-fold.



**Figure 12.4** Increases in *in vivo* clearance or decreases in AUC correlated to *in vitro* fold increases in transactivation or enzyme activity.

As we get closer to being able to link EC50 and  $E_{max}$  values for PXR activation to the prediction of the significance of *in vivo* inductive DDIs, then even *in silico* approaches could also be used. Computer modeling methods are in place for computing EC50 values of transactivators on the basis of their fits to pharmacophore models of the human PXR (hPXR). As these models are refined and the computer-generated estimates of EC50 values begin to match more closely the actual *in vitro* EC50 values generated from reporter-gene transactivation assays, it may become possible to use these values along with, for example, *in vivo*  $C_{max}$  values in order to predict the clinical significance of enzyme induction interactions.

To get a sense of the xenobiotics that are known to cause clinically significant inductive DDIs in humans, check out the inducer table at <http://medicine.iupui.edu/flockhart/table.htm>.

### 12.2.2 Phase II Enzymes and DDIs

More is known about metabolic DDIs associated with CYP enzymes than any other drug-processing enzyme. This no doubt reflects the comparative extent to which these enzymes have been researched, the relative abundance of these enzymes, and their overall contribution to drug metabolism. Phase II enzymes have been less intensively studied, are collectively responsible for the metabolism of fewer drugs than the CYPs, and are often not responsible for the rate-limiting step in drug metabolism. It follows that less is known about their role in DDIs. Having said that, there are some very important Phase II reactions, and among the Phase II enzymes UDP-glucuronosyltransferases (UGTs) mediate more than one-third of the Phase II reactions of all drugs that are subject to Phase II metabolism. More information about UGTs and other Phase II enzymes can be found in Chapter 8.

As a quick reminder, though, the human UGTs comprise a superfamily of enzymes that play a role in the metabolism of numerous endobiotics and

xenobiotics. They utilize UDP-glucuronic acid as a cofactor, and are bound to the internal membrane of endoplasmic reticulum. The human UGT superfamily consists of the UGT1 and UGT2 families and the UGT1A, UGT2A, and UGT2B subfamilies. As is the case with CYP enzymes, the UGTs tend to convert molecules to more hydrophilic metabolites, and—more often than not—less pharmacologically active molecules than their parent molecules. Although there is some evidence of substrate selectivity, in fact, most substrates are glucuronidated by multiple UGTs. The regulation of UGTs bears some similarity to the regulation of CYPs in that AhR, CAR, and PXR have been found to be involved in the increased expression of individual UGTs. Specifically, hPXR and CAR mediate UGT1A1 induction, whereas AhR regulates UGT1A6 and UGT1A9 induction.

UGTs are, like CYPs, inhibitable. However, unlike inhibitors of CYPs, there are very few known UGT inhibitors exhibiting *in vitro*  $K_i$  values  $\leq 1 \mu\text{M}$ . Therefore, although ranitidine, propranolol, and cisapride have been shown to inhibit acetaminophen glucuronidation, it's not clear that this inhibition would be a clinically significant phenomenon. Likewise, although atovaquone, fluconazole, naproxen, and valproic acid have been shown to inhibit the glucuronidation of the antiretroviral drug, AZT, only the effects of fluconazole on the PK parameters of AZT (namely AUC and  $C_{max}$ ) could be characterized as clinically meaningful. Tacrolimus, which elicits a  $K_i$  of  $0.033 \mu\text{M}$  when mycophenolic acid is the UGT substrate in a kidney microsomal system, was shown to cause a 1.5-fold increase in the AUC for mycophenolate in 18 stable renal transplant patients. It's somewhat disconcerting that the two UGT inhibitors eliciting what might be considered clinically significant changes in victim drug PK (i.e., fluconazole inhibition of AZT glucuronidation and tacrolimus inhibition of mycophenolate glucuronidation) exhibit *in vitro*  $K_i$  values of  $163$  and  $0.033 \mu\text{M}$ , respectively. Probenecid, better known for its inhibition of renal OATs, however, has been shown to inhibit UGT *in vitro*, and to significantly slow the clearance of lorazepam in humans.

Rifampin, carbamazepine, phenytoin, phenobarbital, and oral contraceptives have been studied for their ability to induce UGTs and alter PK characteristics of select victim drugs in humans. Rifampin caused a more than five-fold decrease in the AUC of codeine and a comparable increase in the oral clearance of codeine, though it didn't alter the AUC of morphine or either of the morphine glucuronides. Rifampin also doubled the clearance of lamotrigine and reduced its half-life comparably. It also caused a profound reduction in the oral bioavailability of propafenone. Estrogen-containing oral contraceptives caused a 65% increase in acetaminophen clearance, and shortened its half-life by about one-third. However, the effects of the anticonvulsant agents, phenytoin, carbamazepine, and phenobarbital, on acetaminophen PK were in the range of 25 to 33%, making it somewhat equivocal as to whether this represents a clinically significant effect.

### 12.2.3 Inhibition of Xanthine Oxidase

An interesting example of a DDI due to the inhibition of a non-CYP enzyme that can have serious clinical consequences is the inhibition of xanthine oxidase by allopurinol 6-mercaptopurine (6-MP) as an antimetabolite type of antineoplastic drug. One of its indications is in the treatment of inflammatory bowel disease. Actually, 6-MP is a prodrug whose active metabolite, 6-thioguanine (6-TG) is responsible for its therapeutic activity. Some nonresponders to 6-MP do not form sufficient amounts of 6-TG. A complementary pathway of 6-MP metabolism is oxidation to 6-thiouric acid (6TU), which is mediated by xanthine oxidase. Inhibition of this complementary pathway by allopurinol shunts the metabolism of 6-MP favoring increased formation of 6-TG.

### 12.2.4 Pharmacokinetic Considerations

For an inhibitory perpetrator, the magnitude of change in the plasma concentration versus time curve of the victim, or even in the AUC of the victim may not be well portrayed by Equations (12.3) through (12.9) even if the correct values of  $[I]$  and  $K_i$  are used. Likewise, even if Equation (12.11) were ideally suited to estimate the extent of *in vitro* activation by an inducing perpetrator, and if the extent of *in vivo* induction or transactivation could be predicted from an *in vivo*  $C_{max}$  value of the perpetrator drug, the actual clinical impact of the perpetrator on the plasma concentration versus time curve of the victim drug might, nevertheless, be errantly predicted. Errant prediction of *in vivo* PK outcomes from *in vitro* data ( $K_i$  for inhibitory perpetrators and % maximum transactivation for inductive perpetrators) and *in vivo* data about the perpetrator (e.g.,  $C_{max}$ ) most likely would occur for victim drugs with high values for  $CL_{int}$ . These drugs (i.e., high clearance drugs) are subject to such rapid metabolic clearance that their total clearances are limited by organ blood flow rather than by metabolic clearance. The overriding contribution of organ blood flow to total clearance can be seen in the simple, well-stirred model of clearance:

