Chapter 28
Drug–Drug Interactions: What Have We Learned and Where Are We Going?

K. Sandy Pang, Raimund M. Peter, and A. David Rodrigues

Abstract The study of drug–drug interactions (DDIs) has significantly progressed in recent years, sometimes at a considerable pace. The impetus has undoubtedly been due to the much publicized market withdrawal of a number of drugs (terfenadine, mibefradil, astemizole, and cisapride) due to unforeseen drug–drug interactions that placed the patients at considerable health risk or even resulted in fatalities. In response, subsequent actions by regulatory agencies like the FDA generated a more stringent DDI framework and guidance for drugs submitted for market approval. The pharmaceutical industry reacted with a more proactive approach in trying to screen out any undesirable drug–drug interaction liability from candidate drugs already at the early drug discovery stage. Looking forward, however, it is apparent that numerous challenges remain and opportunities still exist to develop an improved and more complete toolbox that can support the preclinical and clinical study of drug transporters and drug-metabolizing enzymes beyond the cytochrome P450s. At the same time, DDI models will have to become more comprehensive and enable the integration of enzyme and transporter data, taking into account the dynamic nature of both "perpetrator" and "victim" pharmacokinetics. This dynamic approach will have to consider the underlying mechanism(s) of the DDI in multiple elimination organs, transporter-mediated transport, as well as aspects like inter-subject variability, pharmacogenetics, or pharmacodynamics. In the future, the industry and regulatory agencies will need to integrate an even larger amount of preclinical and clinical information from different sources, deal with larger numbers of drug combinations, and consider complicated DDI scenarios involving a heterogeneous mix of small molecules and biologics, or when the interacting drug is a ligand of nuclear receptors that results in changes in transporter and enzyme function. Physiologically-based pharmacokinetic (PBPK) modeling approaches hold much promise in this regard and will be one of a number of steps en route to a more

K.S. Pang (✉)
Leslie Dan Faculty of Pharmacy, Department of Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada
e-mail: ks.pang@utoronto.ca

K.S. Pang et al. (eds.), *Enzyme- and Transporter-Based Drug–Drug Interactions*,
DOI 10.1007/978-1-4419-0840-7_28,

systems-based approach within a burgeoning model-based drug development environment. As the areas of molecular and cell biology and pharmacology progress toward such a goal, we can foresee that the study of DDIs, accompanied by the attendant data, will become more mechanistic and complete.

28.1 Drug Interactions Involving Metabolic Enzymes

Enzymatic reactions are quite ordered and are usually very specific toward select substrates. Their susceptibility to induction and inhibition, transcriptional regulation of nuclear receptors, and variation due to pharmacogenetics have been well appreciated (see Part I; Chapters 1, 3, and 4) over the recent years. Consequently, drug metabolism has become one of the most important factors in drug discovery and toxicological studies. In drug discovery, decisions focused on synthesis, testing, and further chemotype progression have to be made rapidly, requiring succinct information related to the metabolism of the compounds in question, the desired modifications based on available methodologies (Part II), in silico predictions (Chapter 6), and assessment of DDI potential (Chapter 23).

One can imagine a scenario where the inhibition of one enzyme will be compensated by another enzyme and thus lead to increased removal of the drug via alternate, metabolic, or excretion pathways (Morris and Pang, 1987; Sirianni and Pang, 1997). The compensation by a seemingly, unimportant pathway may even lead to an increase of a toxic pathway in some cases, and understanding of enzyme inhibition leading to an apparent induction of the alternate metabolic pathway is part and parcel of observations for competitive pathways. Enzyme inhibition can be observed in different types of kinetics: reversible inhibition as competitive, non-competitive, uncompetitive, or mixed type or as irreversible inhibition, which can show a time-dependent or even mechanism-based inactivation of the respective enzyme(s). The latter type involves either the formation of a metabolite intermediate complex or covalent binding (Part III, Chapters 19 and 20). Complex cases of multiple inhibition (Chapter 26) or inhibitory and inductive events (Chapter 21) are occasional observations that can complicate predictions of DDIs even further. Moreover, inhibitors can behave differently in various organ tissues, such that an inhibitor of an intestinal enzyme may not reach sufficiently high enough concentration levels to act as an inhibitor of the same enzyme in the liver. Clearly, there is a need for more comprehensive tools that link in vitro data with in vivo and biomarker data, and support decision making.

Due to the abundance of drug-metabolizing enzymes expressed in hepatic tissue, there has been an overwhelming emphasis on the liver as the metabolizing organ, and little and not enough attention given to extrahepatic organs or tissues. In addition to the liver, drug-metabolizing enzymes responsible for clearance processes are also expressed in significant levels in other organs/tissues, e.g., the intestine and the kidney (Chapters 1, 14, and 17). Organ-specific inhibition has been noted even for the same enzyme. Hence, prediction strategies for drug interactions should be

expanded to include extrahepatic tissues, especially the intestine that is strategically placed as the gateway tissue to the liver in first-pass removal.

28.1.1 P450s Versus Phase II Enzymes

As can be surmised from these proceedings and other text references regarding DDIs, we currently possess ample knowledge on the cytochrome P450s (P450s), their multiplicity, and information related to the regulation, structure, and function of various subfamily members. The duplicity of CYP3A4 and CYP3A5 in the metabolism of common substrates, and a greater propensity of CYP3A4 toward inhibition by all inhibitors, render additional complexity in deciphering the degree of inhibition (McConn et al., 2004). This observation may suggest that one has now to consider *CYP3A5* genotype prior to conducting a CYP3A DDI study. Additionally, we can anticipate that further unknown P450s will be discovered and their functions be defined. The potential activities of orphan P450s have been examined repeatedly (Stark and Guengerich, 2007; Stark et al., 2008b), and some have been implicated in the metabolism of endogenous substrates, e.g., arachidonic acid (Stark et al., 2008a). As many of these P450s catabolize or metabolize pharmacologically active substrates like eicosanoids or steroids, DDIs involving their induction and inhibition should be increasingly taken into consideration.

Drug-metabolizing enzymes that catalyze conjugation reactions (phase II enzymes), such as UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs), share common substrates, albeit each proceeding with differing affinities. There is a general consensus that additional tools are required in order to enable more robust reaction phenotyping of these enzymes in vitro, improve the bridging of in vitro to in vivo, and support the conduct of more mechanistically meaningful clinical DDI studies (Zhang et al., 2007). Various UGTs and SULTs are expressed in the gut and liver, and contribute to first pass metabolism of various drugs, thus they cannot be dismissed in DDI risk assessment. Hence, although a particular drug may not inhibit the P450s, unexpected DDIs with UGT and SULT substrates can dominate in altering the clearance of a particular drug (Schwartz et al., 2009).

Among the three human UGT superfamilies (Tukey and Strassburg, 2000), multiple UGTs can be involved in the conjugation of the same substrate (Kostrubsky et al., 2005). Only a few inhibitors exist, though reaction phenotyping of glucuronidated substrates is currently feasible to some extent (Chapter 8) and online drug-metabolism systems, integrated into capillary electrophoresis that entail the encapsulation of microsomes in tetramethoxysilane (TMOS)-based silica matrices for the determination of UGT inhibitors in a single capillary, are available (Sakai-Kato et al., 2004). For example, retinoids are found to be inhibitors of UGT2B7 mRNA expression (Samokyszyn et al., 2000; Lu et al., 2008), whereas HIV protease inhibitors are UGT1A1 inhibitors (Zhang et al., 2005a).

In the past, the prediction of UGT-dependent clearance was problematic. However, Chapter 8 emphasized that the addition of bovine serum albumin (BSA)

or HSAFAF (fatty acid free human serum albumin) to incubations of human liver microsomes (or recombinant UGTs) may permit the accurate prediction of in vivo clearance parameters and DDI potential. Recently, it has been proposed that cryopreserved hepatocytes may serve as an alternative model to assess UGT activity and DDI potential in vitro (Coughtrie et al., 2009). Irrespective of the model employed, however, prediction of UGT-mediated DDIs is difficult, because many substrates are metabolized by multiple UGTs, and inhibition constants (K_i) tend to be high. The implication of pumps at the endoplasmic reticulum membrane of glucuronides (Battaglia and Golan, 2001; Csala et al., 2004) further complicates the picture. Enterohepatic circulation of glucuronides to reappear as the unconjugated species renders an apparent observation of lessened exposure and therefore formation of the glucuronide metabolite. These complications add to the difficulties in addressing inhibition of the glucuronidation reaction.

