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EXPERT REVIEW

Use of *In Vivo* Animal Models to Assess Pharmacokinetic Drug-Drug Interactions

Cuyue Tang • Thomayant Prueksaritanont

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ABSTRACT Animal models are used commonly in various stages of drug discovery and development to aid in the prospective assessment of drug-drug interaction (DDI) potential and the understanding of the underlying mechanism for DDI of a drug candidate. *In vivo* assessments in an appropriate animal model can be very valuable, when used in combination with *in vitro* systems, to help verify *in vivo* relevance of the *in vitro* animal-based results, and thus substantiate the extrapolation of *in vitro* human data to clinical outcomes. From a pharmacokinetic standpoint, a key consideration for rational selection of an animal model is based on broad similarities to humans in important physiological and biochemical parameters governing drug absorption, distribution, metabolism or excretion (ADME) processes in question for both the perpetrator and victim drugs. Equally critical are specific *in vitro* and/or *in vivo* experiments to demonstrate those similarities, usually both qualitative and quantitative, in the ADME properties/processes under investigation. In this review, theoretical basis and specific examples are presented to illustrate the utility of the animal models in assessing the potential and understanding the mechanisms of DDIs.

KEY WORDS animal models · drug transporters · drug-drug interactions · metabolizing enzymes

ABBREVATIONS

ADME	absorption, distribution, metabolism and excretion
CYP	Cytochrome P450
DDI	drug-drug interaction
i.pv.	intra-hepatic portal vein
MRP	multidrug resistance proteins
OAT/Oat	organic anion transporters
OATP/Oatp	organic anion transport polypeptides
OCT/Oct	organic cation transporters
Pgp	P-glycoprotein

C. Tang • T. Prueksaritanont (✉)
Department of Drug Metabolism and Pharmacokinetics
Merck Research Laboratories, Merck & Co., Inc.
WP75A-203
West Point, Pennsylvania 19486, USA
e-mail: thomayant_prueksaritanont@merck.com

INTRODUCTION

Pharmacokinetic drug interactions, typically characterized by alterations of plasma concentration–time curves, could be mediated *via* changes in processes of absorption, distribution, metabolism and/or excretion (ADME) of a drug substance (victim) by another compound (perpetrator) given concomitantly. In drug discovery and development processes, the assessment of drug-drug interaction (DDI) potential of a drug candidate usually encompasses two main objectives: 1) to help select/design a new chemical entity with least DDI liability potential in humans and 2) to help understand the underlying mechanism for DDIs observed for a drug candidate in humans. The first objective is achieved prospectively with the main focus on the issues during lead optimization in early discovery phase, while the second one is usually accomplished retrospectively throughout the whole drug discovery and development process, including post-marketing. In both cases, *in vitro* studies using human tissue preparations constitute an integral component in DDI assessments (1,2). The 'human only' *in vitro-in vivo* extrapolation (IVIVE) approach has given rise to some successful quantitative predictions for the DDI magnitude in humans due to inhibition of metabolizing enzymes. However, IVIVE approach has not been well established for DDIs mediated through mechanisms beyond Cytochrome P450 (CYP) inhibition, such as CYP induction and

altered drug transport, not to mention the changed interplay of drug transporters and metabolizing enzymes. Even in the case of reversible CYP inhibition, false predictions have been observed (3,4), which may be attributable to the uncertainty of the right inhibitor concentration, a key determinant for the prediction of DDI magnitude with IVIVE approach. The fact that the static, isolated, simplified experimental conditions *in vitro* do not always well reflect the dynamic, integrated, complex *in vivo* human studies has inevitably elicited the quest for some measure of *in vivo* relevance of the *in vitro* findings. This is particularly true in drug candidate selection when a decision has to be made based on prospective predictions. In this regard, *in vivo* animal models can serve as an effective approach to bridging *in vitro–in vivo* findings. Not only will a proper *in vitro-in vivo* preclinical assessment help form/strengthen a basis for extrapolating *in vitro* human data to clinical outcomes (5), but will also provide a mechanistic understanding of clinical interactions (6). The latter could not satisfactorily be addressed on the basis of *in vitro* studies alone.

Conceptually, an appropriate animal model for DDI studies should possess similarities to humans in key physiological or biochemical factors which govern specific ADME characteristics of both victim and perpetrator drugs. Examples of these important factors are organ blood flow, volume or pH, and tissue distribution/localization of drug transporters or metabolizing enzymes. In order to maximize the outcome of the *in vivo* animal DDI studies, there are three important considerations in choosing the animal model: 1) a thorough understanding of underlying mechanisms for specific ADME processes of both a drug candidate and the drug intended to be coadministered in humans and the animal model of choice, 2) information regarding similarities between humans and the animal model in physiological and biochemical parameters relevant to the ADME processes of interest, and 3) evidences or specific experiments to show similarities between animals and humans (ideally both qualitative and quantitative) in a key factor governing the ADME property under investigation. The first and the last considerations are compound specific, constituting the foundation for conducting *in vivo* or *in vitro* experiments, while the second is biological system specific and may be accomplished based on literature data. It is important to emphasize that while qualitative similarities in each of the ADME properties under investigation are prerequisite for the validity of an animal model, quantitative differences are anticipated between the animal model and humans regarding a specific governing physiological factor, such as expression levels of drug transporters or drug metabolizing enzymes. These quantitative variations need to be considered for proper interpretations in gauging the potential differences between the magnitude of DDI observed in the animal model and that anticipated in humans.

In this review, animal models known to possess physiological and biochemical properties common to humans in the four aspects of disposition (i.e. ADME) are described. Presented in detail are illustrations of the utility of these animal models in assessing DDIs and understanding the associated underlying mechanisms, especially at the levels of drug metabolism and drug transport. An attempt is also made to include the effort in understanding the complicated DDI outcomes due to the interplay of drug-metabolizing enzymes and transporters. Not covered in this review is recent DDI work utilizing transgenic animal models with humanized mouse lines to selectively knock in or knock out specific drug transporters and/or metabolizing enzymes. This type of animal model is expected to provide additional valuable mechanistic insights to the underlying mechanisms for DDIs. Interested readers are referred to a recent excellent review article on this topic (7) for more details.

ANIMAL MODELS

Absorption Models

Although extent of drug absorption in humans can generally be extrapolated from animal data (8), there have been reports of marked species differences in absorption due to species variations in physiological and/or biochemical conditions. The important factors known to cause species differences in absorption include gastrointestinal pH as well as expression and localization of drug transporters. It is important to emphasize that the term *absorption* is different from *oral bioavailability*. Oral bioavailability, which is often used as an indirect measure of drug absorption *in vivo*, is dependent on both the extent of absorption and first-pass metabolism in the intestine and liver. While high oral bioavailability would be indicative of good absorption, the opposite is not necessarily true for compounds subjected to extensive intestinal and/or hepatic metabolism. For such a compound, study designs and data interpretations of *in vivo* DDI studies are more complicated and are covered under "Metabolism Model" and "Metabolic Interaction" case examples. Described below are generalities around species similarities and differences in these physiological and biochemical properties to aid in selecting an animal model for studying DDIs during absorption.