$$CL = Q \times \frac{CL_{int}}{(CL_{int} + Q)} \quad (12.12)$$

where  $Q$  is organ blood flow rate and  $CL_{int}$  is intrinsic clearance mediated by the metabolic machinery of the organ (e.g., liver) and is the product of intrinsic unbound clearance, ( $CL_{int,unb}$ ), and the unbound fraction of drug in the blood,  $f_u$ . Now, suppose  $CL_{int}$  of a victim drug was actually 10 times faster than hepatic blood flow, for example,  $CL_{int} = 10Q$ . The total organ clearance could be described as:

$$CL = Q \times \frac{10Q}{(10Q + Q)} \quad (12.13)$$

$$CL = .91Q \quad (12.14)$$

Next, suppose a perpetrator drug was added to the therapeutic regimen such that at its  $C_{max}$  value it was capable of doubling the rate of metabolism of the

victim drug. In this case  $CL_{int}$  would double from  $10Q$  to  $20Q$ . However total clearance would become:

$$CL = Q \times \frac{20Q}{(20Q + Q)} \quad (12.15)$$

or

$$CL = .95Q \quad (12.16)$$

Thus, the increase in  $CL$  would only be between 4 and 5%. If the victim drug were administered intravenously, it's not likely that there would be a detectable change in the plasma concentration versus time curve of the victim drug.

On the other hand, if the victim drug were given orally, there might be a significant change in the plasma concentration versus time curve. That's because the victim's hepatic extraction ratio is given by:

$$E = \frac{CL_{int}}{(CL_{int} + Q)} \quad (12.17)$$

If  $CL_{int}$  of the victim drug before treatment with a perpetrator were  $10Q$  then the baseline extraction ratio,  $E$ , would be 0.91. For a victim drug processed only by the liver, the oral bioavailability,  $F$ , would simply be:

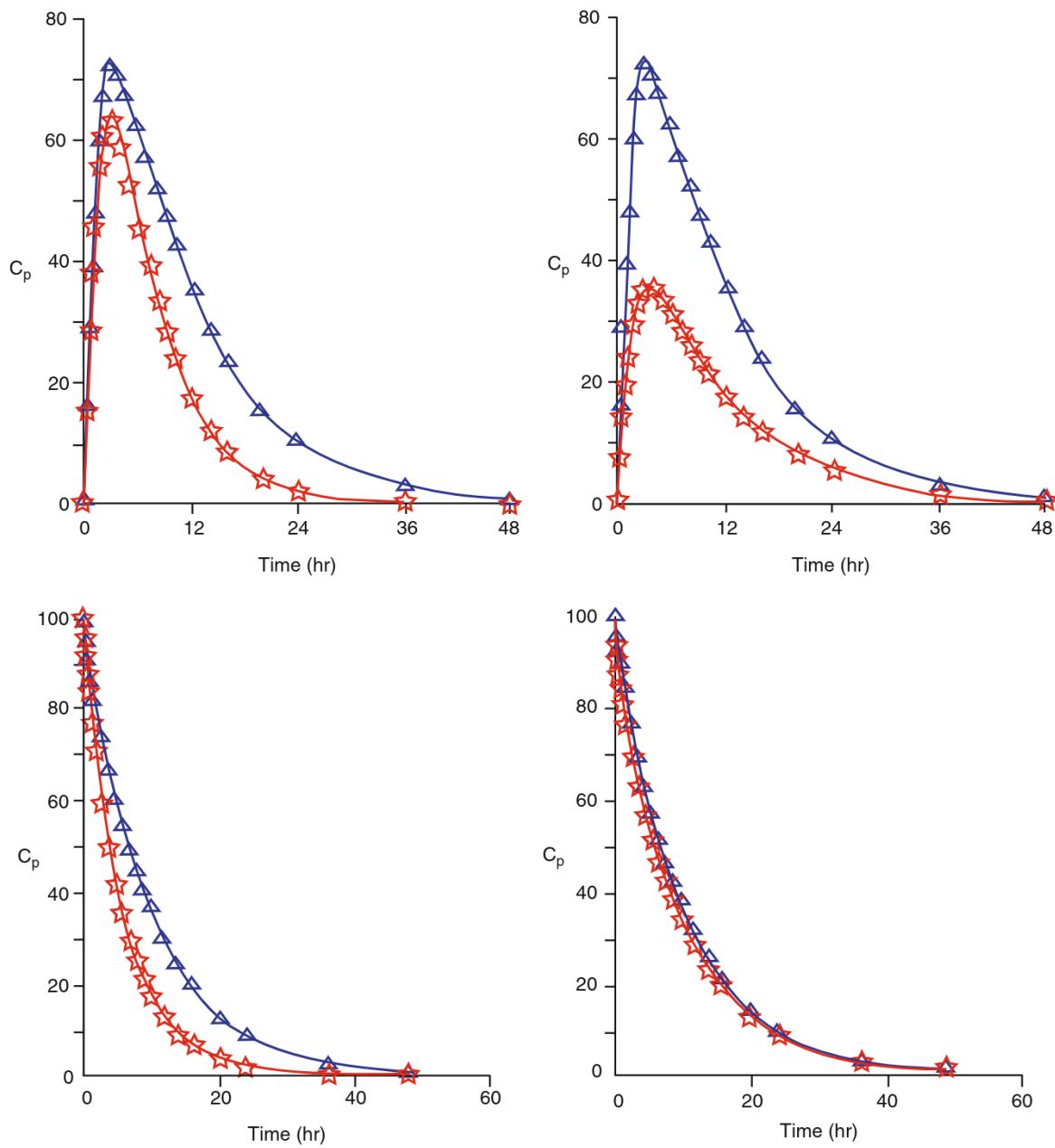
$$F = (1 - E) \quad (12.18)$$

or 9%. As shown earlier, if a perpetrator doubled the  $CL_{int}$  of the victim to  $20Q$ , then according to Equation (12.17),  $E$  would become 0.95, and  $F$  would become approximately 5%. Thus, for this high clearance victim drug a perpetrator capable of doubling the  $CL_{int}$  of the victim drug would decrease the AUC and the  $C_{max}$  of the victim drug by close to 50% if the victim drug were administered orally, but would fail to alter the AUC of the victim if the victim were given intravenously.