In comparison, the sulfotransferase isoforms exert more stringent substrate specificities than the UGTs, and are subject to differential effects of inhibitors. Probes such as acetaminophen or 2-aminophenol (Riches et al., 2007) for phenosulfotransferase, SULT1A1, exist. Pentachloral phenol and mefenamic acid are potent and selective inhibitor of human liver SULT1A1 (SULT1A3 for mefenamic acid). Phthalates (used as plasticizers) inhibited estrogen sulfotransferase, SULT1E1, and hydroxysteroid sulfotransferase, SULT2A1 (Harris and Waring 2008). Hydroxylated polychlorinated biphenyls (PCBs), important persistent environmental contaminants, are substrates and inhibitors of human SULT2A1 (Liu et al., 2006). In addition, SULTs are inhibited by many dietary and environmental chemicals. SULT1A1 is strongly inhibited by flavonoids and a range of environmental chemicals and dietary components (see Chapter 22). Fruit and vegetable cytosols also inhibit SULT isoforms, as do long-chain alkylphenols and chlorinated phenols. Juices and green tea (Tamura and Matsui, 2000; Saruwatari et al. 2008) are known inhibitors of SULTS, and curcuinoids inhibit not only SULTs but UGTs and CYP3A4 (Volak et al., 2008). Quercetin, a flavonoid present in edible fruit, vegetable, and wine, was found to be a potent inhibitor of SULT1A1, and SULT1E1 activities and resveratrol sulfation (Pacifici, 2004).

The area of inhibitors of glutathione *S*-transferases (GST) is sparked by the notion that these GST enzymes are involved in the resistance to anticancer drugs, since elevated levels of GSTs are among the factors associated with an increased resistance of tumors to a variety of antineoplastic drugs. The inverse correlation between expression and prognosis in many tumors has provided a rationale for the design of GST inhibitors to enhance the therapeutic index. A major advancement to overcome GST-mediated detoxification of antineoplastic drugs is the development of GST inhibitors. Human GST inhibitors are multidrug resistance chemomodulators on human recombinant glutathione *S*-transferase (GSTs) activity, GST P1-1 by sulfinpyrazone, GST Al-1 by sulfasalazine, and camptothecin, GST M1-1 by sulfasalazine, camptothecin, and indomethacin, and progesterone as a potent inhibitor of GST P1-1 (Hayeshi et al., 2006). The α-glutamyl moiety plays an important role in modulating the affinity of the ligands to interact with GSH-dependent proteins. The glutathione *S*-conjugate,

L-γ-glutamyl-(S-9-fluorenylmethyl)-L-cysteinyl glycine, has been found to be a highly potent inhibitor of human GSTA1-1 in vitro, with lesser inhibitory activities toward GSTP1-1 and GSTM2-2 isoenzymes (Cacciatore et al., 2005). Thonningianin A (Th A), a novel antioxidant isolated from the medicinal herb, *Thonningia sanguinea*, inhibited rat liver GST and human GST P1-1 (Gyamfi et al., 2004).

28.1.2 Species Differences

The use of preclinical models to assess DDI in metabolism is commonplace but is complicated by species differences. What is encountered is that the expression level, functional activity, and/or tissue distribution differ. An appropriate animal model, when chosen and used properly, could be a valuable tool to provide the basis for extrapolating in vitro human data to clinical outcomes as well as mechanistic insight for the interpretation of interactions observed clinically (see Chapter 11). On occasion, relevance of the in vitro/in vivo animal models and gene-knockout animals is uncertain and needs to be questioned. The knockout animals that lack specific drug-metabolizing enzymes may exhibit altered morphology and flow dynamics in the liver (Schmidt et al., 1996; Lahvis et al., 2000), altered levels of ligands that affect receptors/transcription factors, and exhibit redundant or alternate pathways that are absent in humans (Kimura et al., 1999). Greater progress is needed, so that species differences are understood more fully and results of animal-based DDI models can be translated to man (Chapters 11 and 23).

28.1.3 Transgenic Animal Models

The recent development of transgenic animal models with humanized liver in mouse lines expressing specific drug transporters and/or metabolizing enzymes is of interest (see Chapter 11). Through the pioneering efforts of many investigators, humanized mice are now routinely used to rapidly advance research. Chimeric mice, constructed by transplanting human hepatocytes, are useful for predicting the human metabolism of drug candidates. Some success was seen with the metabolism of *S*-warfarin (Inoue et al., 2009) and induction of rifamycin by SXR (steroid X receptor) (Kim et al. 2008), and in the study of sex and developmental changes of the CYPs (Felmlee et al., 2008). In other instances, higher activities are found expressed in chimeric mice carrying humanized liver CYP1A2 (Uno et al., 2009). However, lots more need to be known about this model since the intra- and inter-organ characteristics as well as changes in hormonal and cytokine levels are unknown. Although quantitative assessments using these animal models are currently limited, it is conceivable that in the next decade, these models could become more valuable and validated in DDI assessments during drug discovery and early development processes.

28.2 Drug Interactions Involving Transporters

In contrast to the stringent specificities of the P450 CYPs and SULTs, a lot of redundancy exists among transporters that share the same substrates. Hence, it begs the question of whether the inhibition of certain transporters is important because of the sharing of substrates and the redundancy. The answer is yes, if transport is the rate-limiting step and especially when uptake or transport is mediated solely via the presence of the transporter. The situation is far more complex for compounds/inhibitors undergoing both metabolism and transport. We should also consider the drug and its metabolite as potential inhibitors (substrate and end product inhibition) as well as levels of the inhibitor and its metabolite, e.g., gemfibrozil glucuronide as inhibitor of OATP for cerivastatin transporter (Shitara et al., 2004).

28.2.1 Proteomics-Based Approach to Define Transporter Abundance in Tissues

Up until recently, we do not have a quantitative feel about how much of the transporter exists. There is a lack of the availability of absolute protein concentration levels in recombinant systems, isolated primary cells, and human tissues. For example, inhibition studies conducted in human hepatocytes ordinarily reflect the overall effect of inhibition (Shitara et al., 2003), and not on inhibition of any particular uptake transporter since the relative contribution of the sinusoidal transporters to uptake is unknown. In order to gain this insight, the relative amounts of the transporters must be known. The use of proteomics, with LC-MS/MS technologies, for example, will aid to decipher the problem. An absolute quantification method for membrane proteins in murine blood–brain barrier, liver, and kidney was determined by liquid chromatography–tandem mass spectrometer (LC/MS/MS). The method resulted in expression levels of 34 transporters in liver, kidney, and blood–brain barrier of mouse that showed an excellent correlation with the values obtained with existing methods using antibodies or binding molecules (Kamiie et al., 2008). The relative importance of transporter and function must now be correlated to the amount of protein present in the tissue or organ to those measured in in vitro systems to more quantitatively assess the relative contribution of transporters to drug transport in vivo, and define the significance of the DDIs.

28.2.2 Species Difference in Transport

An appropriate animal model, when chosen and used properly, could be a valuable tool to provide a basis for extrapolating in vitro human data to clinical outcomes as well as a mechanistic insight for the interpretation of interactions observed clinically. Although human orthologs may be identified among transporters, species difference can exist in drug transport and therefore in vitro data may not be that

relevant in the prediction of DDI. Differing substrate specificities are often found among species and must be noted (Ho et al., 2006). Significant challenges still need to be overcome in terms of studying human drug transport.

28.2.3 Improved Tools to Examine Transporter Function

The identification of transporters involved in drug transport, as described in Chapters 2, 9, and 10, heavily relies on use of expression systems (Kopplow et al., 2005); this information is of vital importance for the determination of DDIs in transport. Otherwise, drug transport is assessed in hepatocytes or cells in culture. First, human (freshly prepared or cryopreserved) hepatocytes that contain the full complement of transporters are expensive and vary greatly from batch to batch for good viability measures. Hepatocytes are rarely prepared fresh and are reliant on the donor characteristics (age, sex, and disease), the source, and method of preparation, and handling of shipment during procurement. Hence, cryopreserved hepatocytes are used (Shitara et al., 2003). Recently, differences in transporter content assayed by LCMS were found between cryopreserved hepatocytes and liver but not between freshly prepared hepatocytes and whole liver, and more importantly, age dependency in MRP2 levels was suggested (Li et al., 2009).

Hepatocytes in culture undergo differentiation (Jigorel et al., 2005), and may not contain the complement of transporters or nuclear receptors in its native state (Nahmias et al., 2006; Ohno et al., 2008). Upon co-culture with sinusoidal endothelial cells, the transport function of low density lipoprotein is regained (Nahmias et al., 2006). Sandwich culture systems, even from human sources, contain reduced or elevated levels of transporters and enzymes, and may require use of dexamethasone, ligand of the glucocorticoid receptor (GR) and SXR (steroid X receptor) for culture. Transporter and enzyme genes in culture may further respond to induction by dexamethasone (1–100 μM) (Turncliff et al., 2004; Hoffmaster et al., 2004). Differential growth or loss of some transporters and enzymes exists in rat hepatocyte sandwich systems (Chandra et al., 2001); P-gp, the Mrp, the Oatps, Bsep, and the P450s are maintained, allowing for uptake, induction, and regulatory studies (Annaert et al., 2001; Zamek-Gliszczynski et al., 2003; Turncliff et al., 2004; Zhang et al., 2005b). Bile acids are secreted and retained within the bile canaliculus and may adversely present a cholestatic model; the accumulation of bile acids may affect enzymes and transporters via regulation by FXR (see Chapter 4). Although the micropatterned co-culture of human hepatocytes and murine fetal fibroblast show some promise (Khetani and Bhatia, 2008) (see Chapter 9), the flow circuitry, hepatocyte heterogeneity as well as acinar regions may not be maintained. For improved utilization of these systems, a thorough comparison of the levels of freshly prepared/whole liver vs. cultured hepatocytes and functional studies needs to be established for validation of the systems.