Dogs

The dog is generally considered as an absorption animal model for humans for compounds with pH-independent

solubility (8). This is attributable to the well-established similarities in major gastro-intestinal physiological features and differences in gastric pH (higher in dogs than in humans) between dogs and humans. In addition to the known absorption differences observed in dogs and humans for compounds with pH-sensitive solubility profiles, small, hydrophilic, and passively transported drugs have also been shown to be better absorbed in dogs than in humans (9). Among commonly utilized preclinical species, dogs have been most frequently used for investigating absorption-related interactions, primarily between food and drugs (10). However, results from these studies should be interpreted with caution, considering that food may affect the gastric emptying time and intestinal transit time differently in dogs than in humans (11). In addition to those aforementioned physiological factors, a variety of efflux and uptake transporters have been characterized, primarily in rats and humans, for their roles in DDIs at the absorption level. To date, little information is available regarding similarities between dogs and humans in drug transporters expressed in the gastro-intestinal tract. Only a couple of reports are presented in literature on a similar regional distribution of multidrug resistance protein 2 (MRP2) mRNA in human and dog intestinal segments (12,13). Reports on using dogs to assess DDIs during absorption due to altered transporters also are scarce, and it remains to be explored prior to using this species for this application.

Rat

Unlike dogs, rodents are categorized as gastric-acid secretor and therefore are used for studying of pH-sensitive absorption. In the early 1970s, altered absorption of dextroamphetamine and salicyclic acid from rat small intestine was reported (14). The differential absorption pattern of the basic and acidic drugs from duodenal and ileal sites suggests that the alterations in absorption are due mainly to pH changes resulting from carbonic anhydrase inhibition by acetazolamide. In addition, SKF 525–A was found to decrease sulfacetamide absorption in rats *via* inhibition of gastric emptying (15). Recently, McConnell *et al.* (16) have reported that the mean intestinal pH in mice and rats was lower than that in man (pH < 5.2 in the mouse; pH < 6.6 in the rat). Also, the water content in the gastrointestinal tract, when normalized for body weight, was higher in mice and rats than in human. In theory, these physiological differences may lead to distinct extent of drug absorption in rats and humans, but thus far experimental evidence supporting this is lacking.

A correlation of intestinal permeability between human and animals can be expected for drugs which traverse enterocytes by passive diffusion. Interestingly, a recent analysis (17) revealed a correlation of drug intestinal permeability between human and rat small intestine ($R^2 = 0.8–0.95$) with both carrier-mediated-absorption and passive-diffusion mechanisms. The molecular basis for this finding is the similar gene expression levels of many transporters in human and rat duodenum. Except for P-glycoprotein (Pgp), multi-drug resistance protein 3 (MRP3), glucose transporter 1 and glucose transporter 3, the overall drug transporters share similar expression levels in both human and rat intestine with similar regional-dependent expression patterns. However, the two species exhibit distinct expression levels and patterns for major metabolizing enzymes in the intestine. Therefore, a rat model may be used to predict oral drug absorption by transporters in the small intestine, but not to assess oral bioavailability for compounds subjected to appreciable intestinal first-pass due to metabolism. Efforts in understanding the mechanism of altered absorption associated with transporters are emerging. Dahan and Amidon recently (18) disclosed a case of rat Mrp2-mediated DDI where indomethacin increases sulfasalazine permeability in the small intestine by competing for the transporter. Of note is the difficulty of isolating the impact of DDI at absorption level from contributions of other key factors to systemic exposure changes observed in *in vivo* studies. This may explain a paucity of information on successful absorption DDI studies in rats, either to assess DDI potential or understand mechanism of DDI mediated at the absorption level.

Non-human Primates

The gastrointestinal physiology in cynomolgus monkeys (*Macaca fascicularis*), i.e., gastric pH, gastric emptying time, gastrointestinal agitation intensity and small intestine transit time, is in general similar to that in humans (19). Thus, in spite of some obstacles in animal supply and experimental handling/study cost, monkeys are viewed to be useful as an animal model to predict the bioavailability of oral dosage forms. However, relative to humans, cynomolgus monkeys have demonstrated higher intestinal first-pass metabolism, mainly mediated by Cytochrome P4503A (CYP3A) enzymes (20,21). This has led to significantly lower bioavailability of some CYP3A substrates in the monkey regardless of the similar hepatic extraction ratios in these two species. Again, compared with humans, higher levels of multi-drug resistance 1 (MDR1), MRP2, and breast cancer resistant protein (BCRP) in cynomolgus monkey intestines appeared to correlate with the lower intestinal permeability of several compounds which are known substrates of these efflux transporters (22). To date, no specific absorption-mediated DDI studies have been reported in cynomolgus monkeys, but based on these known quantitative differences of enzyme/transporter in expression or capacity as well as general similarity of substrate selectivity, it is foreseeable that the cynomolgus monkeys could be used to provide a

mechanistic understanding of transporter- and metabolizing enzyme-mediated interactions at the intestinal absorption level.

Distribution Models

As the major determinant of tissue distribution, non-selective binding to proteins, phospholipids and other components in tissues is thought to be similar across species. Therefore, considerable species difference in tissue binding is not anticipated for compounds that enter the tissues by passive diffusion. For such a compound, appreciable changes in its tissue distribution, as a result of DDI, also are not expected, given the massive capacity of tissue binding. However, transporter-mediated tissue distribution would engender an increased vulnerability to DDIs. It is well known that DDIs at tissue distribution level are mainly derived from altered functions of active transport into or out of a given tissue. In general, species differences in substrate specificity for a drug transporter are more quantitative than qualitative in nature. Namely, it is uncommon to encounter a compound which is highly subjected to the efflux by human Pgp, but not by animal Pgp, or *vice versa*. However, transport kinetics, magnitude of response to inhibitors/inducers and transporter expression levels have been found to be species-dependent (23–26). In this regard, comparative assessments across species of transporter activities and responses to inhibition/induction can be conducted using a variety of *in vitro* systems (27). Encouragingly, recent advances in absolute quantification of various drug transporters across species using LC-MS/MS methodology (26) have shown the promise of overcoming the obstacle in quantitative prediction of human pharmacokinetics and DDIs associated with transporters. Of the tissues where altered transporter functions may be involved in DDIs at the distribution level, the brain and liver deserve special attention considering the relative abundance of transporters in these tissues and potential pharmacological and toxicological consequences.