For the same high clearance victim drug, if a perpetrator were an inhibitor capable of decreasing the  $CL_{int}$  (initially  $CL_{int} = 10Q$ ) of the victim by 50%, then the clearance of the victim drug would fall from  $0.91Q$  to  $0.83Q$  according to Equation (12.12). This would represent about an 8.8% decrease in the total clearance rate of the victim drug. However the extraction ratio,  $E$ , would go from an initial value (absent perpetrator) of 0.91 to a value of 0.83, and the oral bioavailability would just about double from the initial value of 9% to a value of 17%. Here, too, the impact of the perpetrator on the AUC and  $C_{max}$  of the victim would depend upon the route of administration of the victim. If the victim were administered intravenously, even reducing  $CL_{int}$  by 50% would not produce clinically significant changes in victim PK. However, if the victim were given orally, and if a perpetrator reduced the victim's  $CL_{int}$  by 50%, then the AUC of the victim and its  $C_{max}$  would nearly double. These examples are illustrated in the simulations depicted in Figure 12.5.

Dextromethorphan is a widely used cough suppressant. However, it is also known to possess neuroprotectant, anticonvulsant, and antinociceptive effects associated with NMDA receptor antagonism, calcium

Au1



**Figure 12.5** Plasma concentration time curves for DDIs if the victim drug is a low clearance drug (left panels) or a high clearance drug (right panels). The lower graphs depict likely outcomes if the victim drug is given intravenously. The upper panels depict likely outcomes if the victim drug is given orally. For a perpetrator acting as an enzyme inducer, the baseline conditions (before perpetrator) would be depicted by the upper curves, and the effect of the perpetrator would be depicted by the lower curves. For a perpetrator acting as an enzyme inhibitor, the baseline conditions (before the perpetrator) would be depicted by the lower curves, and the effect of the perpetrator would be depicted by the upper curves. Either way, note that in the lower right panel, significant changes in  $CL_{int}$  of the victim elicited by a perpetrator are not reflected in the shape of the plasma concentration versus time curve of the victim or the victim drug's AUC.

channel inhibition, and interactions with sigma-1 sites and voltage-gated sodium channels. However, the neuroprotectant effects that potentially could be useful in treating Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, and neuropathic pain would require plasma concentrations considerably higher than those that develop after typical doses of dextromethorphan for cough. As it turns out, it's not

easy to generate measurable plasma concentrations of dextromethorphan, even when doses many times higher than antitussive doses are given. The main reason is that dextromethorphan is cleared so rapidly by hepatic metabolism mediated largely by CYP2D6. Dextromethorphan clearance has been estimated at approximately 110.25 L/min. If hepatic blood flow is approximately 1.5 L/min, then dextromethorphan

clearance is  $73.5Q$ . Clearly, its metabolic clearance is flow-limited. Accordingly, its oral bioavailability would be expected to be very limited:

$$F = 1 - \left( \frac{73.5Q}{74.5Q} \right) \quad (12.19)$$

or 1–E. Thus, dextromethorphan would be expected to exhibit a bioavailability of about 1.5%. Quinidine is a highly selective and potent inhibitor of CYP2D6. Its use in a fixed dose combination with dextromethorphan is currently under investigation with a view that the combination might be used to generate high enough blood levels of dextromethorphan so that it could be used for its neuroprotectant effects. Since quinidine is such a potent CYP2D6 inhibitor, the dosages of quinidine could be kept low enough to be devoid of eliciting any effects of its own apart from inhibition of CYP2D6. In theory, if quinidine were used in doses that gave rise to plasma quinidine concentrations equal to its  $K_i$  ( $<0.1 \mu\text{M}$  or  $<0.03 \text{ mg/L}$ ) for CYP2D6 inhibition, then  $F$  would become

$$F = 1 - \left( \frac{36.75Q}{37.75Q} \right) \quad (12.20)$$

or 2.7%. It should be noted that the usual therapeutic concentration range of quinidine is approximately 2 to 5 mg/L. Thus the  $C_{max}$  of dextromethorphan would double. In fact, actual clinical trials in which capsules containing 30 mg each of dextromethorphan and quinidine have shown that 30 mg of quinidine for seven days can increase the dextromethorphan  $C_{max}$  from 15.9 ng/ml when dextromethorphan is given by itself, to 95.5 ng/ml when it is given with quinidine. This combination would be an example of a purposefully engineered drug–drug interaction.

Or consider the estrogen receptor blocking drug raloxifene, used in the treatment of breast cancer. Raloxifene exhibits an intrinsic clearance around  $30Q$ . Thus its oral bioavailability is approximately 3%. It is metabolized by UGTs, which are inducible by drugs such as rifampin. If rifampin treatment were capable of doubling the intrinsic clearance of raloxifene to  $60Q$  it only would increase raloxifene's oral clearance by about 1%. However it would decrease its bioavailability by 50%.

## 12.3 DRUG TRANSPORTERS AND DDIs

Just as the discovery of CYP enzymes in the mid twentieth century led to intensive research regarding their role in DDIs, the relatively recent discovery of the role of drug transporters in drug disposition (see Chapters 7 and 9) has led to increasingly intense investigation of transporters in DDIs. However, even before very much was known about the molecular features of drug transporters or the mechanisms by which they affected drug influx or efflux, it was widely recognized that an organic anionic drug transporter in renal proximal tubular epithelial cells played a significant role in the renal secretion of penicillin and most other  $\beta$ -lactam

antibiotics, and that the uricosuric agent, probenecid, was capable of inhibiting renal penicillin secretion by inhibiting the organic anion transporter. This observation led to the purposeful combined use of probenecid with certain  $\beta$ -lactams for the express purpose of slowing their renal tubular secretion into the urine, increasing their blood levels, and extending their half-lives. This engineered DDI was used years before it was recognized that  $\beta$ -lactams kill susceptible bacteria in a time-dependent manner rather than a concentration-dependent manner, and has been used clinically with good effect for many decades. The organic anion transporter that is responsible for renal penicillin secretion and that is inhibited by probenecid is OAT1 (see Chapter 9). Other transporters that are important in mediating the influx or efflux of drugs in ways that affect absorption and excretion are listed in Table 12.3.

### 12.3.1 Interactions Involving P-Glycoprotein (PgP)

PgP is expressed on the apical membrane of intestinal epithelial cells, the luminal membrane of proximal tubular epithelia, the apical (or bile canalicular) membrane of hepatocytes, and on the luminal membrane of brain capillary endothelial cells. Accordingly, it is situated to play a role in intestinal drug absorption, renal drug excretion, biliary drug excretion, as well

Table 12.3

Transporters Playing Key Roles in Drug Absorption and Excretion

Gene or Gene Family	Common Name
<b>ATP Binding Cassette (ABC) Transporters</b>	
ABCB1	PgP (P-glycoprotein)
ABCC1	MRP1
ABCC2	MRP2
ABCC3	MRP3
ABCC4	MRP4
ABCC5	MRP5
ABCC6	MRP6
ABCC11	MRP8, BSEP (bile salt exporter protein)
ABCG2	BCRP (breast cancer resistance protein), MXR
<b>Solute Carrier Family Transporters (SLC)<sup>†</sup></b>	
SLC21	OATP (organic anion transporting polypeptides)
SLC22	OAT (organic ion transporters)
SLC15A1	OCT (organic cation transporters) PEPT-1 (oligopeptide transporter)
SLC28& SLC29	NT (nucleoside transporters)

\*There are 49 genes in the ABC superfamily.