Other complementary tools for additional insights include knockout (KO) animals lacking specific drug transporters, and/or transgenic animal models with

humanized mouse lines expressing specific drug transporters. Knockout animals for drug efflux: P-gp [*mdr1a*(–/–), *mdr1b*(–/–)] and the *mrp*(–/–) have provided some insight as to the involvement of transporters and alteration of ADME and drug and metabolite profiles. One of the drawbacks of the KO animals is the presence of redundant pathways. The *Abca1*(–/–) KOs that lacks the high density lipoprotein (HDL) transporter showed the same hepatic cholesterol, triglyceride, and phospholipid contents, and the same extent of biliary excretion rates of cholesterol, bile salts, and phospholipid, and unchanged uptake of cholesterol and cholesterol esters when fed high fat and cholesterol diet compared to the standard diet (Groen et al., 2001). The *mrp1(–/–)* KOs failed to protect aflatoxin B1 lung toxicity, and the same degree of tumor development was observed for the wildtype and KOs; the redundant pathways may be P-gp and other Mrps (Wijnholds et al., 1997; Lorico et al., 1997; Rappa et al., 1999; Allen et al., 2000; Lorico et al., 2002). In Bsep KOs, less than expected intrahepatic cholestasis was observed: there was 6% excretion and increased hydroxylation of bile salts (Wang et al., 2001). In Mrp2-KOs, increased P450s and Ugt1a were found to compensate for loss in excretory activity (Chu et al., 2006). Reduction in Cyp2b1/2 and Cyp3b1/2 was found in immunoblot analyses in TR^- that lack Mrp2 vs. control Wistar rats (Jäger et al., 1998). In Mrp deficient, Eisai hyperbilirubinemic rats (EHBR), a compensatory increase in Mrp3 was observed in comparison to Sprague Dawley rats, lending to the greater basolateral efflux of substrates (Akita et al., 2001). Many aspects of this have been covered in Chapter 9. Despite that the humanized liver is being used to examine drug transport (Okumura et al., 2007), many attendant changes are obscure and validation of this model as a useful tool remains unknown. It is envisioned that bioinformatics and in vitro/in vivo approaches are needed to assess the functional and regulatory differences between the human and mouse genes be characterized in these model to ensure a more complete picture

28.2.4 Improved Probes and Inhibitors to Examine Transporter Function

Due to the redundancy in transporters, there is a need for better/improved selective probes or inhibitors of exclusive or high selectivity to segregate the roles of each transporter in drug transport for the basolateral and apical membranes. Many inhibitors are not specific enough for single transporters, especially for the ATP-binding cassette proteins (Matsson et al., 2009). Rather, they serve as inhibitors of multiple transporters: for example, MK571 inhibits P-gp (Honda et al. 2004), MRP1 (Jedlitschky et al., 1996), MRP2 (Leier et al., 2000), MRP3 (Zelcer et al., 2003), MRP4 (Reid et al., 2003), and MRP4 (Chen et al., 2005). GF120918 is an inhibitor of both MDR1 (Tang et al., 2002; Taipalensuu et al. 2004) and BCRP (Maliepaard et al., 2001). Mitoxantrone inhibits P-gp (Polli et al., 2001), BCRP (Volk and Schneider 2003), and MRP1 (Morrow et al., 2006). Recently, the broad specificity of inhibitors has been summarized (Matsson et al., 2009). The lack of

availability of specific inhibitors adds complexity to the identification of the relative contribution of transporters in drug uptake in hepatocytes and renders quantitative predictions difficult.

Among the transfection or expression systems available thus far, it is difficult to assess the relative contribution of uptake and efflux transporters in the net transepithelial flux of drugs. Normally, influx or uptake is studied within a short time frame within which drug efflux is negligible. However, drug efflux is seldom studied, except in indicator dilution (Schwab et al., 1990, 1992) or washout experiments (Akita et al., 2001). Inhibition of efflux would lend to increased accumulation of the victim substrate. In like fashion, estimation of apical efflux is reliant on initial permeation of substrates prior to utilization of available transporters. This does not pose as a problem for lipophilic substrates of P-gp. For polar substrates that utilize the MRP pumps, this presents more difficulty, and may require use of prodrugs (Nezasa et al., 2006 Reid et al., 2003; Dallas et al., 2004; Chen et al., 2005) that undergo intracellular cleavage to furnish the requisite substrate. Some high-throughput screens with facile fluorescent probes are needed.

28.3 Improved Methods for the Interpretation of Drug Interaction Data

28.3.1 PBPK Modeling

There has been a constant improvement in the way to interpret data. The equation on the ratio of area under the curve under inhibited and uninhibited conditions proposed by Rowland and Matin (1973) had served as the initial basis and underwent several modifications to correct for the presence of competing pathways and competing organs (Ito et al., 2005; Brown et al., 2005). An uncertain term that was debated over and over again is the appropriate estimate of the inhibitor concentration, [*I*]. The FDA suggests use of the steady state C_{max} value of the inhibitor, whereas the Japanese counterpart emphasizes use of the unbound value. In a comprehensive analysis, Obach and colleagues compared the appropriateness of total or unbound concentration of the estimated hepatic inlet concentrations, or systemic concentration as $[I]_{max}$ in vivo and concluded that the unbound hepatic inlet C_{max} during the absorptive phase yielded the more accurate prediction of the magnitude of DDI (Obach et al., 2006). While this remains an outstanding issue, many recognized that these terms are fictitious correlates of [*I*] since DDI occurs within the involved eliminating organ, and the corresponding concentration of the inhibitor at the locale of the enzyme or transporter is the only pertinent one.

There is common consensus for a push for improved predictions in an interactive and dynamic fashion. The physiologically based pharmacokinetic (PBPK) modeling and simulation approach is recognized to be the most appropriate method and ideal tool so far to more reliably integrate in vitro data to in vivo (see Chapters 5, 7, 13, 21, and 26). The PBPK model has been found to be superior compared to other

methods in predicting drug kinetics and behavior in man (Parrott et al., 2006). Both transporters and enzymes and their inter-individual variations may be accommodated. The soundness of the PBPK model predicates on how the ADME parameters are fed into the model. In order to achieve good results, strategies of model representation, model parameterization, model simulation, and model evaluation must be used (Nestorov et al., 1998; Luttringer et al., 2003; Jones et al., 2006). Preexisting animal PBPK models would lend insight to the development of a useful PBPK model for humans. The approach is to select the parts of the model that are pertinent; and the lumping of compartments of similar blood flow or partition (Nestorov et al., 1998). To tackle the distributional aspects, one needs to examine the tissue to plasma partition coefficients, and differences in protein binding need to be estimated (Lin et al., 1982; Poulin and Theil, 2000; Grime and Riley, 2006; Ito and Houston, 2005). Lastly, for input of human metabolic/elimination data, direct scaling factors or hepatocellularity per gram liver tissue need to be applied to in vitro human hepatocyte data ($CL_{int,in\ vitro} \times$ cell density number $\times$ liver weight) to arrive at the intrinsic clearance in vivo, $CL_{int,in\ vivo}$ (Howgate et al., 2006; Barter et al., 2007). Then the blood to plasma ratio needs to be known to correlate blood clearance to blood flow, and binding to tissues at the target site should be considered. Some success was achieved in this kind of modeling and simulations on the appraisal of how inter-individual variations in metabolism can affect concentration–time profiles (Rostami-Hodjegan and Tucker, 2007).

In PBPK-DDI modeling, all physiological and ADME properties of both the "perpetrator" and "victim" drugs and their metabolites should be considered, together with information on study design. Mutual inhibition, if present, would be easily demonstrable. This type of modeling has been concentrated around the liver as the only eliminating organ. The efforts would reveal the temporal changes of the drug and inhibitor and their metabolites; the inhibiting species whether the inhibitor or its metabolite in transport or metabolism (modification of the K_m or V_{max}) may be included. Time-dependent destruction or induction of enzymes may further be modeled.