DDI at the Blood Brain Barrier (BBB)

Efflux transport has been demonstrated to be the key element that limits the entry of many endogenous and exogenous molecules into the brain, thus rendering a protective mechanism for this very vital organ. Among the efflux transporters located on the endothelium of the BBB, Pgp is the major player in modulating brain penetration for numerous therapeutic agents, although BCRP and MRP2 have been reported to play a role in efflux of some drugs (28). Obviously, altered efflux by any factors that modulate the function or expression of these transporters will change the exposure of their substrates. Of important note is that altered distribution into brain tissues usually does not accompany noticeable systemic exposure changes. It is not unusual to see more than 10-fold increase in brain level of Pgp substrates in Pgp-knockout or -deficient mice relative to the wild-type group, but the systemic exposures are comparable in both groups (29). Similarly differential effects (brain *vs* plasma levels) have also been reported with a potent, selective Pgp inhibitor, elacridar (GF120918) (30).

Several DDI-related aspects at the BBB level, including *in vitro-in vivo* correlation and extrapolation of animal results to clinical outcomes, have been the focus of extensive research over the past two decades. Transport studies for 12 drugs with stable transformants expressing human, monkey, canine, rat (MDR1a and MDR1b), and mouse (mdr1a and mdr1b) Pgp in LLC-PK1 revealed slight interspecies and interisoforms differences in the substrate recognition (31), with some quantitative differences across species (24). Thus, rodents may serve a reasonable model for mechanistically assessing the potential of Pgp-mediated DDIs at the BBB level. However, quantitative information may be more reliably obtained with monkeys given the similarity of this species to humans in efflux ratio correlation and kinetic parameters. In support of this notion is the amino acid homology in these species (human~monkey>rodents) as described by Kim at al. (32).

Generally, in studies dealing with transporter inhibition, animals are concomitantly dosed with the perpetrator and victim compounds. In the cases of transporter induction, the perpetrators should be given several days prior to the administration of the victim drugs to allow transporter proteins for maximal expression. The interaction magnitude is reflected by the ratio of brain or cerebral spinal fluid (CSF) to plasma concentrations to offset the change of systemic exposures which could be the result of altered absorption and/or metabolism. Terminal sampling for compound levels in the respective matrixes is usually practiced in the pharmaceutical industry for the intended throughput, but more sophisticated imaging methodologies (PET and MRI) are also available for information of distribution to specific regions. Because of the proximity in physiological and biochemical properties to humans, non-human primates have been utilized to substantiate the findings from rodents. The model of cisterna magna ported rhesus monkeys enables serial sampling of CSF, thus providing a useful tool to monitor the time-course of drug concentrations in this fluid. With this model, we have demonstrated the impact of Pgp-mediated efflux on CSF concentrations for a number of Pgp substrates (33). As in rodents, imaging methods can also be applied to large animals, as the reported effects of tariquidar on lopademide (34) and cyclosporine on verapamil (35) in monkeys. With this method, the effect of Pgp inhibition by cyclosporine on verapamil brain penetration in humans and rats was

quantitatively compared (36). It was found that at identical pseudo steady-state cyclosporine blood concentrations, the brain-to-blood concentration ratio of total verapamil-radioactivity in the rat was increased by 75%, virtually identical to that obtained in PET imaging study in humans (78%). The progress and challenges in addressing the two key questions in this area have been thoroughly reviewed recently (37).

DDIs in Hepatic Distribution

Given the great variety and large quantity of drug transporters in the liver, transporter-mediated DDIs in this organ have been extensively investigated, along with the interplay with metabolizing enzymes. For the former, numerous drugs are transported from the blood into intracellular space through the sinusoidal membrane of hepatocytes by carrier-mediated processes rather than by passive diffusion. Some drugs can also be transported out of hepatocytes through the canalicular membrane by efflux processes (38). Thus, the extent of hepatic distribution of these drugs is largely governed by the relative efficiency of the uptake and efflux mechanisms. In addition to the potential pharmacological and toxicological outcomes of altered hepatic distribution, a recent analysis of literature data has indicated that inhibition of hepatic uptake generally lead to a significant decrease in steady state volume of distribution (Vd_{ss}) because of the lessened tissue accumulation attributed to the inhibited uptake (39). Such a change in Vd_{ss} could have an impact on the half-life, depending on the direction and magnitude of the clearance shift as a net effect derived from the interplay of transporters and metabolizing enzymes during DDIs. The inhibitory effect of rifampin on the hepatic uptake of digoxin in rats attested to the importance of transporter-mediated DDIs in volume of distribution. In rats, digoxin is subjected to organic anion transport polypeptide 1a4 (Oatp1a4)-mediated hepatic uptake and unlike in humans, is almost completely metabolized (40). The inhibition of the hepatic uptake mediated by this transporter by rifampin after intravenous administration resulted in significantly decreased Vd_{ss} and clearance (by 71 and 54%, respectively) with no change in the half-life (41). A similar finding in humans was also obtained with atorvastatin as the victim drug upon inhibition of OATP1B1-mediated hepatic uptake by rifampin (42). To date, rats and mice are the animal models used to elucidate the mechanism of drug interactions in tissue distribution in most cases, but extrapolation of animal pharmacokinetic property changes to human situations has to be practiced with caution because many other factors may potentially confound the predictability of animal models (39). Nevertheless, studies in these animal models could offer valuable insights into a probable *in vivo* consequence as a result of the transporter modulation, as shown in the above digoxin case.

Metabolism Models

The most encountered and most pronounced DDIs are typically the consequences of altered activities or expression levels of metabolizing enzymes, especially CYPs (43,44), due to enzyme inhibition and/or induction. While the liver generally has been considered the major site where metabolism-mediated DDIs take place, the intestine has drawn increasing attention recently as another location that plays a role in the occurrence of DDIs, particularly for CYP3A substrates (45).

Species similarities and differences in drug metabolizing enzymes, particularly CYPs, have been extensively studied. In the early 1990s, most comparisons were made based on enzyme kinetics and catalytic activities (46,47). In fact, no single animal species is identical to humans at the functional level for any drug-metabolizing enzyme, but more similarities are found in higher species. Recent advancements in molecular biology have provided valuable insights into these observed similarities and differences at the molecular level. A detailed species comparison in CYP-mediated metabolism, inhibition and induction can be found in a recent review by Martignon *et al.* (48). As for CYP isoforms extensively involved in drug metabolism, higher species generally exhibit a higher degree of amino acid sequence identity to humans (Table I) and, consistently, a greater similarity in substrate specificity. For instance, human CYP3A4, rhesus CYP3A64 and dog CYP3A12 are more selective than other CYP3A isoforms of the respective species for testosterone 6β-hydroxylation, midazolam 1′-hydroxylation and nifedipine oxidation, but the selectivity diminishes between rat CYP3A1 and 3A2 (49). However, slight differences in protein sequence homology also can lead to enormous changes in catalytic efficiency, as exemplified by diclofenic hydroxylation by human CYP2C9 and monkey orthologs which share ≥ 92% amino acid sequence homology (50). The catalytic efficiency of diclofenac 4′-hydroxylation (μL/min/pmol CYP) follows the rank order of human CYP2C9 (15)>rhesus CYP2C75 (2.4)>African green monkey CYP2C9 (0.15). Interestingly, the order appears to be reversed with CYP3A substrates, and the magnitude of difference becomes less significant (49).