<sup>†</sup>There are 43 families and ca. 300 genes in the SLC superfamily.

as drug extrusion across the blood–brain barrier. In a manner analogous to the CYPs in general and to CYP3A4 in particular, PgP exhibits a broad substrate specificity (i.e., nonspecificity), creating great opportunities for its involvement in DDIs. Digoxin is an inotropic cardiac steroid that is still used in the management of heart failure. It has a relatively narrow therapeutic margin of safety, and postdistribution serum or plasma concentrations above 2 ng/ml tend to be associated with serious ADRs including cardiac arrhythmias. Digoxin is essentially free from hepatic or intestinal metabolism by CYP enzymes. However, it is transported by PgP, therefore it has become a useful probe of PgP activity *in vivo*. Quinidine, as we have seen, is a very potent inhibitor of CYP2D6. It is also an inhibitor of PgP. The ability of quinidine to significantly increase blood levels of digoxin leading to serious ADRs has been known for many years, though the central role of PgP inhibition by quinidine is a more recent discovery. It is now recognized that quinidine can increase the absolute bioavailability of digoxin by at least 15% as a consequence of inhibiting intestinal PgP and bile canalicular PgP (which in the absence of quinidine would efflux digoxin into the intestinal lumen and the bile canaliculus, respectively). It also inhibits renal epithelial PgP, thus slowing digoxin's renal secretion. The net consequence can mean an increase in digoxin AUC by as much as 75%. Other drugs that have been reported to increase digoxin bioavailability and/or decrease its renal and biliary excretion are itraconazole and ritonavir.

Studies in *mdr1a*(*-/-*) mice who do not express PgP in brain capillary endothelial cells comprising the blood–brain barrier, exhibit much higher brain levels of numerous drugs compared to their wild type counterparts that do express PgP. In humans, brain endothelial PgP can be inhibited by quinidine, ketoconazole, or the immunosuppressant drug, cyclosporine A. Quinidine was shown to increase the respiratory depressant effects of loperamide in humans, and the effect was not due to the inhibition of loperamide metabolism by quinidine. Ketoconazole was shown to actually increase CSF levels of ritonavir and saquinavir by three- or four-fold in men and women with HIV. Elegant studies using positron emission tomography have shown that cyclosporine A can increase the penetration ratio of verapamil into the brain by 88%. Penetration ratio is defined as the  $AUC_{\text{brain}}/AUC_{\text{blood}}$ . However, the cyclosporine A levels required to limit the efflux of verapamil from the brain were supratherapeutic.

As occurs with the CYP enzymes, PgP expression is regulated by the PXR. Thus, PXR agonists such as rifampin and St. John's wort can be expected to increase PgP expression and the PgP-dependent efflux of its substrates. Accordingly, rifampin has been shown to decrease digoxin bioavailability approximately by half, and St. John's wort has been shown to decrease digoxin's AUC by almost 20%. Both of these effects could lead to therapeutic failure, since digoxin has a relatively narrow therapeutic range. Indeed, hyperforin in St. John's wort was shown to decrease cyclosporine AUC and  $C_{\max}$  values by more than 50%, and

these changes have been associated with therapeutic failure of cyclosporine in renal transplant patients.

### 12.3.1.1 Predicting Clinical DDI Outcomes from In Vitro DDI Data for PgP

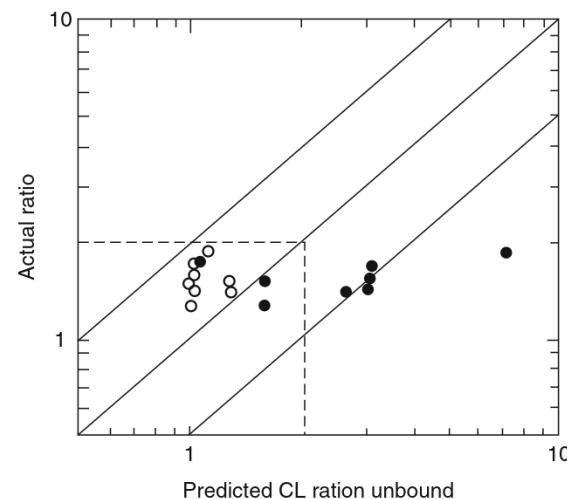
The general approach to these *in vitro/in vivo* predictions parallels the strategy used to predict the clinical significance of inhibitory metabolic DDIs from  $[I]/K_i$  data. Here, too, it can be shown that if a transporter is responsible for < 50% of a victim drug's total clearance, then its inhibition will not increase the AUC ratio by two-fold. Endres and colleagues at the University of Washington, Seattle used the same model as shown in [Equation \(12.7\)](#), replacing  $f_{m_{op}}$  with  $f_{tr}$  denoting fraction cleared by transport, and representing both passive diffusion as well as transporter-mediated transport. Thus [Equation \(12.7\)](#) becomes:

$$AUCratio = \frac{1}{\frac{f_{tr}}{1 + \frac{[I]}{K_i}} + (1 - f_{tr})} \quad (12.21)$$

And if the victim drug's clearance were mediated entirely by transport (e.g., biliary or renal excretion), then [Equation \(12.21\)](#) would simplify to its [Equation \(12.3\)](#) counterpart as follows:

$$AUCratio = 1 + \frac{[I]}{K_i} \quad (12.22)$$

Only now the  $K_i$  would be the inhibition constant for perpetrator inhibition of transporter function *in vitro*, and  $[I]$  would still denote some appropriate *in vivo* concentration of the perpetrator, for example,  $C_{\max}$ . Log transformations of the AUC ratios based on their data are plotted in [Figure 12.5](#) with calculations referenced to both total  $[I]$  and unbound  $[I]$  (i.e.,  $f_u * [I]$ ). The standard two-fold prediction ranges are shown parallel



**Figure 12.6** Actual versus predicted AUC ratios for transporter inhibition. Open symbols denote predictions based on unbound  $[I]$ . Closed symbols denote predictions based on total  $[I]$ .