Various PBPK-DDI models have been used to examine in vivo kinetic consequences of mechanism-based inhibition (MBI) of CYP2D6 by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). A PBPK model with physiologically based components of drug metabolism, taking account of change in the hepatic content of active CYP2D6 due to enzyme inactivation with formation of a metabolic inhibitory complex that resulted in auto-inhibition, was used to explain dose and time dependence observed in the in vivo kinetics of MDMA (Yang et al. 2006). In other interactions comprising of midazolam/macrolides, triazolam/erythromycin, and 5-fluorouracil/sorivudine temporal changes of inhibitors were addressed and somewhat predicted using a semi-PBPK model, where the gut wall metabolism and interaction was accounted simplistically by incorporating a fixed F_g term to denote the fraction of the dose that is metabolized in gut wall (Kanamitsu et al., 2000; Ito et al., 2003). Further model development by Hall and colleagues demonstrated elegantly that excellent prediction of both reversible inhibition and MBI could be achieved with monitoring of the parent drug as well as the metabolite (Gorski et al., 1998; Pinto et al., 2005; Quinney et al., 2008a,b),

including the case that intestinally formed metabolite may act as the inhibitor and exert downstream inhibitory effects on the liver. A semi-PBPK-DDI model that took into consideration the temporal changes in concentrations of the inhibitor, diltiazem (NTZ), gut wall interaction, and contribution from the inhibitory metabolite, *N*-desmethyl-DTZ (ND-NTZ), successfully predicted the nonlinear disposition of DTZ and the interaction between DTZ and midazolam (Zhang et al., 2009). These results considered the temporal disposition of the inhibitor and DDIs at the gut wall and changes in intestinal and hepatic enzymes into model development. Simulation results undertaken were demonstrative that both DTZ and ND-DTZ contributed to the overall inhibitory effect observed following the administration of DTZ. However, success of the method was highly dependent on precise determinations of k_{inact} and K_{i}. The improved predictions from this clinical study showed that DTZ treatment resulted in 4.1-fold and 1.6-fold increases in MDZ exposure following oral and intravenous MDZ administration, respectively, and divulged that the DDI in the gut wall played an important role in the DTZ/MDZ interaction. This improved model is superior compared to other models that examined midazolam/verapamil interaction with a single inhibitor concentration (the unbound average plasma concentration of the inhibitor at the steady state), when the intestinal intrinsic clearance of CYP3A4 was applied in an attempt to account for the gut wall metabolism (Wang et al., 2004).

However, the PBPK-DDI models so far have seldom included transporters into consideration. Pang and colleagues have made some progress in inclusion of transporter parameters for basolateral influx and efflux as well as excretion in PBPK models for organs to understand the role of transporters in areas under the curve of drug and metabolite (Pang et al., 2008; Sun and Pang, 2009b). Combined physiologically based models of different eliminating organs incorporating both uptake transporters and enzymes should provide a comprehensive prediction tool to explore and accommodate the range of possible outcomes and the added complexity that may result. There is the urgent need, therefore, to consider improved intestinal models for drug absorption and metabolism. The segregated flow model (SFM) consisting of reduced flow to the enterocyte region that was developed during the turn of the century to describe route-dependent intestinal removal (Cong et al., 2000; Pang, 2003, Sun and Pang, 2009a) has not been routinely adopted for improved PBPK modeling for orally administered drugs. The fact that greater inhibition was achieved resulted for oral over the intravenous dosing of the DTZ–midazolam interaction (Zhang et al., 2009) and the greater impact for the oral dose is suggestive that the SFM may better describe the DDI data over traditional, physiological models. Since segmental distribution of transporters and enzymes will further affect bioavailability (Tam et al., 2003), improved PBPK modeling for the intestine, achieved through recognition of segmental differences in enzymes and transporters, will further address the interplay of enzymes and transporters of the intestine. There is a recent development toward modeling segmental P-gp and CYP3A in the intestine in light of the segmental traditional PBPK model (Tam et al., 2003), and may have sparked resultant simulations on drugs of varying permeability to reveal the impact of competing metabolism and P-gp efflux (Badhan et al., 2009). The resultant PBPK-DDI model would reveal the interactions between transporters and enzymes, and show

how these would affect the rise and fall of the drug and metabolite profiles and those of the inhibitor and its metabolite. However, implementation would require a quantitative definition of the contribution of transporters and enzymes, as well as the flow rate to eliminating organs. It is highly recommended that metabolites be monitored and considered in the PBPK-DDI model.

28.3.2 In Vitro Estimates For In Vivo Extrapolation (IVIVE)

From the previous section (Section 28.3.1), it is recognized that modeling and simulation is the way to gain an improved understanding of DDI. The important task is how to implement this. Better success needs to be achieved with good in vitro estimates for in vivo extrapolation (IVIVE). Much more efforts have been devoted to IVIVE to address DDI potential. But many difficulties remain (see Chapters 7–10). First, recent investigations revealed that protein–protein interactions may further complicate IVIVE. Several examples were found. For the cytochromes in a purified, reconstituted enzyme system, CYP2D6 was found to decrease the substrate-binding affinity and rates of catalysis of CYP2C9 and inhibited the CYP2C9-mediated *S*-flurbiprofen metabolism in a protein concentration-dependent manner (Subramanian et al., 2009). UGT2B7 was reported to interact with UGT1A enzymes, affecting their kinetics in human liver microsomes and underscoring the complexities in glucuronidations in human liver (Fujiwara et al., 2009). Another example existed between CYP3A4 and UGT2B7, enzymes in close proximity to each other; the Leu331-to-Lys342 domain or the surrounding region of CYP3A4 played a role in the interaction with UGT2B7 and glucuronidation (Takeda et al., 2009). Second, it is difficult, and maybe impossible, to obtain the in vivo K_i value from DDI clinical studies. What is widely appreciated is that in vitro K_i values in PBPK model-based analyses do not necessarily reflect the result observed in vivo. In almost all cases, it is predicted that when the inhibitory potency is ≤ 1 μM, an in vivo drug–drug interaction would be observed; however, if the inhibitory potency is ≥ 10 μM, there is still the possibility that the drug would cause an interaction. Even with available programs such as Simcyp®, the program currently uses the method of predicting the in vivo K_i value based on correlations of clogP (calculated logP) with the ratio of the in vivo to in vitro K_i values. Improved accuracy in the estimation of in vivo K_i values must be achieved in the future. Simulation programs (e.g., Simcyp® or Simulation Plus®) must aim toward use of in vitro data for prediction purposes by allowing the incorporation of inter-individual variability and the easy exploration of various model options.

28.3.3 Software for Modeling

There are some attempts for Simcyp® to develop improved PBPK-DDI models that can account for the segregated flow effect, by employing only 40–59%

of intestinal flow for the assessment of intestinal clearance (Yang et al., 2007). Similar attempts have been made for Simulation Plus® (personal communication, Dr. Michael Bolger). It would be of interest to view how the above concepts on segregated flow of the intestine (Cong et al., 2000) and segmental distribution of transporters and enzymes (Tam et al., 2003), when incorporated into modeling and software, improve the level of prediction of drug absorption and bioavailability in clinical DDI studies. It was further stressed that the unbound fraction obtained in vitro in the incubation mixture, as well as the unbound and not total concentration of the inhibitor should be inputted for proper estimation of the intrinsic clearance of drug (see Chapter 7).

Present softwares that examine PBPK modeling or PBPK-DDI have yet to include transporters. It is hoped that some of the recently developed theoretical works on organ clearance concepts (Pang et al., 2008; Sun and Pang, 2009b) would fuel further development in this area. Such progress is sorely needed, because current models fail to fully rationalize long-standing and well-documented perpetrator–victim pairs such as gemfibrozil–repaglinide (involving CYP2C8 and/or organic anion transporting peptide 1B1 inhibition), 17α-ethinyl estradiol–selegiline (involving CYP2B6 inhibition), 17α-ethinyl estradiol–melatonin (involving CYP1A2 inhibition), and capecitabine–warfarin (involving CYP2C9 inhibition) (Chang et al., 2009; Hinton et al., 2008; Yildirim et al., 2006; Janney and Waterbury, 2005).

28.4 Difficulties Remaining

The inclusion of transporter activities (as influx or efflux intrinsic clearances) for the liver and kidney, and the combination of more physiologically relevant intestinal models would improve the IVIVE. Remaining challenges of accounting for variability in patient populations due to age, ethnicity, and genetic makeup on IVIVE need attention in simulation packages. The slowest process (or rate-limiting step) that affords the greatest change (or sensitivity) should be emphasized in the DDI reaction. The complex metabolic drug interactions such as inductive, mechanism-based, and allosteric DDIs would add more complexity and difficulty for prediction in vivo. Consideration should be given to the interactions between a drug ligand and nuclear receptors or cytokines that result in changes in transporters and enzymes (Le Vee et al., 2009; Fardel and Le Vee, 2009). These may be overcome by modeling and simulations, and development of softwares may be advanced to predict these kinds of occurrences and understand rate-determining steps. Metabolites as culprits should be seriously considered, and metabolite information is paramount (see Chapter 5). It is envisaged that proper validation of the simulation model with in vivo data would greatly strengthen the methodology and allow future classifications to be made.