In principle, if the change in metabolic efficiency does not lead to a metabolic switch, the animal model of interest may still be of value in assessing DDI potential, provided other factors are desirable. However, different responses to perpetrator drugs would exclude the opportunity of using an animal model in this regard. We have shown recently the lack of inhibitory potency against rhesus CYP2C75 by

Table I Amino Acid Sequence Homology of P450s Extensively Involved in Drug Metabolism in Rats, Dogs and Monkeys

		Human				
		2C8	2C9	2C19	3A4	3A5
Rat	2C11[a]		76			
	2C12[a]					
	2C13[a]		66			
	3A1				73	72
	3A2[a]				72	71
Dog	2C21	66	69	70		
	2C41	70	75	74		
	3A12				79	78
	3A26				77	77
Cyno-monkey	2C20	92				
	2C43		93	91		
	2C75		93			
	3A8				93	
	3A5					91
Rhesus monkey	2C74	92				
	2C75		94	92		
	3A64				93	83
	3A66					92
AG monkey	2C8	92				
	2C9		92			
	3A4				94	

[a] Gender-specific CYP isoforms

Data sources

Published references: 63, 101–103

In-house data (Rhesus and AG monkey CYPs) are provided by Dr. B. Carr

the potent, selective human CYP2C9 inhibitors sulfaphenazole (reversible) and tienilic acid (mechanism-based) (50). Further studies are needed to understand if this observation is CYP2C-specific. Similar observations have also been reported for rat CYP1A, 2C and 2D (51). However, potent reversible human CYP3A inhibitors, such as ketoconazole, appear to have reasonable cross-activity in many species, as shown by the comparable K_i or IC_{50} values determined in multiple species (51,52). Some mechanism-based CYP3A4 inhibitors also are effective for CYP3A of cynomolgus monkeys (53) and rats (54). Thus, DDIs caused by CYP3A inhibition may be reasonably reflected by multiple animal models. On the other hand, DDIs caused by CYP induction may be best assessed with both rhesus monkeys (*Macaca mulatta*) and cynomolgus monkeys in light of the recent extensive studies of DDIs in these species after treatment with standard CYP3A inducers (55,56) and increased mechanistic understanding from those endeavors. Likewise, beagle dogs may have similar potential to estimate CYP induction based on the information collected from a number of studies (57–59). It has been reported that the induction of dog CYP1A, CYP2B, and CYP3A exhibits characteristics that are intermediate to those of rodent and human (59). Kyokawa *et al.* (60) also reported the induction of beagle dog intestinal CYP3A12 by rifampin at a dose (10 mg/kg/day) comparable to the clinical dose for humans (600 mg/man/day). On the contrary, rodents may have limited value in this regard because of the known different response to CYP inducers (61).

In addition to the above factors, species-dependent metabolizing enzyme expression levels and tissue distribution/localization can further complicate the extrapolation of DDI potential from the animal to humans. Komura and Iwaki (62) showed that species differences exist in intestinal CYP3A enzymes. Namely, the identical CYP3A4 enzyme is expressed in human intestine and liver, but different CYP3A isoforms are expressed in intestines (CYP3A62) and liver (CYP3A1/2) of the rat. Thus, depending on the relative intrinsic efficiency of CYP3A1/2 and 3A62 for a given drug, the contribution of intestinal metabolism to the total clearance in rats may vary following systemic administration, even more after oral administration. As a result, it stands to reason that inhibitors with different selectivity to CYP3A1/2 and 3A62 may elicit varying degrees of DDIs for compounds known to undergo extensive intestinal first-pass metabolism. This potential occurrence would complicate the assessment of human DDI propensity for CYP3A4 substrates with a rat model.

Studies on monkey CYPs have attracted more attention; both rhesus and cynomolgus monkeys are generally considered to be more appropriate animal models for metabolism-related DDI assessments. In addition to the recent effort on rhesus monkey CYP3A64, which is most similar to human CYP3A4 in protein sequences and functional activities (49), several cynomolgus monkey CYPs have also been cloned and characterized (63). Eleven members of CYP1A, CYP2A, CYP2C, CYP2D, CYP2E, and CYP3A subfamilies from this species exhibited a high degree of homologies (more than 90%) in cDNA and amino acid sequences with corresponding human CYPs and catalysed typical reactions of corresponding human CYPs. However, one member of the cynomolgus CYP2C subfamily, CYP2C76, exhibited a lower homology (around 70%) in amino acid sequences than other cynomolgus monkey and human CYP2C subfamilies. CYP2C76 catalyzed typical CYP2C substrates with low activities and has not been found in humans. This cynomolgus monkey-specific CYP2C76 has been shown to be at least partly responsible for the different metabolism of pivastatin between cynomolgus monkeys and humans (64). Interestingly, we also observed species differences between rhesus monkeys and humans in the metabolism of one compound in the early development. This compound is metabolized

extensively by CYP2C75 in rhesus monkeys (65) but CYP3A4 in humans (unpublished information). These findings underscore the need to have a thorough understanding of the underlying mechanism for specific ADME processes of a drug candidate in the animal model *versus* in humans before conducting preclinical *in vivo* DDI studies.

The chimpanzee (*Pan troglodytes*) has also been characterized as a surrogate for drug oxidation and glucuronidation in humans and as a pharmacokinetic model for the selection of drug candidates (66,67). Similarities in the *in vivo* and *in vitro* metabolism of acetaminophen, oestradiol and morphine have been reported between chimpanzees and humans (67). Western immunoblot analysis of chimpanzee liver microsomes revealed a single immunoreactive band when probed with anti-human UGT1A1, anti-human UGT1A6, and anti-human UGT2B7. Levels of CYP2D-and 1A-like enzyme activities appear to be higher (10-fold) in the chimpanzee, consistent with the darker immunoreactive protein bands in chimpanzee than in human liver microsomes. Also, chimpanzees have levels of CYP3A- and 2C9-like enzyme activities similar to those of humans. Most recently, Williams *et al.* (68) have shown 99.7% nucleotide similarity of CYP3A5 clone between the two species and chimpanzee CYP3A67 most closely related to human CYP3A7, with the mRNA expression of CYP3A67 comparable to the expression of CYP3A4. It is conceivable that the chimpanzee may be useful as an animal model for assessment of DDI potential of a drug candidate undergoing UGT and CYP-mediated metabolism.

