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Scientific Perspectives on Drug Transporters and Their Role in Drug Interactions†

Lei Zhang, John M. Strong, Wei Qiu, Lawrence J. Lesko, and Shiew-Mei Huang*

Office of Clinical Pharmacology and Biopharmaceutics, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland 20993

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Abstract: Recently, increased interest in drug transporters and research in this area has revealed that drug transporters play an important role in modulating drug absorption, distribution, and elimination. Acting alone or in concert with drug metabolizing enzymes they can affect the pharmacokinetics and pharmacodynamics of a drug. This commentary will focus on the potential role that drug transporters may play in drug−drug interactions and what information may be needed during drug development and new drug application (NDA) submissions to address potential drug interactions mediated by transporters.

Keywords: Transporter; drug−drug interaction; regulatory; guidance; new drug application; drug development; exposure−response relationship; labeling; risk management

Introduction

The frequency of possible drug interactions increases with the number of concomitantly administered drugs, and these interactions can lead to serious adverse events resulting in harm to the patients, early termination of development, prescribing restrictions, and withdrawal of drugs from the market. In fact, five of 12 drugs withdrawn from the U.S. market from 1997 to 2002 exhibited metabolic drug−drug interactions.[1] Drug metabolism enzymes and their role in drug−drug interactions have been intensively investigated. Several documents are available to provide guidance to industry and FDA reviewers regarding the use of various methodologies to address metabolic drug−drug interaction issues: "Guidance for Industry: Drug Metabolism/Drug Interaction Studies in the Drug Development Process—Studies in Vitro";[2] "Guidance for Industry: In Vivo Drug Metabolism/Drug Interaction Studies—Study Design, Data Analysis, and Recommendations for Dosing and Labeling";[3] "Guidance for Industry: Population Pharmacokinetics";[4] and "Guidance for Industry: Exposure−Response".[5]

Various publications are also available that discuss the current best practices for conducting in vitro and in vivo metabolic drug interaction studies[6−8] designed to identify potential metabolic drug−drug interactions and how to avoid

* Corresponding author: Shiew-Mei Huang, Ph.D., FCP, Deputy Office Director for Science, Office of Clinical Pharmacology and Biopharmaceutics, Center for Drug Evaluation and Research, Food and Drug Administration, CDER Office I, Bldg 21, Rm 4550, White Oak, 10903 New Hampshire Avenue, Silver Spring, MD 20993. Phone: 301-796-1541. Fax: 301-796-9738. E-mail: shiewmei.huang@fda.hhs.gov.

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(2) Guidance for Industry: Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies in vitro. http://www.fda.gov/cder/guidance, **1997**.

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(5) Exposure-Response Relationships—Study Design, Data Analysis, and Regulatory Applications. http://www.fda.gov/cder/guidance, **2003**.

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them. However, unexpected drug−drug interactions do occur.[9] One of the confounding factors may involve interactions mediated by transporters. For example, inhibition of P-gp may be partially responsible for the quinidine−digoxin,[10,11] ketoconazole−fexofenadine, and erythromycin−fexofenadine interactions.[12,13] The potential involvement of both transporters and metabolic enzymes responsible for a drug's disposition complicates the interpretation of in vitro data and attempts to predict drug−drug interactions in vivo.[14,15]

This commentary will focus on the role that drug transporters may play in drug−drug interactions and the Agency's current thinking on what information may be relevant during drug development to address potential drug interactions mediated by transporters. In addition to this commentary, a concept paper was published to facilitate the discussion of study design, data analysis, and implication for dosing and labeling.[16] A draft guidance including additional discussions on emerging areas such as drug transporters will soon be available at http://www.fda.gov/cder/guidance for public comment and when finalized will eventually replace the existing in vitro and in vivo guidances.[2,3]

Major Human Drug Transporters and Clinical Relevance

Over the past 15 years, a number of important human drug transporters have been identified that are expressed at the apical or basal side of the epithelial cells in various tissues.[17−26] Most drug transporters belong to two superfamilies, ABC (ATP-binding cassette) and SLC (solute-linked carrier), including both cellular uptake and efflux transporters as shown in Figure 1.[27] Examples of drugs reported to be substrates, inhibitors, or inducers of these transporters are listed in Table 1. The data shown in Table 1 and reports in the literature[28,29] indicate lack of specificity for many of the transporters, substrates, and inhibitors studied.