Table 12.4

Examples of Clinically Significant DDIs Associated with Inhibition of Transporters Other Than PgP

Transporter	Perpetrator	Victim	Outcome	Mechanism
BCRP (ABGC2)	GF120918	Topotecan	> 2-fold increase in bioavailability and AUC	Inhibition of intestinal BCRP and PgP
OATP1B1 (SLCO)	Gemfibrozil	Rosuvastatin Pravastatin Simvastatin Lovastatin	~ 2-fold increase in AUC	Inhibition of hepatic sinusoidal OATP1B1 and also inhibition of MRP2?, BCRP?
OATP1B1 (SLCO)	Gemfibrozil	Cerivastatin	> 5-fold increase in AUC	Inhibition of hepatic sinusoidal OATP1B1 & also inhibition of MRP2? & BCRP? & CYP2C8
OATP1B1 (SLCO)	Cyclosporine A	Rosuvastatin	7-fold increase in AUC	Inhibition of hepatic sinusoidal OATP1B1 & and also inhibition of CYP2C? & BCRP?
OAT1 (SLC22A6)	Probenecid	Penicillins Cephalosporins	~ 2-fold increase in AUC	Inhibition of renal proximal tubular basolateral OAT1
OAT1 (SLC22A6)	Probenecid	Cidofovir	Decrease renal CL by 27%, which is sufficient to reduce cidofovir nephrotoxicity	Inhibition of renal proximal tubular basolateral OAT1
OCT1 (SLC22A1) and OCT2 (SLC22A3)	Cimetidine	Procainamide Metformin	34–43% increase in AUC	Inhibition of renal proximal tubular basolateral OCTs

to the identity line for the AUC ratios. The bounded region denotes a true negative region in which both predicted and actual AUC ratios are <2. Notice that when unbound [I] was used in the predictions, none of the predicted AUC ratios reached or exceeded a value of 2. However when total [I] was used two-thirds of the predicted AUC ratios were greater than 2 even though none of the actual AUC ratios exceeded 2.

### 12.3.2 DDIs Due to Inhibition of Other Transporters

The study of the influence of transporters on drug disposition is still pretty much in its infancy; however, it is becoming increasingly clear that the inhibition or induction of transporters other than PgP can significantly influence the absorption and/or excretion of many (victim) drugs. Table 12.4 provides some examples of clinically significant DDIs associated with the alteration of transporter function by some perpetrator drugs.

### 12.3.3 Predicting DDIs When Victim Drugs Are Processed Both by CYPs and PgP, and When Perpetrators Inhibit Both

We have learned that it is possible to predict with about 80% accuracy (i.e., true positive and true negative predictions) clinically significant inhibitory metabolic DDIs arising from *in vitro* determination of the perpetrator's  $K_i$  for a specific CYP and a reasonable estimate of the perpetrator's *in vivo* concentration, [I]. Furthermore, it is likely that the accuracy rate can be increased by refining

the selection of [I] (e.g., bound vs. unbound), accounting for parallel metabolic pathways and the fraction of metabolic clearance for each CYP involved, and also accounting for (if possible) molecular cooperativity within the broader enzyme binding site (see Eq. 12.9). The same general strategy can be applied to the prediction of clinically significant outcomes associated with the inhibition of transporters as shown for PgP. It should be noted that all throughout this chapter (and generally throughout the literature as well) the term “clinically significant” implies a two-fold change in the victim drug’s AUC in the presence of a perpetrator (i.e., a two-fold increase in the AUC in the presence of an inhibitor or a 50% decrease in the presence of an enzyme inducer). However, even changes of this magnitude may not truly be clinically significant for drugs that have very large therapeutic ranges and/or wide margins of safety. That is to say, doubling the AUC for a relatively nontoxic drug will not likely increase the likelihood of ADRs.

In any case, to what extent can these *in vitro*-to-*in vivo* predictions be relied upon if a drug is both metabolized by CYP enzymes and effluxed by PgP, and if a perpetrator inhibits both of these pathways? We might envision that the combined inhibitory effects could be estimated from:

$$AUCratio = \frac{1}{\left[ \frac{fm_{cyp}}{1 + \frac{[I]}{Ki_{cyp}}} + [1 - fm_{cyp}] \right]} \cdot \frac{1}{\left[ \frac{f_{TR}}{1 + \frac{[I]}{Ki_{TR}}} + [1 - f_{TR}] \right]} \quad (12.23)$$

where  $fm_{cyp}$  denotes the fraction of victim drug clearance due to CYP metabolism,  $K_{icyp}$  is the corresponding metabolic  $K_i$  elicited by the perpetrator,  $fm_{TR}$  denotes the fraction of victim drug clearance due to transport (e.g., biliary excretion), and  $K_{iTTR}$  is the corresponding  $K_i$  for the perpetrator inhibition of the transporter. However, these two processes may not be entirely independent with inhibition of efflux transporter function giving rise to greater enzyme activity (liver) or less enzyme activity (intestine) in the presence of perpetrator than would be expected if the perpetrator inhibited only the enzyme and not the efflux transporter. The issue becomes more complex and it becomes accordingly more difficult to predict outcomes if a perpetrator inhibits enzymes and transporters in both the intestines and the liver, or if a perpetrator inhibits enzymes, efflux transporters, and influx transporters and does so in more than one clearing organ.

### 12.3.4 Using the Biopharmaceutics Classification System (BCS) to Assist in the Prediction of the Clinical Significance of CYP-based and Transporter-based DDIs

In the past few years a substantial effort has been invested in understanding the combined complex roles of transporters and drug metabolizing enzymes in *in vitro-to-in vivo (iv/iv)* predictions of drug clearance and DDIs. Some of these efforts have been described in the work of Leslie Benet and colleagues at the University of California, San Francisco; K. Sandy Pang and colleagues at the University of Toronto; and Yuichi Sugiyama and colleagues at the University of Tokyo; some of which is cited in the bibliography at the end of this chapter. Adapting the BCS to create a Biopharmaceutics Drug Disposition Classification System that segregates drugs by their permeability and solubility characteristics, Wu and Benet at UCSF defined the relative roles of transporters and metabolism on a drug's disposition (Table 12.5). One of the values in segregating drugs by this classification system is that it becomes easy to discern, *a priori*, the types of drugs

for which *iv/iv* predictions about the clinical significance of DDIs should be the easiest. Class 1 substances (high permeability and high solubility compounds) tend to be extensively metabolized, but not particularly subject to processing by transporters. Therefore, *iv/iv* DDI predictions should be relatively uncomplicated for Class 1 substances. Likewise *iv/iv* DDI predictions for Class 3 substances (low permeability and high solubility) that are likely to be processed chiefly by influx transporters (e.g., penicillin influx via OATs in the kidney) should be relatively uncomplicated. On the other hand, *iv/iv* DDI predictions may not be quite so easy for Class 2 substances (high permeability and low solubility) that are both extensively metabolized (e.g., CYP3A4) and subject to significant cellular efflux (e.g., PgP). Likewise, since Class 4 substances (low permeability and low solubility) are subject to both influx and efflux transport, *iv/iv* prediction of DDIs may be somewhat complicated.