28.4.1 Multiple Interactions

Unlike the DDIs that involve a single mechanism for pair of drugs, the effect of multiple drug interactions is difficult to design and evaluate properly in vivo. The use of

modeling and simulation, especially with a PBPK approach, again appears promising to understand these complex drug interactions (see Chapter 26). Future studies need to focus on better defining key parameters required to quantitatively evaluate multiple factors and mechanistic understanding of the combined effects. Besides study design, the utility of modeling and simulation in labeling recommendations for the safe and effective use of a drug needs to be explored

28.4.2 Drug Interactions Involving Biologic Agents

In recent years, the pharmaceutical industry has employed increasing numbers of biologic agents, such as monoclonal antibodies (Mabs), domain antibodies (Dabs), inhibitory ribonucleic acid (RNAi), and protein or peptide-based derivatives (Mascelli et al., 2007; Zhou, 2007). Many of these agents are administered intravenously (or subcutaneously) and circulate at high concentrations, because of "pharmacokinetic enhancement" enabled by pegylation or coupling to proteins such as serum albumin. Unlike their small molecule counterparts, many biologics are characterized by a long plasma half-life of "days" rather than "hours," with clearance and distribution largely determined by the binding to, and turnover rate of, the biological target itself.

Although the manufacturing and safety of biologic agents has received considerable attention, the different aspects of DDIs have yet to be explored. For example, it is only recently that the possibility of DDIs involving therapeutic antibodies was considered and examined (Seitz and Zhou, 2007; Mahmood and Green, 2007). This is important, because many therapeutic antibodies interact with target proteins on the surface of cells and modulate circulating and intracellular cytokines. In turn, such cytokines can impact the expression of drug-metabolizing enzymes and transporters in tissues (Le Vee et al., 2009; Fardel and Le Vee, 2009). How would one go about assessing such DDI potential preclinically? What type of models and screening funnels would have to be in place? In the absence of validated in vivo (animal) and in vitro models, this would prove challenging. It is clear that the industry will have to adapt its current small molecule-oriented paradigms to encompass biologics. Moreover, regulatory agencies will have to more fully address this topic in future guidance documents.

28.4.3 Future Improvement of Prediction Strategies

From an industrial standpoint, a considerable amount of resources has been spent over the last decade building up databases (see Chapters 15 and 16) and developing fully automated (high-throughput) screening platforms. This has reached the point where most mid- to large-sized pharmaceutical companies are able to generate in vitro data for large numbers of compounds employing various (e.g., 384- and 1536-well) assay formats. For example, in vitro P450 inhibition and induction assays have

become routine and the data are being used to screen out compounds, drive lead optimization campaigns, enable development of structure–activity relationships (SARs), and prioritize and guide the design of follow-up clinical DDI studies (Chapter 23). Although most groups can generate such data, the major challenge is what to do with it, how to integrate it, "bring it all together," and enable decision making.

Fortunately, the various members of industry organizations such as PhRMA (Pharmaceutical Research Manufacturers of America) have made the first steps in reaching a consensus on best practices for the *conduct of* preclinical and clinical DDI studies (Bjornsson et al., 2003; Chu et al., 2009; Grimm et al., 2009). In most cases, however, the focus has been on the implementation of empirical approaches (e.g., $[I]/K_i$ ratio guidance or the rank order approach) with little detail related to the actual modeling of DDIs. It is hoped that such consensus will pave the way for additional progress in the years to come, because DDI models in the future will need to be much more mechanistic and incorporate data from an ever increasing array of sources. For example, as microsomal-binding and protein-binding assays continue to become standard approaches, in silico methods for the prediction of free fractions and protein–protein interactions will become as common for DDI predictions as they are currently used for unbound clearance predictions. Similarly, as one P450 is inhibited, in silico models will eventually be able to predict the fraction metabolized (f_m) shift to another clearance mechanism, and the predicted impact on exposure in terms of AUC, C_{max}, and associated pharmacokinetic parameters. With greater emphasis being placed on pharmacokinetic–pharmacodynamics (PK-PD), it is also likely that DDI models will have to incorporate such information and enable assessment of likely impact on PD, not just PK. More broadly, the application of a systems biology approach to PK-ADME (absorption-distribution-metabolism-excretion) research will also enable improvements in DDI modeling and predictions (Ekins et al., 2007). Despite the obvious challenges, if the progress of the last two decades is anything to go by, the field of DDI research will continue to mature and develop in ways that cannot be imagined today.

Acknowledgments This work supported in part by CIHR grant (MOP89850 awarded to KSP).

References

Akita H, Suzuki H and Sugiyama Y (2001) Sinusoidal efflux of taurocholate is enhanced in Mrp2-deficient rat liver. *Pharm Res* **18**:1119–1125.

Allen JD, Brinkhuis RF, van Deemter L, Wijnholds J and Schinkel AH (2000) Extensive contribution of the multidrug transporters P-glycoprotein and Mrp1 to basal drug resistance. *Cancer Res* **60**:5761–5766.

Annaert P, Turncliff RZ, Booth CL, Thakker DR and Brouwer KLR (2001) P-Glycoprotein-mediated in vitro biliary excretion in sandwich-cultured rat hepatocytes. *Drug Metab Dispos* **29**:1277–1283.

Badhan R, Penny J, Galetin A, and Houston JB (2009) Methodology for development of a physiological model incorporating CYP3A and P-glycoprotein for the prediction of intestinal drug absorption. *J Pharm Sci* **98**:2180–2197.

Barter ZE. Bayliss MK, Beaune PH, Boobis AR, Carlie DJ, Edwards RJ, Houston JB, Lake BG, Lipscomb JC, Pelkonen OR, Tucker GT and Rostami-Hodjegan A (2007) Scaling factors for the extrapolation of in vivo metabolic drug clearance from in vitro data: reaching a consensus on values on human microsomal protein and hepatocellurity per gram of liver. *Curr Drug Metab* **8**:33–45.

Battaglia E and Gollan J (2001) A unique multifunctional transporter translocates estradiol-17β-glucuronide in rat liver microsomal vesicles. *J Biol Chem* **276**:23492–23498.

Bjornsson TD, Callaghan JT, Einolf HJ, Fischer V, Gan L, Grimm S, Kao J, King SP, Miwa G, Ni L, Kumar G, McLeod J, Obach RS, Roberts S, Roe A, Shah A, Snikeris F, Sullivan JT, Tweedie D, Vega JM, Walsh J, and Wrighton SA Pharmaceutical Research and Manufacturers of America (PhRMA) Drug Metabolism/Clinical Pharmacology Technical Working Group; FDA Center for Drug Evaluation and Research (CDER) (2003) The conduct of in vitro and in vivo drug–drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metab Dispos* **31**:815–832.

Brown HS, Ito K, Galetin A and Houston JB (2005) Prediction of in vivo drug–drug interactions from in vitro data: impact of incorporating parallel pathways of drug elimination and inhibitor absorption rate constant. *Br J Clin Pharmacol* **60**:508–518.

Cacciatore I, Caccuri AM, Cocco A, De Maria F, Di Stefano A, Luisi G, Pinnen F, Ricci G, Sozio P and Turella P (2005) Potent isozyme-selective inhibition of human glutathione S-transferase A1-1 by a novel glutathione S-conjugate. *Amino Acids* **29**:255–261.

Chandra P, LeCluyse EL and Brouwer KLR (2001) Optimization of culture conditions for determining hepatobiliary disposition of taurocholate in sandwich-culture rat hepatocytes. In Vitro *Cell Dev Biol – Animal* **37**:380–385.

Chang SY, Chen C, Yang Z and Rodrigues AD (2009) Further assessment of 17α-ethinyl estradiol as an inhibitor of different human cytochrome P450 forms in vitro. *Drug Metab Dispos* **38**:1667–1675.

Chen ZS, Guo Y, Belinsky MG, Kotova E and Kruh GD (2005) Transport of bile acids, sulfated steroids, estradiol 17-beta-D-glucuronide, and leukotriene C4 by human multidrug resistance protein 8 (ABCC11). *Mol Pharmacol* **67**:545–557.

Chu XY, Strauss JR, Mariano MA, Li J, Newton DJ, Cai X, Wang RW, Yabut J, Hartley DP, Evans DC and Evers R (2006) Characterization of mice lacking the multidrug resistance protein MRP2 (ABCC2). *J Pharmacol Exp Ther* **317**:579–589.

Chu V, Einolf HJ, Evers R, Kumar GN, Moore D, Ripp SL, Silva J, Sinha V, Sinz M and Skerjanec A (2009) In vitro and in vivo induction of cytochrome P450: a survey of the current practices and recommendations: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metab Dispos* **37**:1339–1354.

Cong D, Doherty M and Pang KS (2000) A new physiologically-based segregated flow model to explain route-intestinal metabolism. *Drug Metab Dispos* **28**:224–235.

Coughtrie M, Riches Z, Bloomer J, Patel A and Nolan A (2009) Assessment of cryopreserved human hepatocytes as a model system to investigate sulfation and glucuronidation and to evaluate inhibitors of drug conjugation. *Xenobiotica* **11**:1–8.