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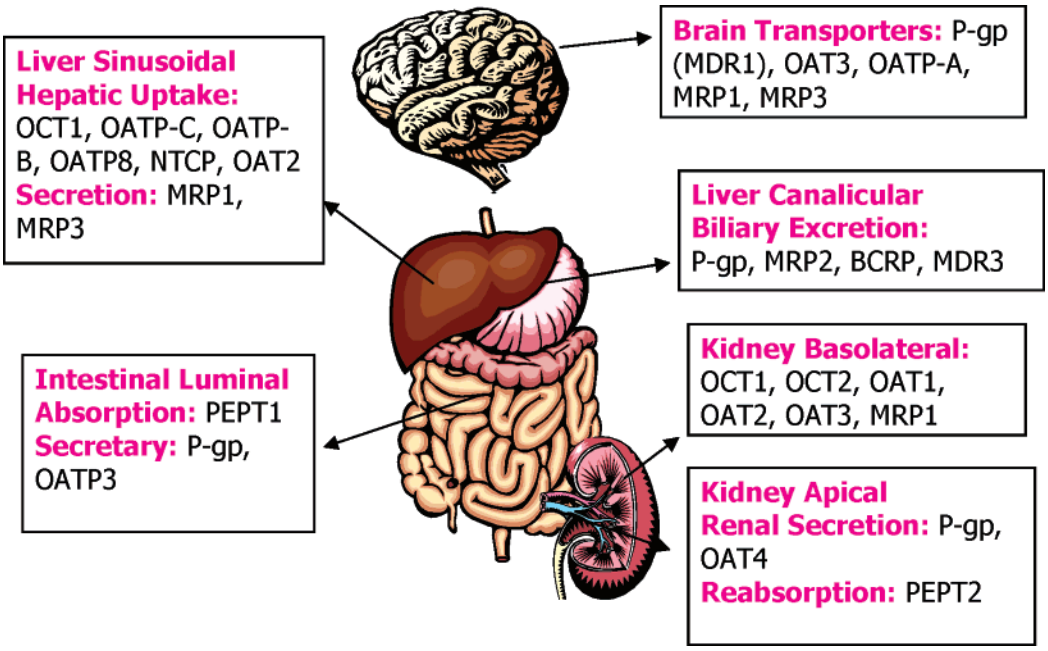

Figure 1. Tissue localization of transporters and their role in drug disposition (adapted from Figure 1 in ref 27).

Analogous to drug−drug interactions mediated by P450 (CYP) enzymes, co-administration of a drug that is an inhibitor or an inducer of a drug transporter may affect the kinetics of another drug that is a substrate for the same transporter.[23,29−31] For example, digoxin is a P-gp substrate that is eliminated mainly unchanged via renal and biliary excretion. Its AUC has been found to increase with co-administration of several P-gp inhibitors, e.g., quinidine,[10,11] itraconazole,[32] and atorvastatin,[33] and decrease with co-administration of P-gp inducers, e.g., rifampin[34] and St. John's wort.[35] Another recent example suggests a transporter-mediated interaction between rosuvastatin, a known substrate for OATP1B1 (gene: *SLCO1B1*), and cyclosporine, identified as an effective inhibitor of the same transporter.[36] When rosuvastatin was co-administered with cyclcosporin A in heart transplantation patients, its AUC increased 7-fold.[36,37] Other transporter-based interactions reported include probenecid−cephalosporin antibiotics (OAT) and cimetidine−dofetilide (OCT) interactions.[38,39] These examples support the increasingly recognized view that metabolism alone does not adequately account for the individual variation in absorption, distribution, and elimination of drugs and that transporters may play a role in these processes.

Human Transporter Polymorphisms

Recently, numerous polymorphisms have been identified in transporter genes and allele frequencies determined in various populations.[40−42] Unlike polymorphisms observed for

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Table 1. Major Human Drug Transporters