Currently, the practice of DDI assessment with animal models is largely qualitative in nature due to the difficulties in obtaining quantitative information on some factors. However, some mathematic models have evolved in an attempt to quantitatively predict the magnitude of DDIs in humans that are caused by reversible or irreversible inhibition of CYPs (69). Of note is the value of animal models in substantiating this 'human-only' *in vitro-in vivo* extrapolation (IVIVE) approach of quantitative prediction. Clearly, the prerequisite for a valid animal model is the aforementioned similarities to humans in the target orthorlogous CYP, substrate specificity, response to the inhibitor, and disposition mechanism. Other factors, whether perpetrator/victim specific or enzyme specific, can be experimentally determined and reasonably scaled up. A recent report (54) illustrates the application of this approach in predicting the DDI magnitude in humans with the rat as the animal model. In this case, the investigating compound irreversibly inhibits both human and rat CYP3A-mediated metabolism of indinavir that is cleared in both species with CYP3A-mediated metabolism as the major elimination mechanism. The fold of indinavir AUC increase observed in rats correlates well with the predicted value. Thus, this good *in vivo-in vitro* correlation (IVIVC) in rats has strengthened the human prediction. Mathematical models for quantitative prediction of the magnitude of DDIs caused by CYP induction have been emerging recently (70–72). It is imperative to confirm the validity of this IVIVE approach with proper animal models for a variety of inducers and victim compounds.

Excretion Model

Biliary and renal excretions are two major pathways of eliminating a variety of compounds that escape metabolism. Active transport in canalicular membranes and tubular epithelium involves both uptake and efflux transporters. Drug interactions at the excretion level are mostly the result of altered functions and expression of these transporters. As discussed in metabolism-related DDIs, the validity of an animal model would mainly depend on the similarities of substrate specificity, responses to the affecting agents and the primary mechanism of elimination.

Biliary Excretion

It is well known that the amount of xenobiotics, especially those with the molecular size less than 700 daltons, excreted in bile varies widely among species (8). The underlying mechanism for this species differences has not been well investigated. Recently, characterization of MRP2/Mrp2, an ABC transporter (ABCC2/Abcc2) extensively expressed on the canalicular membrane of hepatocytes has revealed the important role of this transporter in biliary excretion and the difference in its expression level, function, and responses to various modulators across species (73,74). This information may shed some light on the variable interspecies difference in biliary excretion and assist in identifying an appropriate animal model to assess DDIs associated with this transporter. For instance, the absolute protein amount of MRP2/Mrp2 in liver tissues and isolated hepatocytes was found to be ~10-fold greater in rats than in humans (75). The ~10-fold difference in expression level of this transport in rat and dog livers appeared to correlate well with the reported rank order of *in vivo* biliary excretion clearance of temocaprilat (76). Moreover, the function of a transporter, either by substrate specificity or by efficiency, is another important determinant of species difference in biliary excretion. By and large, the substrate specificity of MRP2/Mrp2 among different species is similar; but the transport efficiency (intrinsic transport activity) of a transporter could vary with species. Analogous to the case of CYP enzymes, this species difference is substrate dependent. Fortunately, the function of a transporter can readily be characterized with a number of established *in vitro*

systems. Therefore, once comparative information is available regarding the responsible transporter, its substrate specificity, intrinsic transport activity and expression level, depending on the objective of the investigation, a proper animal model could be readily selected. Currently, rodents have been used for mechanistic understanding of drug interactions of biliary excretion, but prediction of human situation with animal models remains to be explored.

Renal Excretion

The rate of renal excretion (renal clearance) is dependent on renal blood flow, glomerular filtration rate (GFR), and tubular secretion and reabsorption. The GFR values vary considerably among species, dependent on the number of nephrons, whereas both the GFR and number of nephrons show a good allometric relationship. Thus, for compounds with GFR and passive reabsorption as major mechanism for renal excretion, any species could be considered as good animal models for humans (8). In contrast, compounds subjected to significant tubular secretion may display marked variation in renal excretion if the secretion proceeds *via* species-dependent drug transporters. The widely investigated transporters responsible for renal uptake belong to the families of organic anion transporters (OATs/Oats) and organic cation transporters (OCTs/Octs). Like CYP enzymes, isoforms of each family of transporters have been identified in animals and humans. Recent studies from Sugiyama's group have revealed species-dependent function and distribution of these isoforms. Namely, Oct1 and Oct2 are both important in renal uptake of organic cations in rodents, while OCT2 is the predominant isoform in human kidney. For the OAT/Oat family, Oat1 and Oat2 are found in rodents, but OAT1, OAT2 and OAT3 in humans with OAT3 as the abundant one. Interestingly, OAT1/Oat1 substrates have shown a good correlation of transport activity in human, cynomolgus monkey and rat, while such a correlation only exists for OAT3/Oat3 substrates between human and monkey, not rat (77). These results suggest that there is a minimal species difference in the OAT1/Oat1-mediated transport. This is in a good agreement with a successful allometric scaling reported for 2,4-dichlorophenoxyacetate in the mouse, rat, pig, calf and human (78). This compound is eliminated predominantly by the kidney (78), and its basolateral uptake has been suggested to be predominantly accounted for by rat Oat1 (79). On the contrary, cynomolgous monkeys may serve a proper model for renal OAT3-mediated DDIs. Furthermore, the response to inhibitors is also isoform selective, as illustrated by the potent inhibition by probenecid of OAT3/Oatp3, but not of other OAT/Oat isoforms (80). As will be described in detail in the section of case examples, characterization of species differences of these transporters in substrate specificity, intrinsic transport activity and response to inhibitors has provided some mechanistic understanding of the observed different effect of probenecid on famotidine renal excretion in humans and rats.

Drug Transporter—Metabolism Interplay

Many drugs are subjected to both metabolism and transport processes, and their disposition and susceptibility to DDIs are thus governed by how the responsible metabolizing enzymes and transporters work in concert. The interplay of these two mechanisms has often been shown to take place in the intestine and liver, where the transport takes the form of either uptake or efflux mediated by the respective transporters on the membrane of enterocytes or hepatocytes, while metabolism proceeds through a variety of membrane-bound or cytosolic metabolizing enzymes in those cells. It has been recognized recently that such interplay represents one of the emerging confounding factors that contributes to complex drug interactions for compounds subjected to both transport and metabolism. These complications may be different in magnitude, direction, or time-dependency from the cases where only one mechanism operates (81). Clearly, the complexity attributed to this interplay would be very difficult to predict from *in vitro* studies. In this sense, the net outcome from alterations of multiple factors may be reasonably reflected from *in vivo* studies using an appropriate animal model. Then, based on animal *in vitro* and *in vivo* findings, the relevance of human *in vitro* data can be interpreted in the context of the relative *in vivo* importance of each factor. Furthermore, animal *in vitro-in vivo* relationship can help validate the result from modeling and simulations, which are considered as an effective tool to integrate human transporter and metabolizing enzyme characteristics obtained from separate *in vitro* methods (82). An increasing application of animal models in this regard is foreseeable. At present, the rat has been used to investigate the impact of CYP3A enzyme and efflux/uptake transporter interplay on pharmacokinetics and drug interactions.