Csala M, Staines AG, Bánhegyi G, Mandl J, Coughtrie MW and Burchell B (2004) Evidence for multiple glucuronide transporters in rat liver microsomes. *Biochem Pharmacol* **68**:1353–1362.

Dallas S, Schlichter L and Bendayan R (2004) Multidrug resistance protein (MRP) 4- and MRP 5-mediated efflux of 9-(2-phosphonylmethoxyethyl)adenine by microglia. *J Pharmacol Exp Ther* **309**:1221–1229.

Ekins S, Ecker GF, Chiba P and Swaan PW (2007) Future directions for drug transporter modeling. *Xenobiotica* **37**:1152–1170.

Fardel O and Le Vee M (2009) Regulation of human hepatic drug transporter expression by pro-inflammatory cytokines. *Expert Opin Drug Metab Toxicol* September 28 [epub]

Felmlee MA, Lon HK, Gonzalez FJ and Yu AM (2008) Cytochrome P450 expression and regulation in CYP3A4/CYP2D6 double transgenic humanized mice. *Drug Metab Dispos* **36**:435–441.

Fujiwara R, Nakajima M, Oda S, Yamanaka H, Ikushiro SI, Sakaki T and Yokoi T (2009) Interactions between human UDP-glucuronosyltransferase (UGT) 2B7 and UGT1A enzymes. *J Pharm Sci* May 27. [Epub ahead of print].

Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara EM Jr and Hall SD (1998) The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. *Clin Pharmacol Ther* **64**:133–143.

Grime K, Riley RJ (2006) The impact of in vitro binding on in vitro-in vivo extrapolations, projection of metabolic clearance and clinical drug–drug interactions. *Curr Drug Metab* **7**:251–264.

Grimm SW, Einolf HJ, Hall SD, He K, Lim HK, Ling KH, Lu C, Nomeir AA, Seibert E, Skordos KW, Tonn GR, Van Horn R, Wang RW, Wong YN, Yang TJ and Obach RS The conduct of in vitro studies to address time-dependent inhibition of drug metabolizing enzymes: a perspective of the Pharmaceutical Research and Manufacturers of America (PhRMA). *Drug Metab Dispos* **37**:1355–1370.

Groen AK, Bloks VW, Bandsma RH, Ottenhoff R, Chimini G and Kuipers F (2001) Hepatobiliary cholesterol transport is not impaired in Abca1-null mice lacking HDL. *J Clin Invest* **108**: 843–850.

Gyamfi MA, Ohtani II, Shinno E and Aniya Y (2004) Inhibition of glutathione S-transferases by thonningianin A, isolated from the African medicinal herb, Thonningia sanguinea, in vitro. *Food Chem Toxicol* **42**:1401–1408.

Harris RM and Waring RH (2008) Sulfotransferase inhibition: potential impact of diet and environmental chemicals on steroid metabolism and drug detoxification. *Curr Drug Metab* **9**:269–275.

Hayeshi R, Chinyanga F, Chengedza S and Mukanganyama S (2006) Inhibition of human glutathione transferases by multidrug resistance chemomodulators in vitro. *J Enzyme Inhib Med Chem* **21**:581–587.

Hinton LK, Galetin A and Houston JB (2008) Multiple inhibition mechanisms and prediction of drug–drug interactions: status of metabolism and transporter models as exemplified by gemfibrozil-drug interactions. *Pharm Res* **25**:1063–1074.

Ho RH, Tirona RG, Leake BF, Glaeser H, Lee W, Lemke CJ, Wang Y and Kim RB (2006) Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology* **130**:1793–1806.

Hoffmaster KA, Turncliff RZ, LeCluyse EL, Kim RB, Meier PJ and Brouwer KLR (2004) P-glycoprotein expression, localization, and function in sandwich-cultured primary rat and human hepatocytes: relevance to the hepatobiliary disposition of a opiod peptide. *Pharm Res* **21**: 1294–1302.

Honda Y, Ushigome F, Koyabu N, Morimoto S, Shoyama Y, Uchiumi T, Kuwano M, Ohtani H and Sawada Y (2004) Effects of grapefruit juice and orange juice components on P-glycoprotein- and MRP2-mediated drug efflux. *Br J Pharmcol* **143**:856–864.

Howgate EM, Rowland Yeo K, Proctor NJ, Tucker GT and Rostami-Hodjegan A (2006) Prediction of in vivo drug clearance from in vitro data. I: Impact of inter-individual variability. *Xenobiotica* **36**:473–497.

Inoue T, Sugihara K, Ohshita H, Horie T, Kitamura S and Ohta S (2009) Prediction of human disposition toward S-^{3}H-warfarin using chimeric mice with humanized liver. *Drug Metab Pharmacokinet* **24**:153–160.

Ito K and Houston JB (2005) Prediction of human drug clearance from in vitro and preclinical data using physiologically based and empirical approaches. *Pharm Res* **22**:103–112.

Ito K, Ogihara K, Kanamitsu S and Itoh T (2003) Prediction of the in vivo interaction between midazolam and macrolides based on in vitro studies using human liver microsomes. *Drug Metab Dispos* **31**:945–954.

Ito K, Hallifax D, Obach RS and Houston JB (2005) Impact of parallel pathways of drug elimination and multiple cytochrome p450 involvement on drug–drug interactions: CYP2D6 paradigm. *Drug Metab Dispos* **33**:837–844.

Jäger W, Sartori M, Herzog W and Thalhammer T (1998) Genistein metabolism in liver microsomes of Wistar and mutant TR(-)-rats. *Res Commun Mol Pathol Pharmacol* **100**: 105–116.

Janney LM and Waterbury NV (2005) Capecitabine-warfarin interaction. *Ann Pharmacother* **39**:1546–1551.

Jedlitschky G, Leier I, Buchholz U, Barnouin K, Kurz G and Keppler D (1996) Transport of glutathione, glucuronate, and sulfate conjugates by the MRP gene-encoded conjugate export pump. *Cancer Res* **56**:988–994.

Jigorel E, Le Vee M, Boursier-Neyret C, Bertrand M and Fardel O (2005) Functional expression of sinusoidal drug transporters in primary human and rat hepatocytes. *Drug Metab Dispos* **33**:1418–1422.

Jones HM, Parrott N, Jorga K and Lavé T (2006) A novel strategy for physiologicallly based predictions of human pharmacokinetics. *Clin Pharmacokinet* **45**:511–542.

Kamiie J, Ohtsuki S, Iwase R, Ohmine K, Katsukura Y, Yanai K, Sekine Y, Uchida Y, Ito S and Terasaki T (2008) Quantitative atlas of membrane transporter proteins: development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel in-silico peptide selection criteria. *Pharm Res* **25**:1469–1483.

Kanamitsu SI, Ito K, Okuda H, Ogura K, Watabe T, Muro K and Sugiyama Y (2000) Prediction of in vivo drug–drug interactions based on mechanism-based inhibition from in vitro data: inhibition of 5-fluorouracil metabolism by (E)-5-(2-bromovinyl)uracil. *Drug Metab Dispos* **28**:467–474.

Kim S, Pray D, Zheng M, Morgan DG, Pizzano JG, Zoeckler ME, Chimalakonda A and Sinz MW (2008) Quantitative relationship between rifampicin exposure and induction of Cyp3a11 in SXR humanized mice: extrapolation to human CYP3A4 induction potential. *Drug Metab Lett* **2**:169–175.

Kimura S, Kawabe M, Ward JM, Morishima H, Kadlubar FF, Hammons GJ, Fernandez-Salguero P and Gonzalez FJ (1999) CYP1A2 is not the primary enzyme responsible for 4-aminobiphenyl-induced hepatocarcinogenesis in mice. *Carcinogenesis* **20**:1825–1830.

Khetani SR and Bhatia SN (2008) Microscale culture of human liver cells for drug development. *Nat Biotechnol* **26**:12012–12016.

Kopplow K, Letschert K, König J, Walter B and Keppler D (2005) Human hepatobiliary transport of organic anions analyzed by quadruple-transfected cells. *Mol Pharmacol* **68**:1031–1038.

Kostrubsky SE, Sinclair JF, Strom SC, Wood S, Urda E, Stolz DB, Wen YH, Kulkarni S and Mutlib A (2005) Phenobarbital and phenytoin increased acetaminophen hepatotoxicity due to inhibition of UDP-glucuronosyltransferases in cultured human hepatocytes. *Toxicol Sci* **87**:146–155.

Lahvis GP, Lindell SL, Thomas RS, McCuskey RS, Murphy C, Glover E, Bentz M, Southard J and Bradfield CA (2000) Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. *Proc Natl Acad Sci USA* **97**:10442–10447.

Le Vee M, Lecureur B, Stieger B and Fardel O (2009) Regulation of transporter expression in human hepatocytes exposed to the proinflammatory cytokines tumor necresis factor-α or interleukin-6. *Drug Metab Dispos* **37**:685–593.