gene	aliases	tissue	substrate	inhibitor	inducer
ABCB1	P-gp, MDR1	intestine, liver, kidney, brain, placenta, adrenal, testes	digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel, talinolol, loperamide	ritonavir, cyclosporine, verapamil, erythromycin, ketoconazole, itraconazole, quinidine, elacridar (GF120918) azithromycin, valspodar	rifampin, St. John's wort
ABCB11	BSEP	liver	vinblastine		
ABCC1	MRP1	intestine, liver, kidney, brain	adefovir, indinavir		
ABCC2	MRP2, CMOAT	intestine, liver, kidney, brain	indinavir, cisplatin		
ABCC3	MRP3, CMOAT2	intestine, liver, kidney, placenta, adrenal	etoposide, methotrexate, tenoposide		
ABCC6	MRP6	liver, kidney	cisplatin, daunorubicin		
ABCG2	BCRP	intestine, liver, breast, placenta	daunorubicin, doxorubicin, topotecan, rosuvastatin	elacridar (GF120918)	
SLCO1B1	OATP1B1, OATP-C, OATP2	liver	rifampin, rosuvastatin, methotrexate, pravastatin, thyroxine	cyclosporine rifampin	
SLCO1B3	OATP1B3, OATP8	liver	digoxin, methotrexate, rifampin,		
SLCO2B1	SLC21A9, OATP-B	intestine, liver, kidney, brain	pravastatin		
SLC15A1	PEPT1	intestine, kidney	ampicillin, amoxicillin, captopril, valacyclovir		
SLC15A2	PEPT2	kidney	ampicillin, amoxicillin, captopril, valacyclovir		
SLC22A1	OCT-1	liver	acyclovir, amantadine, desipramine, ganciclovir, metformin	disopyramide, midazolam, phenformin, phenoxy-benzamine, quinidine, quinine, ritonavir, verapamil	
SLC22A2	OCT2	kidney, brain	amantadine, cimetidine, memantine	desipramine, phenoxy-benzamine, quinine	
SLC22A3	OCT3	skeletal muscle, liver, placenta, kidney, heart	cimetidine	desipramine, prazosin, phenoxy-benzamine	
SLC22A4	OCTN1	kidney, skeletal muscle, placenta, prostate, heart	quinidine, verapamil		
SLC22A5	OCTN2	kidney, skeletal muscle, prostate, lung, pancreas, heart, small intestine, liver	quinidine, verapamil		
SLC22A6	OAT1	kidney, brain	acyclovir, adefovir, methotrexate, zidovudine	probenecid, cefadroxil, cefamandole, cefazolin	
SLC22A7	OAT2	liver, kidney	zidovudine		
SLC22A8	OAT3	kidney, brain	cimetidine, methotrexate, zidovudine	probenecid, cefadroxil, cefamandole, cefazolin	

some drug metabolizing enzymes and their effects on drug disposition,[43] the clinical relevance of transporter genetic variations are less well established. There are published reports on the importance of genetic variations in the MDR1 transporter;[42] however, in many cases, the reports have been inconsistent and in some cases conflicting.[44] Many challenges remain in our understanding of the clinical relevance of genetic variations in transporters to drug disposition and drug−drug interactions.

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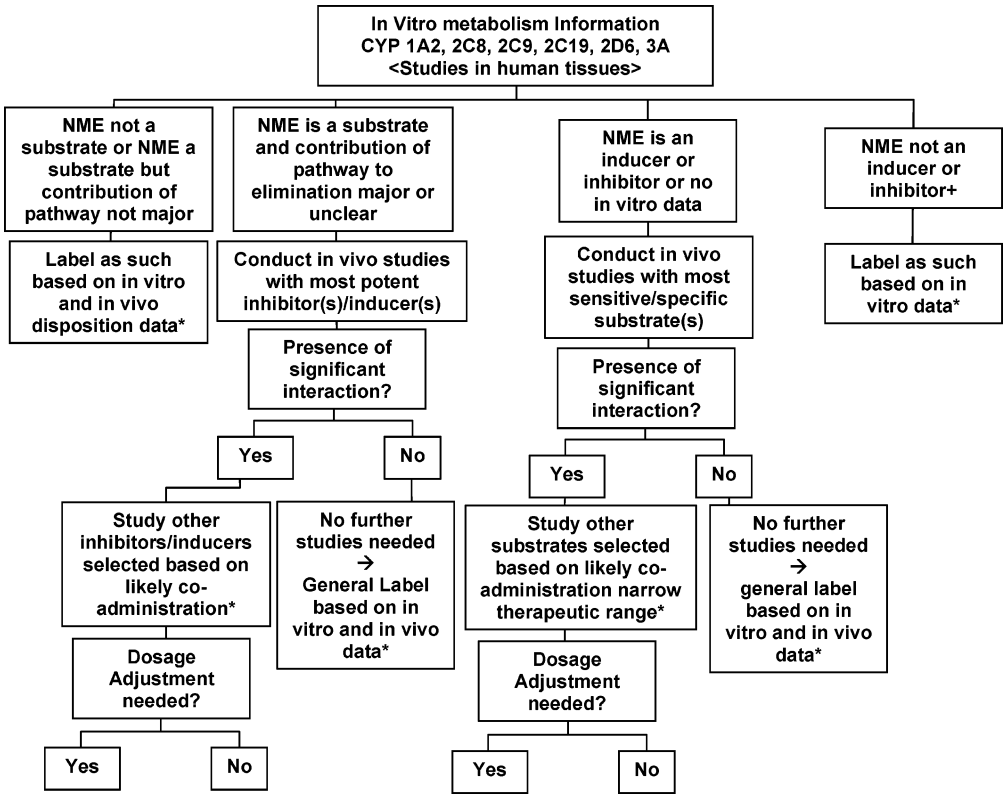