## 12.4 DDIs ASSOCIATED WITH PROTEIN BINDING

The drug interaction literature is replete with warnings about the potential increase in pharmacological activity or ADRs associated with the displacement of a victim from its plasma protein binding sites by a perpetrator that also has affinity for the same binding sites. The major plasma proteins to which most drugs bind are albumin and  $\alpha$ 1-acid glycoprotein; the former typically binds acidic, anionic drugs whereas the latter typically favors basic drugs (see Chapter 7). Dr. James Gillette working at the NIH was one of the first people to explore the clinical significance of so-called binding-displacement interactions; interactions in which a perpetrator was postulated to interfere with the binding of a victim drug, thereby increasing the circulating unbound concentration of the victim. Since the pharmacodynamic effects of drugs correlate with their unbound concentrations, it follows that increases in unbound concentrations will be associated with increases in desired therapeutic effects or with ADRs. Dr. Gillette formulated a very simple analysis that

**Table 12.5** Transporter and Metabolic Contribution to Drug Disposition Depending upon the Drug's Solubility and Permeability

Characteristic	Class 1 Substances	Class 2 Substances	Class 3 Substances	Class 4 Substances
Permeability	High	High	Low	Low
Solubility	High	Low	High	Low
Metabolism	Extensive*	Extensive*	Poor	Poor
Renal or biliary excretion	Minor	Minor	Major	Major
Significant efflux transporter effects	No	Yes	No	Yes
Significant influx transporter effects	No	No	Yes	Yes

\*at least 70% metabolized

stipulated how extensively a victim drug had to be bound to plasma proteins in order for there to be a significant change in its pharmacodynamic effects on account of the phenomenon of protein binding displacement by some perpetrator (binding displacer). The very simplest case of his explanation follows.

Suppose that a victim drug bound only to the protein, albumin, in the blood. Its volume of distribution throughout the body would be described by its distribution in the albumin space,  $\sim 5.5$  liters, plus its distribution throughout total body water,  $\sim 50$  liters.

The total body burden of the drug could then be described by the following:

$$A_T = C_b \times 5.5 + C_u \times 50 \quad (12.24)$$

where  $A_T$  is the total amount of drug in the body,  $C_b$  is the concentration of drug bound to albumin in the blood (assuming virtually all albumin binding occurs in the blood), and  $C_u$  is the unbound concentration of the drug in all body water. Now, if the fraction of the victim drug circulating in the blood as unbound drug ( $f_u$ ) is 50%, then the fraction bound ( $f_b$ ) will also be 50%. The unbound concentration will be  $C \times f_u$ , and bound concentration will be  $C \times f_b$ . Substituting  $C \times f_u$  for  $C_u$  and  $C \times f_b$  for  $C_b$  in Equation (12.24), and using 0.5 for both  $f_u$  and  $f_b$  (the drug exhibits a free fraction of 50%), then:

$$A_T = (C \times 0.5 \times 5.5) + (C \times 0.5 \times 50) \quad (12.25)$$

Now, the percent of the total amount of bound drug in the body (not the plasma) would be:

$$\frac{(C \times 0.5 \times 5.5)}{(C \times 0.5 \times 5.5) + (C \times 0.5 \times 50)}$$

or approximately 10%. Therefore the total amount of unbound drug in the body (not the plasma) would be about 90%. Assuming the perpetrator were capable of displacing every molecule of victim drug from its albumin binding sites in the plasma, then the very most that the pharmacodynamic effect could be expected to increase would be 10% as virtually all the drug in the body became unbound. Furthermore, it is likely that in order to effectively displace all the victim from its albumin binding sites, the perpetrator drug would have to achieve a concentration in the plasma of about  $0.6 \mu\text{M}$ , which is approximately the molar concentration of albumin. According to this line of reasoning it would seem that for a victim drug that exhibits a bound fraction (in blood or plasma) of 50%, and for a perpetrator that could elicit the complete binding displacement of the victim drug, there would not be much expected change in the pharmacodynamic effect of the victim drug. This same approach can be used to predict outcomes for other extents of victim binding to plasma proteins. Table 12.6 is also based on Equation (12.24).

According to this simple analysis, a victim drug would have to approach exhibiting a bound fraction of 90% ( $f_u \sim 10\%$ ) before a perpetrator likely would cause a large enough change in the unbound amount

Table 12.6

Possible Increase That Plasma Protein Binding Displacement Could Make in a Victim Drug's Pharmacodynamic Effect as a Function of the Extent of Plasma Protein Binding of the Victim Drug

Maximum Fraction Bound in Plasma ( $\beta_{max}$ )	Fraction of Total Drug Bound in the Body	Maximum Possible Increase in Pharmacodynamic Effect Due to Complete Binding Displacement
50%	10%	10%
90%	49.6%	$\sim$ two-fold
99%	91.5%	$\sim$ 12-fold

of drug in the body to cause a significant alteration in the victim drug's pharmacodynamic effect.

There's also a pharmacokinetic way to analyze the likely outcome of binding displacement interactions on pharmacodynamic outcomes. Steady-state unbound plasma concentrations can be defined in terms of a drug's clearance ( $CL$ ), the fraction of drug absorbed ( $F$ ), the dose ( $D$ ), and the dosing interval,  $\tau$ ,

$$\bar{C}_{ss} = \frac{FD}{CL \times \tau} \quad (12.26)$$

The total organ clearance (e.g., liver) will depend upon the intrinsic clearance (e.g., metabolic clearance) and the unbound fraction of the drug,  $f_u$ .

$$CL = CL_{int} \times f_u \quad (12.27)$$

Substituting into Equation (12.26),

$$\bar{C}_{ss} = \frac{FD}{CL_{int} \times f_u \times \tau} \quad (12.28)$$

now, the average steady-state unbound concentration,  $\bar{C}_{SSunb}$ , is related to the total average steady-state concentration as follows:

$$\bar{C}_{SSunb} = \bar{C}_{ss} \times f_u \quad (12.29)$$

Notice in Equation (12.28), that if you multiply both sides by  $f_u$  the following expression develops:

$$\bar{C}_{ss} \times f_u = \frac{FD}{CL_{int} \times \tau} \quad (12.30)$$

or

$$\bar{C}_{SSunb} = \frac{FD}{CL_{int} \times \tau} \quad (12.31)$$

This means that  $\bar{C}_{SSunb}$  is strictly a function of metabolic intrinsic clearance, the dose, the fraction of dose absorbed, and the dosing interval. Furthermore,

$$\bar{C}_{SSunb} = \frac{AUC_{unb}}{\tau} \quad (12.32)$$

Therefore the unbound AUC will only be a function of metabolic intrinsic clearance, dose, and fraction of dose absorbed as follows:

$$AUC_{unb} = \frac{F \times D}{CL_{int}} \quad (12.33)$$

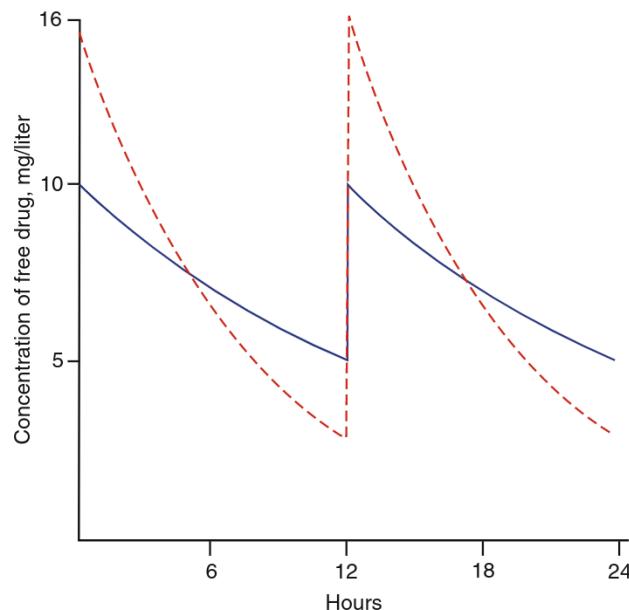
It will not at all depend upon the fraction of drug that is unbound in the blood.

This means that overall exposure to unbound drug can't be affected by plasma binding or plasma protein binding displacement interactions. Furthermore, if the drug is a high hepatic extraction-ratio drug, such that  $CL_{int} \gg Q$ , then  $Q$ , hepatic blood flow rate, becomes limiting, and [Equation \(12.31\)](#) becomes:

$$\bar{C}_{SSunb} = \frac{FD}{Q \times \tau} \quad (12.34)$$

Thus, for a high (hepatic) extraction ratio victim drug the  $AUC_{unb}$  will be solely a function of hepatic blood flow rate, fraction of dose absorbed, and dose. Again, plasma protein binding, and therefore changes in plasma protein binding associated with binding displacement, will not affect total exposure to unbound drug.

The total exposure to unbound drug ( $AUC_{unb}$ ) is not a function of the fraction of drug unbound,  $f_u$ , but rather the fraction of dose absorbed, the dose, and  $CL_{int}$  for low clearance drugs or hepatic blood flow rate,  $Q$ , for high clearance drugs, therefore changes in  $f_u$  caused by protein-binding displacement interactions would not be expected to have significant outcomes. Even though  $AUC_{unb}$  would not be affected by  $f_u$  there could be times within each dosing cycle when exposure to unbound drug would, in fact, be determined by the unbound fraction of drug. Dr. Gerhard Levy at SUNY, Buffalo illustrated this many years ago. Suppose a highly bound low clearance drug exhibited very rapid distribution throughout the body. Its unbound plasma concentrations at steady-state might be depicted by [Figure 12.6](#). Suppose, too, that if this victim drug were coadministered with another drug (i.e., perpetrator) that in fact was capable of displacing the victim drug from plasma protein binding sites,  $f_u$  for the victim drug increased. This is shown for the dashed curve in [Figure 12.6](#). The increase in  $f_u$  for the victim drug subsequent to protein-binding displacement by the perpetrator drug gives rise to (1) an increase in the clearance of the victim as evidenced by a steeper disappearance curve; (2) an increase in the peak plasma concentration of unbound drug; and (3) a decrease in the trough plasma concentration of unbound drug. So, the  $AUC_{unb}$  (i.e., total exposure to unbound victim drug) would not be altered fundamentally (as shown in [Equations 12.26–12.34](#)). But it is nevertheless possible that the peak plasma concentrations of unbound drug at steady-state would be higher than prior to the addition of the perpetrator (dashed lines vs. solid lines), and also that the trough concentrations would



**Figure 12.7** Steady-state unbound plasma concentrations of an extensively bound low-clearance victim drug before (solid line) and after (dotted line) its displacement (decrease in plasma protein binding) caused by a perpetrator.

be lower. Even though the total exposure to unbound drug would not have changed, it is possible that ADRs could develop soon after the dose of the victim drug (peak), and it is simultaneously possible that therapeutic failure could occur immediately before each next dose of the victim drug (trough). It's actually possible that a binding displacement interaction involving a low clearance, extensively bound victim drug could result in both an increased chance of ADRs and also therapeutic failure.

## 12.5 CONCLUSIONS AND KEY POINTS

Drug–drug interactions constitute a significant fraction of avoidable ADRs. The frequency with which they occur depends, in part, on the number of different drugs that are used simultaneously. Since the population is aging, and therefore it is expected that the number of drugs used per person in an aging population will increase, it may well be that the risk of DDIs leading to ADRs will increase sharply. On the other hand, some DDIs can be engineered purposefully to improve dosing schedules or therapeutic outcomes. For the purpose of drug management in general and in an aged population in particular, it is important to be able to identify DDIs that are likely to lead to clinically significant adverse outcomes marked either by ADRs or therapeutic failure. To assist clinicians in identifying risks associated with the combined use of two drugs, drug-interaction books and searchable drug-interaction databases are available. These resources exhibit varying degrees of sophistication and accuracy, and both include some interactions that have never been validated by controlled clinical trials.

Pharmaceutical scientists engaged in drug development require predictive tools to enable them to ascertain whether a drug under development is likely to cause clinically significant drug interactions with marketed drugs. Recognizing the likelihood of clinically significant drug interactions is an important determinant in decisions about continuing further with drug development. Though predictions might be made from analogies to what is known about drug interactions of currently marketed drugs, these analogous predictions can be completely wrong. For example, the anti-infective drug, ciprofloxacin, is a fluoroquinolone and a known inhibitor of CYP1A2. It can sufficiently slow the clearance of theophylline, a drug with a relatively narrow margin of safety, to cause ADRs including seizures. On the other hand, apart from enoxacin, most of the fluoroquinolones that have been marketed since ciprofloxacin do not inhibit CYP1A2, and would not be expected to interact with theophylline. Placing all fluoroquinolones into a probable risk category for an adverse reaction with theophylline based on the findings with ciprofloxacin would lead to bad predictions. Along these lines the macrolide antibiotic, triacetyloleandomycin (TAO), is a reasonably good inhibitor of CYP3A4. Erythromycin and clarithromycin are less effective inhibitors, but have been shown to be capable of causing serious clinical adverse effects associated with CYP3A4 inhibition. On the other hand, neither dirithromycin nor azithromycin, both of which are also macrolide antibiotics, inhibit CYP3A4. They do not share the same risks of DDI-related ADRs as erythromycin, clarithromycin, or TAO.