Leier I, Hummel-Eisenbeiss J, Cui Y and Keppler D (2000) ATP-dependent para-aminohippurate transport by apical multidrug resistance protein MRP2. *Kidney Int* **57**:1636–1642.

Lin JH, Sugiyama Y, Awazu S and Hanano M (1982) In vitro and in vivo evaluation of the tissue-to-blood partition coefficient for physiological pharmacokinetic models. *J Pharmacokinet Biopharm* **10**:637–647.

Li N, Zhang Y, Hua F and Lai Y (2009) Absolute difference of hepatobiliary transporter multidrug resistance-associated protein (MRP2/Mrp2) in liver tissues and isolated hepatocytes from rat, dog, monkey, and human. *Drug Metab Dispos* **37**:66–73.

Liu Y, Apak TI, Lehmler HJ, Robertson LW and Duffel MW (2006) Hydroxylated polychlorinated biphenyls are substrates and inhibitors of human hydroxysteroid sulfotransferase SULT2A1. *Chem Res Toxicol* **19**:1420–1425.

Lu Y, Bratton S, Heydel JM and Radominska-Pandya A (2008) Effect of retinoids on UDP-glucuronosyltransferase 2B7 mRNA expression in Caco-2 cells. *Drug Metab Pharmacookinet* **23**:364–372.

Lorico A, Nesland J, Emilsen E, Fodstad O and Rappa G (2002) Role of the multidrug resistance protein 1 gene in the carcinogenicity of aflatoxin B1: investigations using mrp1-null mice. *Toxicology* **171**:201–205.

Lorico A, Rappa G, Finch RA, Yang D, Flavell RA and Sartorelli AC (1997) Disruption of the murine MRP (multidrug resistance protein) gene leads to increased sensitivity to etoposide (VP-16) and increased levels of glutathione. *Cancer Res* **57**: 5238–5242.

Luttringer O, Theil F-P, Poulin P, Schmitt-Hoffmann AH, Guentert TW, Lavé T (2003) Physiologically based pharmacokinetic (PBPK) modelling of disposition of epiroprim in humans. *J Pharm Sci* **92**:1990–2007.

Mahmood I and Green MD (2007) Drug interaction studies of therapeutic proteins or monoclonal antibodies. *J Clin Pharmacol* **47**:1540–1554.

Mascelli MA, Zhou H, Sweet R, Getsy J, Davis HM, Graham M and Abernethy D (2007) Molecular, biologic, and pharmacokinetic properties of monoclonal antibodies: impact of these parameters on early clinical development. *J Clin Pharmacol* **47**:553–565.

Matsson P, Pedersen JM, Norinder U, Bergström CA and Artursson P (2009) Identification of novel specific and general inhibitors of the three major human ATP-binding cassette transporters P-gp, BCRP and MRP2 among registered drugs. *Pharm Res* **26**:1816–1831.

Maliepaard M, van Gastelen MA, Tohgo A, Hausheer FH, van Waardenburg RC, de Jong LA, Pluim D, Beijnen JH and Schellens JH (2001) Circumvention of breast cancer resistance protein (BCRP)-mediated resistance to camptothecins in vitro using non-substrate drugs or the BCRP inhibitor GF120918. *Clin Cancer Res* **7**:935–941.

McConn DJ 2nd, Lin YS, Allen K, Kunze KL and Thummel KE (2004) Differences in the inhibition of cytochromes P450 3A4 and 3A5 by metabolite-inhibitor complex-forming drugs. *Drug Metab Dispos* **32**:1083–1091.

Morris ME and Pang KS (1987) Competition between two enzymes for substrate removal in liver: modulating effects due to substrate recruitment of hepatocyte activity. *J Pharmacokinet Biopharm* **15**:473–496.

Morrow CS, Peklak-Scott C, Bishwokarma B, Kute TE, Smitherman PK and Townsend AJ (2006) Multidrug resistance protein 1 (MRP1, ABCC1) mediates resistance to mitoxantrone via glutathione-dependent drug efflux. *Mol Pharmacol* **69**:1499–1505.

Nahmias Y, Casali M, Barbe L, Berthiaume F and Yarmush ML (2006) Liver endothelial cells promote LDL-R expression and the uptake of HCV-like particles in primary rat and human hepatocytes. *Hepatology* **43**:257–265.

Nestorov IA, Aarons LJ, Arundel PA and Rowland M (1998) Lumping of whole-body physiologically-based pharmacokinetic models. *J Pharmcokinet Biopharm* **26**: 21–46.

Nezasa K, Tian X, Zamek-Gliszczynski MJ, Patel NJ, Raub TJ and Brouwer KL (2006) Drug Altered hepatobiliary disposition of 5 (and 6)-carboxy-2′,7′-dichlorofluorescein in Abcg2 (Bcrp1) and Abcc2 (Mrp2) knockout mice. *Drug Metab Dispos* **34**:718–723.

Obach RS, Walsky RL, Venkatakrishnan K, Gaman EA, Houston JB and Tremaine LM (2006) The utility of in vitro cytochrome p450 inhibition data in the prediction of drug–drug interactions. *J Pharmacol Exp Ther* **316**:336–348.

Ohno M, Motojima K, Okano T and Taniguchi A (2008) Up-regulation of drug-metabolizing enzyme genes in layered co-culture of a human liver cell line and endothelial cells. *Tissue Eng Part A* **14**:1861–1869.

Okumura H, Katoh M, Sawada T, Nakajima M, Soeno Y, Yabuuchi H, Ikeda T, Tateno C, Yoshizato K and Yokoi T (2007) Humanization of excretory pathway in chimeric mice with humanized liver. *Toxicol Sci* **97**:533–538.

Pacifici GM (2004) Inhibition of human liver and duodenum sulfotransferases by drugs and dietary chemicals: a review of the literature. *Int J Clin Pharmacol Ther* **42**:488–495.

Pang KS (2003) Modeling of intestinal drug absorption: roles of transporters and metabolic enzymes (For the Gillette Review Series). *Drug Metab Dispos* **31**:1507–1519.

Pang KS, Morris ME and Sun H (2008) Formed and preformed metabolite: facts and comparisons. *J Pharm Pharmacol* **60**:1247–1275.

Parrott N, Parquerau N, Coassolo P and Lavé T (2006) An evaluation of the utility of physiologically-based models of pharmacokinetics in early drug discovery. *J Pharm Sci* **94**:2327–2343.

Pinto AG, Wang Y-H, Chalasani N, Skaar T, Kolwankdar D, Gorski JC, Lngpunsakul S, Hamman MA, Arefayene M and Hall SD (2005) Inhibition of human intestinal wall metabolism by macrolide antibiotics: effect of clarithromycin on cytochrome P450 3A4/5 activity and expression·. *ClinPharmacol Ther* **77**:178–188.

Polli JW, Wring SA, Humphreys JE, Huang L, Morgan JB, Webster LO and Serabjit-Singh CS (2001) Rational use of in vitro P-glycoprotein assays in drug discovery. *J Pharmacol Exp Ther* **299**:620–628.

Poulin P and Theil F-P (2000) A priori prediction of tissue:plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery. *J Pharm Sci* **89**:16–35.

Quinney SK, Galinsky RE, Jiyamapa-Serna VA, Chen Y, Hamman MA, Hall SD and Kimura RE (2008a) Hydroxyitraconazole, formed during intestinal first-pass metabolism of itraconazole, controls the time course of hepatic CYP3A inhibition and the bioavailability of itraconazole in rats. *Drug Metab Dispos* **36**:1097–1101.

Quinney SK, Haehner BD, Rhoades MB, Lin Z, Gorski JC and Hall SD (2008b) Interaction between midazolam and clarithromycin in the elderly. *Br J Clin Pharmacol* **65**:98–109.

Rappa G, Finch RA, Sartorelli AC and Lorico A (1999) New insights into the biology and pharmacology of the multidrug resistance protein (MRP) from gene knockout models. *Biochem Pharmacol* **58**:557–562.

Reid G Wielinga P, Zelcer N, De Haas M, Van Deemter L, Wijnholds J, Balzarini J and Borst P (2003) Characterization of the transport of nucleoside analog drugs by the human multidrug resistance proteins MRP4 and MRP5. *Mol Phamracol* **63**:1094–1103.

Riches Z, Bloomer JC and Coughtrie MW (2007) Comparison of 2-aminophenol and 4-nitrophenol as in vitro probe substrates for the major human hepatic sulfotransferase, SULT1A1, demonstrates improved selectivity with 2-aminophenol. *Biochem Pharmacol* **74**:352–358.

Rostami-Hodjegan A and Tucker GT (2007) Simulation and prediction of in vivo drug metabolism in human populations from in vitro data. *Nature Rev* **6**:141–148.

Rowland M and Matin SB (1973) Kinetics of drug–drug interactions. *J Pharmacokinet Biopharm* **1**:553–567.

Sakai-Kato K, Kato M and Toyóoka T (2004) Screening of inhibitors of uridine diphosphate glucuronosyltransferase with a miniaturized on-line drug-metabolism system. *J Chromatgor A* **1051**:261–266.