The role of drug metabolism and transport interplay in DDIs has been subjected to extensive investigations, with the work pioneered in Dr. Benet's Laboratory and extended in many different laboratories (83–85). This is well exemplified by the recent study of hepatic transporter effect on erythromycin metabolism by correlating *in vitro* and *in vivo* findings in rats (86). Erythromycin is subjected in OATP/Oatp-mediated hepatic uptake. This drug and its major metabolite are also substrates of Pgp (87,88). In humans, erythromycin is partially metabolized by CYP3A4 to its major metabolite, *N*-demethyl-erythromycin, but itself is excreted primarily unchanged in the bile. In rats,

erythromycin is subjected to more extensive metabolism, and the biliary excretion appeared to be Oatp2-mediated (86). Thus, the rat serves as a good substrate for investigating both hepatic uptake and efflux transporter interplay with metabolic enzymes. Using rifampin as a general inhibitor of Oatps and Oats, and GF120918 as a potent inhibitor of Pgp, comprehensive information from both *in vitro* and *in vivo* rat studies has shed light on how the metabolism and transport operated in concert to respond to altered functions of uptake and efflux in rats, and potentially also in humans.

SPECIFIC CASE EXAMPLES

In this section, we present a number of examples to help illustrate how an animal model has been used to either assess DDI potential during lead optimization or help understand the underlying mechanism for DDI observed for drug candidates during efficacy/safety evaluations of drug candidates. The emphasis is given to commonly encountered DDI cases, namely metabolic and renal excretion interactions, due to alterations of drug-metabolizing enzymes and drug transporters, respectively. Through these examples, it should become clear that a thorough understanding of similarities and differences in the disposition of affected drug, the target enzyme/transporter, and the mechanism of elimination is the key in selecting an appropriate animal for successful assessments of drug interactions. Where applicable, we also highlight potential limitations of the chosen animal model, as well as key experiments connecting findings between animals and humans for meaningful interpretations.

Metabolic Interactions

CYP3A-Mediated DDIs in Monkeys

Recently, we have evaluated the rhesus monkey as an animal model for CYP3A-mediated DDI assessments (55). The rhesus was chosen based on our previous work (49) showing similarities between rhesus CYP3A64 and human CYP3A4 with respect to their ability to metabolize known human CYP3A substrates, including midazolam. To further qualify this species for induction and inhibition studies, we compared the recombinant orthologous CYP3A (rhesus CYP3A64 *versus* human CYP3A4) and isolated hepatocytes from rhesus and human liver tissues with respect to their susceptibility to induction and inhibition by a known CYP3A4 inducer, rifampin, and a known inhibitor, compound A. We also showed the similarity of midazolam disposition in rhesus monkeys to that in humans. We found that midazolam was metabolized extensively in rhesus following intravenous administration, with 1′-hydroxy midazolam as the major metabolite, but relative to humans, rhesus monkeys exhibited a higher systemic blood clearance (80–90% *vs.* 30–40% of hepatic blood flow) and a lower hepatic availability (16% *vs.* ~40%). Consistent with the induction of hepatic metabolism of a high clearance compound, pretreatment with rifampin (for 5 days to achieve plasma concentrations comparable to therapeutic concentrations in humans) did not significantly affect the intravenous kinetics of midazolam, but caused a pronounced reduction (~10-fold) in its systemic exposure. Consequently, the hepatic availability of midazolam was also significantly decreased following intra-hepatic portal vein (i.pv.) administration of midazolam. The magnitude of systemic exposure after i.pv. administration ($AUC_{i.pv}$) agreed well with the finding that midazolam displayed a clearance higher in rhesus than in humans. It is important to note that the i.pv. administration was chosen in this study to allow a direct comparison with the *in vitro* hepatocyte studies and to avoid potential complications from incomplete absorption and intestinal enzyme induction or inhibition. The latter consideration was deduced from the fact that, unlike the liver, where extensive research efforts have been devoted, there is little information on the exact identity of CYP3A enzymes in monkey intestine except for comparative functional enzyme activities using CYP3A probes between human and monkey intestines (47). Overall, our results suggested that the rhesus monkeys could be used as an animal model to evaluate propensity of a compound to induce CYP3A substrate or estimate its susceptibility to induction by a potent CYP3A inducer, provided that appropriate key experiments connecting animal to human findings be performed to aid in proper interpretations. A very recent report (56) discloses the similar efforts with cynomolgus monkeys as the animal model to predict human CYP3A4 induction. The orthologous enzyme in this species is CYP3A8, as opposed to CYP3A64 in rhesus monkeys, but they both share the identical deduced amino acid sequence (49). Compared with humans, cynomolgus monkeys showed similar amino acid sequence of PXR (~96%) and CYP3A (~93%), and comparable responses to known human CYP3A4 inducers measured by both PXR activation and enzyme induction. More importantly, *in vivo* induction of CYP3A8 by rifampin and hyperforin was shown by significant reductions of midazolam exposure that were comparable with those in humans. These results demonstrate that the cynomolgus monkey can be a predictive *in vivo* animal model of PXR-mediated induction of human CYP3A4 and can provide a useful assessment of the resulting pharmacokinetic changes of affected drugs (56).

In the case of inhibition, we used compound A, a potent and mechanism-based inhibitor of human CYP3A4, to evaluate the validity of rhesus monkeys as an *in vivo* model

in this scenario. With both recombinant CYP3A enzymes and liver microsomal preparations of rhesus and humans, compound A showed a comparable inhibitory potency as measured by K_I and k_{inact}. This finding laid a foundation for predicting the magnitude of DDI in humans based on the unbound concentration of compound A determined at the relevant site. As expected, the *in vivo* relevance of the *in vitro* inhibition of CYP3A64 was demonstrated in rhesus monkeys. A single intravenous dose of compound A (6 mg/kg) markedly increased the systemic exposure of midazolam following i.pv. infusion. Notably, this inhibitory effect was observed at plasma concentrations of compound A over the 6-h period of about 0.5 to 1 μM, which corresponded to the unbound plasma concentrations of approximately 0.1 to 0.2 μM, exceeding its K_I value obtained *in vitro* with CYP3A64. Although the single dosing regimen used in this study may not be sufficient to produce the maximal level of enzyme inactivation by compound A, the magnitude increase in midazolam $AUC_{i,pv}$ by compound A was nevertheless close to the anticipated range based on the values of k_{inact} and K_I obtained in this study and using a quantitative prediction method proposed for mechanism-based inactivators by Ernest *et al.* (89). Encouragingly, similar observations were also made by other researchers in cynomolgus monkeys, whether by reversible or mechanism-based inhibition (53,90).