Beginning at the start of this century an attempt was made to bring together regulatory scientists, those in the pharmaceutical industry, and those in academia to agree upon predictive strategies for characterizing the clinical significance of DDIs. These efforts focused primarily on predicting the clinical significance of inhibitory metabolic and inhibitory transporter-based DDIs, and established the paradigm of using  $[I]/K_i$  ratios for this purpose. In this paradigm, DDIs with a high probability of being clinically significant would be those in which the AUC ratio ( $AUC_i/AUC$ )  $\geq 2$ , and an  $[I]/K_i$  ratio  $\geq 1$  would predict an AUC ratio  $\geq 2$ . Likewise, an AUC ratio  $\leq 1$  would suggest that there would be little or no likelihood of a clinically significant DDI, and an  $[I]/K_i$  ratio  $\leq 0.1$  would predict an AUC ratio  $\leq 1.1$ . There are many subtleties that must be taken into consideration, however, including the matrix or system in which  $K_i$  is estimated, the type of *in vitro* victim or substrate that is used, nonspecific binding of the victim, the most representative value for the *in vivo* concentration of  $[I]$  for the perpetrator, metabolism of the victim drug by parallel pathways, the issue of cooperativity, and others. Remarkably, even when these subtleties are simply ignored, the predictive accuracy of the  $[I]/K_i$  paradigm is between 70 and 85%. The same strategy can be applied to predicting the clinical significance of DDIs associated with transporter inhibition. In view of the relatively high level of predictive accuracy of the  $[I]/K_i$  paradigm,

flaws notwithstanding, it may not be too soon to begin applying it to the evaluation of DDIs when the only data at hand is case report data. For example, authors of case reports of putative inhibitory DDIs should include  $[I]/K_i$  data when it is available, to support any contention of an inhibitory DDI.

Other factors currently limit the predictive accuracy of inhibitory DDIs. For example, although there is some consensus about the drugs that should be used as representative victim drugs of each of the CYP enzymes, such a consensus about representative victim drugs for transporters or for non-CYP enzymes doesn't exist. The exception to this might be for the transporter, P-glycoprotein, for which digoxin may be the victim of choice. By comparison, the study of the function of OATs, OCTs, MRPs, OATPs, and other transporters, their substrates, and their inhibition, is still in its early stages. In addition, some drugs can be substrates for both influx and efflux transporters as well as for CYP or other enzymes. Some drugs can inhibit both CYP enzymes and transporters, for example, PgP, whereas other drugs can induce the activity of CYP enzymes and transporters. The multiplicity of dispositional processes to which a given victim drug might be subject and the multiplicity of processes that a perpetrator drug might affect promise to make our ability to improve on the predictive accuracy of inhibitory DDIs a significant challenge.

Strategies for predicting the clinical significance of enzyme-inducing or transporter-inducing DDIs are not quite as far advanced as for the inhibitory DDIs. To be sure, many of the same issues that limit the predictive accuracy of inhibitory DDIs suffuse predictions for inductive DDIs. However, early work in this field suggests that relatively few perpetrators capable of stimulating, for example, the PXR, will cause clinically meaningful (victim) exposure (AUC) decreases in humans. Moreover, there are currently very few examples of clinical increases in victim clearance that are much greater than two-fold, regardless of the extent of PXR agonism or CYP induction *in vitro*. Unless a victim has a very narrow therapeutic range, it could be that these types of interactions are simply overrated.

One of the issues that has not been explored extensively in connection with *iv/iv* predictions of the clinical significance of DDIs is the impact of the victim drug's clearance (before the interaction) on the clinical significance of an interaction in humans. Whether the interaction is one of inhibition of the victim drug's metabolism or whether it is induction of the victim drug's metabolism, even a predicted outcome based, for example, on  $[I]/K_i$  ratios might errantly predict real-life clinical consequences if the victim's baseline clearance isn't considered. Remember, it is possible that the pharmacokinetics of a high clearance drug given intravenously will not be substantially affected even in the face of an  $[I]/K_i$  ratio of  $\sim 1$ , although the pharmacokinetics of the same drug given orally might be substantially affected.

Finally, it would appear that most putative protein-binding displacement interactions should not have meaningful clinical consequences, since the  $AUC_{unb}$

will not be affected by changes in protein binding. However, there could still be instances in which the displacement of an extensively bound drug could result in both higher peak plasma concentrations of unbound drug and also lower trough plasma concentrations of unbound drug. Detecting the clinical consequences would take very careful clinical observations, since under these circumstances it is possible for such a DDI to cause both an increase in intermittent ADRs and some evidence of intermittent therapeutic failure.

## REVIEW QUESTIONS

- What are the essential features about two drugs that you would have to know in order to determine whether there was a chance that they could (1) interact pharmacodynamically and (2) interact pharmacokinetically?
- How can the *in vitro* determination of a perpetrator drug's  $K_i$  value along with knowledge about its  $C_{max}$  in humans permit a prediction as to the clinical significance of its interaction with a victim drug?
- Use of  $[I]/K_i$  ratios to predict the clinical significance of inhibitory metabolic DDIs in humans (qualitative zoning) has been shown to be over 80% accurate for interactions involving CYP3A4 inhibition and approximately 70% accurate for interactions involving CYP2C9 inhibition. What are some of the reasons why the accuracy rates aren't, say, 95%?
- How can *in vitro* transactivation assays be used to predict the clinical significance of inductive DDIs? What are some of the drawbacks or limits to this strategy?
- How could the  $[I]/K_i$  strategy for predicting the clinical significance of DDIs be used to strengthen or refute case reports about the significance and/or mechanism of inhibitory DDIs in humans?
- How would you use the bipartite classification system (BCS) to enable you to predict the clinical significance of a pharmacokinetic DDI from *in vitro* data?
- A new chemical entity has the following properties: (1) it has been shown to inhibit CYP enzymes in heterologous expression systems expressing human CYP enzymes; (2) it has been shown to be selective for CYP2C9; and (3) though three different substrates were used, the mean (range) for its  $K_i$  for CYP2C9 inhibition was 0.5 (0.2–0.8)  $\mu\text{M}$ . The  $C_{max}$  in early clinical trials was 4  $\mu\text{M}$ . Would a clinically significant interaction with the anticonvulsant drug, phenytoin (Dilantin®) be anticipated based on the foregoing data? Why or why not?
- Valproic acid is an anticonvulsant drug that binds principally to plasma albumin, and at therapeutic plasma concentrations the extent of its binding is 93%. Gemfibrozil is a drug used to lower LDL cholesterol and triglycerides. Its peak plasma concentration at usual doses is about 100  $\mu\text{M}$ , and it also binds

principally to plasma albumin. Would you expect that gemfibrozil would promote a binding-displacement interaction with valproic acid? Why or why not? What would the consequences be of such an interaction?

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