Samokyszyn VM, Gall WE, Zawada G, Freyaldenhoven MA, Chen G, Mackenzie PI, Tephly TR and Radominska-Pandya A (2000) 4-hydroxyretinoic acid, a novel substrate for human liver microsomal UDP-glucuronosyltransferase(s) and recombinant UGT2B7. *J Biol Chem* **275**:6908–6914.

Saruwatari A, Okamura S, Nakajima Y, Narukawa Y, Takeda T and Tamura H (2008) Pomegranate juice inhibits sulfoconjugation in Caco-2 human colon carcinoma cells. *J Med Food* **11**: 623–628.

Schmidt JV, Su GH, Reddy JK, Simon MC and Bradfield CA (1996) Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc Natl Acad Sci USA* **93**:6731–6736.

Schwab AJ, Barker III F, Goresky CA and Pang KS (1990) Transfer of enalaprilat across rat liver cell membranes is barrier-limited. *Am J Physiol* **258**:G461–G475.

Schwab AJ, de Lannoy IAM, Poon K, Goresky CA and Pang KS (1992) Enalaprilat handling by the rat kidney: barrier-limited cell entry. *Am J Physiol* **263**:F858–F869.

Schwartz J, Hunt T, Smith WB, Wong P, Larson P, Crumley T, Mehta A, Gottesdiener K and Agrawal N (2009) The Effect of etoricoxib on the pharmacokinetics of oral contraceptives in healthy participants. *J Clin Pharmacol* **49**:807–815.

Seitz K and Zhou H (2007) Pharmacokinetic drug–drug interaction potentials for therapeutic monoclonal antibodies: reality check. *J Clin Pharmacol* **47**:1104–1118.

Shitara Y, Hirano M, Sata H and Sugiyama Y (2004) Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug–drug interaction between cerivastatin and gemfibrozil. *J Pharmacol Exp Ther* **311**:228–236.

Shitara Y, Itoh T, Sato H, Li AP and Sugiyama Y (2003) Inhibition of transporter-mediated hepatic uptake as a mechanism for drug–drug interaction between cerivastatin and cyclosporin A. *J Pharmacol Exp Ther* **304**:610–616.

Sirianni G and Pang KS (1997) Organ clearance concepts: new perspectives on old principles. *J Pharmacokinet Biopharm* **25**:449–470.

Stark K and Guengerich FP (2007) Characterization of orphan human cytochromes P450. *Drug Metab Rev* **39**:627–637.

Stark K, Dostalek M and Guengerich FP (2008a) Expression and purification of orphan cytochrome P450 4X1 and oxidation of anandamide. *FEBS J* **275**:3706–3717.

Stark K, Wu ZL, Bartleson CJ and Guengerich FP (2008b) mRNA distribution and heterologous expression of orphan cytochrome P450 20A1. *Drug Metab Dispos* **36**:1930–1937.

Subramanian M, Low M, Locuson CW and Tracy TS (2009) CYP2D6-CYP2C9 Protein-protein interactions and isoform-selective effects on substrate binding and catalysis. *Drug Metab Dispos* **37**:1682–1689.

Sun H and Pang KS (2009a) Disparity in intestine disposition between formed and preformed metabolites and implications: a theoretical study. *Drug Metab Dispos* **37**:187–202.

Sun H and Pang KS (2009b) Physiological modeling to understand the impact of enzymes and transporters on drug and metabolite data and bioavailability estimates. Submitted.

Taipalensuu J, Tavelin S, Lazorova L, Svensson AC and Artursson P (2004) Exploring the quantitative relationship between the level of MDR1 transcript, protein and function using digoxin as a marker of MDR1-dependent drug efflux activity. *Eur J Pharm Sci* **21**:69–75.

Takeda S, Ishii Y, Iwanaga M, Nurrochmad A, Ito Y, Mackenzie PI, Nagata K, Yamazoe Y, Oguri K and Yamada H (2009) Interaction of cytochrome P450 3A4 and UDP-glucuronosyl-transferase 2B7: evidence for protein-protein association and possible involvement of CYP3A4 J-helix in the interaction. *Mol Pharmacol* **75**:956–964.

Tam D, Tirona RG and Pang KS (2003) Segmental intestinal transporters and metabolic enzymes on intestinal drug absorption. *Drug Metab Dispos* **33**:373–383.

Tamura H and Matsui M (2000) Inhibitory effects of green tea and grape juice on the phenol sulfotransferase (SULT1A1) activity of human colon carcinoma cell line, Caco-2. *Biol Pharm Bull* **23**:695–699.

Tang F, Horie K and Borchardt RT (2002) Are MDCK cells transfected with the human MRP2 gene a good model of the human intestinal mucosa? *Pharm Res* **19**:773–779.

Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* **40**:581–616.

Turncliff RZ, Meier PJ, Brouwer KLR (2004) Effect of dexamethasone treatment on the expression and function of transport proteins in sandwich-culture rat hepatocytes. *Drug Metab Dispos* **32**:834–839.

Uno S, Endo K, Ishida Y, Tateno C, Makishima M, Yoshizato K and Nebert DW (2009) CYP1A1 and CYP1A2 expression: comparing ′humanized′ mouse lines and wild-type mice; comparing human and mouse hepatoma-derived cell lines. *Toxicol Appl Pharmacol* **237**:119–126.

Volak LP, Ghirmai S, Cashman JR and Court MH (2008) Curcuminoids inhibit multiple human cytochromes P450, UDP-glucuronosyltransferase, and sulfotransferase enzymes, whereas piperine is a relatively selective CYP3A4 inhibitor. *Drug Metab Dispos* **36**:1594–1605.

Volk EL and Schneider E (2003) Wild-type breast cancer resistance protein (BCRP/ABCG2) is a methotrexate polyglutamate transporter. *Cancer Res* **63**:5538–5543.

Wang R, Salem M, Yousef IM, Tuchweber B, Lam P, Childs SJ, Helgason CD, Ackerley C, Phillips MJ and Ling V (2001) Targeted inactivation of sister of P-glycoprotein gene (spgp) in mice results in nonprogressive but persistent intrahepatic cholestasis. *Proc Natl Acad Sci USA* **98**:2011–2016.

Wang YH, Jones DR and Hall SD (2004) Prediction of cytochrome P450 3A inhibition by verapamil enantiomers and their metabolites. *Drug MetabDispos* **32**:259–266.

Wijnholds J, Evers R, van Leusden MR, Mol CA, Zaman GJ, Mayer U, Beijnen JH, van der Valk M, Krimpenfort P and Borst P (1997) Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking the multidrug resistance-associated protein. *Nature Med* **3**:1275–1279.

Yang J, Jamei M, Yeo KR, Tucker GT and Rostami-Hodjegan A (2007) Prediction of intestinal first-pass drug metabolism. *Curr Drug Metab* **8**:676–684.

Yang J, Jamei M, Yeo KR, de la Torre R, Farré M, Tucker GT and Rostami-Hodjegan A (2006) Implications of mechanism-based inhibition of CYP2D6 for the pharmacokinetics and toxicity of MDMA. *J Psychopharmacol* **20**:842–849.

Yildirim Y, Ozyilkan O, Akcali Z and Basturk B (2006) Drug interaction between capecitabine and warfarin: a case report and review of the literature. *Int J Clin Pharmacol Ther* **44**:80–82.

Zamek-Gliszczynski M, Xiong H, Patel NJ, Turncliff RZ, Pollack GM and Brouwer KLR (2003) Pharmacokinetics of (5 and 6)-carobyx-2'-7'-dichlorofluorescein and its diacetate promoiety in the liver. *J Pharmacol Ep Ther* **304**:801–809.

Zelcer N, Saeki T, Bot I, Kuil A and Borst P (2003) Transport of bile acids in multidrug-resistance-protein 3-overexpressing cells co-transfected with the ileal Na^+-dependent bile-acid transporter. *Biochem J* **369**(Pt 1):23–30.

Zhang D, Chando TJ, Everett DW, Patten CJ, Dehal SS and Humphreys WG (2005a) In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. *Drug Metab Dispos* **33**:1729–1739.

Zhang P, Tian X, Chandra P and Brouwer KLR (2005b) Role of glycosylation in trafficking of Mrp2 in sandwich culture hepatocytes. *Mol Pharmacol* **67**:1334–1341.

Zhang H, Davis CD, Sinz MW and Rodrigues AD (2007) Cytochrome P450 reaction-phenotyping: an industrial perspective. *Expert Opin Drug Metab Toxicol* **3**:667–687.

Zhang X, Jones DR and Hall SD (2009) Prediction of the effect of erythromycin, diltiazem, and their metabolites, alone and in combination, on CYP3A4 inhibition. *Drug Metab Dispos* **37**:150–160.

Zhou H (2007) Biologics in the pipeline: large molecules with high hopes or bigger risks? *J Clin Pharmacol* **47**:550–552.