Taken together, our work, along with findings by other researchers, indicates that monkeys are suitable for the assessment of CYP3A-mediated DDI in humans. Table II summarizes the key considerations and information acquired in this study to qualify the rhesus monkey as the animal model for studying DDI between midazolam-rifampin. The same principle could also be applied for studying other pairs of perpetrator/victim of interest.

It is worth noting that species closer to humans on the evolutionary tree do not always reflect the disposition of a drug in humans. The following case investigated in our lab (65) exemplifies the divergent outcome of auto-induction in rhesus monkeys and humans, and the mechanic investigation of the event. A potent bradykinin B_1 receptor antagonist was primarily eliminated *via* biotransformation in rhesus monkeys, with oxidation on the chlorophenyl ring as one of the major metabolic pathways. The two major oxidative metabolites derived from this pathway are M11 and M13. Repetitive daily treatment of rhesus monkeys with this compound led to a decrease of its systemic exposure (C_{max} and AUC) by 2-3-fold with concomitant increased formation of M11 and M13 in liver microsomes from those treated monkeys. The results therefore indicated the occurrence of metabolism-related auto-induction. While potential drug interactions due to CYP3A induction in both species was implicated because this compound significantly activated human and rhesus PXR and induced CYP3A4/3A64 expression *in vitro*, rhesus monkeys did not turn out to be a proper model to assess the potential of auto-induction in humans in this case. As proven by further metabolism studies using recombinant human and rhesus CYPs and immuno-inhibition with monoclonal antibodies,

Table II Examples of Key Considerations for Choosing an Animal Model for Metabolism-Mediated DDI Studies

	Midazolam—Victim drug		Rifampin—Perpetrator drug	
	Human	Monkey	Human	Monkey
Relevant ADME porperties	Oxidative metabolism as the major clearance mechanism	Same	At therapeutic dose, C_{max} ~ 10 μM Plasma protein binding ~30%.	At the dose used in the study, C_{max} ~15 μM Plasma protein binding ~20%
Key metabolizing enzyme	CYP3A4	CYP3A64—proven to have amino acid sequence and catalytic activity similar to human CYP3A4	Not critical	Not critical
Additional supporting evidence (qualitative and quantitative)	Moderate clearance E_h ~40%	High clearance E_h~80%	Induced CYP3A4 mRNA by~6-14-fold Induced midazolam 1'-hydroxylation activity by ~2-fold	Induced CYP3A64 mRNA by~5-10-fold Induced midazolam 1'-hydroxylation activity by ~3-10-fold
Outcomes of DDI studies	~2-fold change in iv midazolam kinetics, consistent with moderate clearance characteristics 20-fold change in oral midazolam AUC	No change in iv midazolam kinetics, consistent with high clearance characteristics ~10-fold change in AUC after i.pv. administration; expected a higher magnitude following an oral dose		

oxidation of this compound was proven to be mediated by CYP3A4 in humans but by CYP2C75 in rhesus. Through activation of PXR, in addition to CYP3A64, another CYP isoform induced by this compound is CYP2C75, as subsequently confirmed by a concentration-dependent increase of CYP2C75 mRNA in rhesus hepatocytes, along with the enhanced CYP2C proteins and catalytic activities toward CYP2C75 probe substrates. We concluded that this compound, both a substrate and inducer for CYP2C75, caused auto-induction of its own metabolism in rhesus monkeys by increasing the expression of this enzyme. The results from this work, together with the understanding of multiple metabolic pathways in humans and anticipated clinical exposures, helped rationalize the auto-induction potential of this compound in the clinic (unpublished data). Therefore, once again, the similarity of key factors defining the disposition of the affecting and the affected compounds between human and the animal need to be evaluated on a case-by-case basis to determine the suitability of an animal model.

Evaluation of the Validity of Diclofenac as a CYP2C9 Probe in Humans

Based on their *in vitro* metabolism studies, Kumar *et al.* (91) have recently proposed that the direct glucuronidation of diclofenac is a more important component to the *in vivo* clearance than the oxidation pathway in rats, dogs and humans. If confirmed, the utility of diclofenac as an *in vivo* CYP2C9 probe in humans would no longer be valid. We then used rhesus monkey as an animal model to show that the CYP2C-mediated oxidative metabolism of diclofenac is not the major determinant for its *in vivo* clearance in monkeys, and unlikely in humans either (92). This conclusion was based on a couple of *in vitro* and *in vivo* results. First, in both monkey and human liver microsomes and hepatocytes, diclofenac underwent glucuronidation predominantly and oxidation modestly; the intrinsic clearance value for the glucuronidation pathway accounted for >90% of the total intrinsic clearance. Second, effects of rifampin on *in vitro* oxidative metabolism and *in vivo* pharmacokinetics of diclofenac were investigated in rhesus monkeys. Although rifampin markedly induced diclofenac 4′-hydroxylase activity in monkey hepatocytes as well as human hepatocytes, pretreatment with rifampin did not alter diclofenac pharmacokinetics following either intravenous or i.pv. administration of diclofenac to monkeys. At the dose studied, plasma concentrations of rifampin reached 10 μM, far exceeding the *in vitro* EC_{50} values for the diclofenac 4′-hydroxylase activity (0.2–0.4 μM). Finally, under similar conditions, rifampin was previously shown to induce midazolam 1′-hydroxylation in rhesus monkey hepatocytes and markedly affected the *in vivo* pharmacokinetics of midazolam in this animal species (89). Based on these *in vitro* and *in vivo* results from rhesus monkeys, together with the *in vitro* findings in humans, we concluded that rifampin may also elicit modest effects on the diclofenac pharmacokinetics *via* induction of CYP2C9 in humans as well as in monkeys. However, this modest effect may be obscured by the presence of the predominant glucuronidation *in vivo*. Collectively, the results provided convincing evidence that diclofenac is not a suitable *in vivo* probe for CYP2C9-mediated DDI studies in humans.

Assessment of the Susceptibility of Drug Candidates to DDIs Caused by Potent CYP3A Inhibitors

It still remains very challenging to estimate the DDI potential for victim drugs because in most cases the information of their disposition, such as the fraction of metabolism (f_m) and intestinal first-pass metabolism (F_g), is not available, especially at the drug discovery stage. In the first attempt at coping with this issue, Mandlekar *et al.* (93) used the rat as an *in vivo* screening model to rank order compounds for their potential liability to interact with ketoconazole. Based on the relative magnitude of pharmacokinetic interaction observed with ketoconazole in the rat, the compounds were prioritized for further preclinical development. To qualify the rat as an appropriate animal model, they conducted *in vitro* reaction phenotyping using individual human and rat cDNA-expressed CYP enzymes and human or rat liver microsomes in the presence of ketoconazole to demonstrate similarities between rats and humans regarding the main drug metabolizing enzyme, CYP3A. The authors acknowledged that the degree of pharmacokinetic interaction with ketoconazole would also be dependent on their fraction metabolized (f_m) in the rat relative to other disposition pathways and that this value may be different between rats and humans. It is also important to note that, as highlighted above, species differences exist in intestinal CYP3A proteins between rats and humans and that while oral administration was used in this study, comparative *in vitro* metabolism was not conducted with rat and human intestinal tissues. These factors may impact different compounds to various extents; therefore, the successful uses of the rat *in vivo* screening model may be limited to a selected group of compounds and not applicable for an early broad screening.

Interaction at Renal Excretion

Effects of Probenecid on Renal Elimination of H_2 Receptor Antagonists

H_2 receptor antagonists are mainly eliminated by the kidney as the intact form by tubular secretion as well as

glomerular filtration (94). It has been known for more than 20 years that the renal secretion clearance of famotidine in humans was significantly reduced by oral coadministration of probenecid (95), whereas this interaction has not been reproduced in rats, although the plasma concentration of probenecid achieved a similar level to that in clinical studies (96). Recent work from Sugiyama's laboratory on the characterization of the transporters responsible for famotidine uptake in human, rat and cynomolgus monkey kidneys has shed light on this different DDI outcomes from human and rat studies. Their findings also suggest cynomolgus monkeys as a good animal model to assess DDIs caused by renal OAT3 function or expression alterations.

In this case study, Tahara *et al.* (77,97,98) characterized human and rat transporters capable of famotidine uptake and evaluated the inhibitory potency of probenecid against those transporters. There existed a striking difference between human and rat in the transporter important to famotidine uptake. Namely, famotidine was primarily transported by OAT3 in humans, but by Oct1 in rats. Further evaluation of probenecid inhibition revealed that it was a potent inhibitor to human OAT3 and rat Oat3, but not effective to rat Oct1 and Oct2. Since rat Oat3 was not a major player in famotidine renal uptake, probenecid would not be expected to exert an appreciable effect on famotidine renal elimination in rats. The different key transporters responsible for famotidine renal uptake in human and rats as well as transporter isoform selectivity of probenecid inhibition reasonably accounted for the observed disparity between human and rat DDI results. In contrast to the case in the rat, famotidine was transported only by OAT3 in monkeys, similar to the situation in humans, regardless of the lower intrinsic activity in relation to human OAT3. Given the fact that probenecid also strongly inhibited monkey OAT3-mediated famotidine transport, it was anticipated that the monkey was likely to mirror the interaction observed in humans. This hypothesis was confirmed by an *in vivo* study in cynomolgus monkeys. It was found that probenecid treatment caused a 65% reduction in the renal clearance and a 90% reduction in the tubular secretion clearance of famotidine following intravenous administration. The 2-fold increase in famotidine AUC is consistent with the previous findings in humans.

The fundamental understanding of the involved transporters and their responses to probenecid also helped elucidate another initially perplexing observation with another H_2 receptor antagonist, cimitidine. In both rats and humans, probenecid at the same dose only caused slight reduction (ca. 20%) in renal clearance of cimitdine (94,99). It turned out that cimitidine was preferentially transported by OCT2 over OAT3 in humans and by Oat3, Oct1 and Oct3 at a comparable efficiency in rats. Thus, the OAT3/Oat3-selective probenecid would not significantly change cimitidine renal clearance to which other transporters also contributed.

Coupled with other findings, such as a good correlation of reference compound transport by OAT3 between monkeys and humans, as opposed to the poor correlation between rats and human, and similarly high expression levels of monkey and human OCT1 and OCT2, the authors concluded that monkeys, rather than rodents, can be used to predict drug-drug interactions involving tubular secretion, particularly when multiple transporters are involved.

Rat and Rhesus Models to Assess Potential Renal Transporter-Mediated DDI

In this example, the mechanism of renal excretion of compound B, a potent and selective $\alpha_v\beta_3$ integrin antagonist, and its renal transporter-mediated DDI potential were investigated (100). In both rats and rhesus, renal excretion of compound B involved tubular secretion as ratios of renal clearance (corrected for unbound fraction in plasma (CLr, u) to glomerular filtration rate (GFR)) were greater than unity. In rats, the tubular secretion of compound B was inhibited significantly, although modestly (~2-fold), by relatively high plasma concentrations of the organic anion *p*-amino hippuric acid and the cation cimetidine, but not by the Pgp inhibitor quinidine (~50 μM). In rhesus monkeys, the renal secretion of compound B was not affected by either cimetidine or *p*-amino hippuric acid. In both species, compound B had a minimal effect on the renal tubular secretion of both cimetidine and *p*-amino hippuric acid. *In vitro*, compound B was not a substrate for Pgp in the Caco-2 and human *MDR1*/mouse *mdr1a* transfected LLC-PK1 cell lines. However, with rat Oat1 and Oat3-transfected HEK cell lines, compound B was shown to be a substrate for rat Oat3 (K_m=15 μM), but not for rat Oat1. These results suggest that the tubular secretion of compound B is not mediated by Pgp but rather, at least in part, by the organic anion transporter Oat3 (the renal transporter capable of transporting both the organic anion *p*-amino hippuric acid and the organic cation cimetidine). Unfortunately, information regarding renal transporters in rhesus monkeys and species differences in the transporters between rats and monkeys just starts to emerge in the literature. It should have provided more understanding of the mechanism of the observations if available a few years earlier. Nevertheless, given the relatively low magnitude of interaction observed in both species, we concluded that the magnitude of interaction between compound B and substrates or inhibitors of OAT3, at the renal excretion level, would likely be modest in humans at clinically relevant doses.

CONCLUSIONS

Preclinical assessment of DDI is a complex issue that has been discussed extensively among pharmaceutical, academic and regulatory scientists. The main underlying reason for this complexity is species differences commonly encountered in the expression level, functional activity, and tissue distribution of drug-metabolizing enzymes and drug transporters, major determinants of ADME processes. Although this issue can theoretically be addressed by utilizing *in vitro* systems using human tissue preparations, the *in vivo* relevance of such *in vitro* systems is uncertain in some cases and needs to be validated. In this review, we describe an *in vivo* animal model approach to help bridge this gap. 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, complementing the 'human-only' *in vitro-in vivo* extrapolation approach, as well as a mechanistic insight for the interpretation of interactions observed clinically. Other complementary tools for additional insights include knockout animals lacking specific drug transporters or drug-metabolizing enzymes and/or transgenic animal models with humanized mouse lines expressing specific drug transporters and/or metabolizing enzymes of interest. Together, it is conceivable that in the next decade these animal models could become more valuable in DDI assessments during drug discovery and early development processes.

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