Figure 2. CYP-based drug−drug interaction studies. Decision tree (refer to *J. Clin. Pharmacol.* **1999**, *39*, 1006−1014). NME: new molecular entity. (*) Additional population pharmacokinetic analysis may assist the overall evaluation. (+) Negative results from an in vivo cocktail study would preclude further evaluation to determine whether an NME is an inhibitor or an inducer of a particular CYP enzyme.

Current Status and Challenges in Predicting in Vivo Drug Interactions

Evaluation of an NME's drug−drug interaction potential is an integrated part of the drug development and regulatory review prior to its market approval.[45] In general, three basic questions need to be answered: (1) Will other drugs alter exposure to an NME? (2) Will an NME alter exposure to other drugs? (3) Are these alterations in exposure significant enough to warrant adjustment of the usual dose?

To date, P450-mediated drug interactions have attracted the most attention and our understanding of their role in altering drug exposure has matured. In vitro metabolic studies have been suggested as a critical first step in the assessment of drug interactions[2,3,16] and are now widely accepted by the pharmaceutical industry. The results of these studies can be used to establish the need for further in vivo assessment of potential drug−drug interactions.[2,5,7] On the basis of increased information recently obtained from these types of studies, our understanding of the relationship between in vitro and in vivo drug−drug interactions and our ability to predict these interactions has improved dramatically. Figure 2 suggests a decision tree for determining when clinical drug metabolism/drug interaction studies are indicated. Depending on the study results, recommendations can then be made whether dosage adjustment is required including suitable language in the labeling.

In spite of these advances, unexpected drug−drug interactions do occur and, in some cases, may represent the involvement of drug transporters. Although progress is being made, the tools for identifying and evaluating substrates, inhibitors, and inducers of drug transporters in vitro and the ability to predict potential drug−transporter interaction in vivo are much less advanced than those for metabolizing enzymes. In addition, prediction is confounded when both metabolizing enzymes and transporters are involved in a drug's disposition.[14,15]

Antiretroviral agents are one of the therapeutic drug classes where complex drug interactions involving both metabolic enzymes and transporters have been observed.[46,47] For example, the effect of the recently approved HIV protease inhibitor, tipranavir, on drugs that are both CYP3A and P-gp substrates is difficult to predict.[48,49] In vitro and in vivo data suggest that tipranavir is a CYP3A and P-gp inducer while ritonavir is a CYP3A and P-gp inhibitor. Tipranavir, co-

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administered with low-dose ritonavir at the recommended dosage (500 mg/200 mg), is a net inhibitor of CYP3A but appeared to be an inducer for P-gp at steady state. Tipranavir/ritonavir may increase plasma concentrations of agents that are primarily metabolized by CYP3A (e.g., midazolam) and could increase or prolong their therapeutic and adverse effects. On the other hand, tipranavir/ritonavir may decrease plasma concentrations of agents that are primarily transported by P-gp, e.g., loperamide. It is difficult to predict the net effect of tipranavir/ritonavir on drugs that are dual substrates of CYP3A and P-gp.

What is the present state of our ability to predict drug transporter interactions, and where do we go from here? This topic was discussed at a recent FDA Advisory Committee for Pharmaceutical Science—Clinical Pharmacology subcommittee meeting, on November 3−4, 2004.[16] It was generally agreed that our knowledge of P-gp and the tools to study it are more advanced compared to other transporters. Digoxin was agreed to be a suitable in vivo probe for studying P-gp inhibition; however, identification of P-gp inhibitors for in vivo studies requires further studies. Ritonavir, cyclosporine, and verapamil could prove useful as P-gp inhibitors, although certain restrictions may apply due to safety concerns following their administration to healthy volunteers. Clinical studies of transporters other than P-gp cannot presently be routinely recommended, as no standardized methods, probe substrates, or inhibitors have been established. Similar to the experience with drug metabolizing enzymes, as tools are developed for evaluating drug transporter interactions in vitro and in vivo, the knowledge gained will provide a basis for predicting potential drug-transport interactions in vivo.

Proposals for Evaluating Drug Transporter Mediated Interactions

In recent years a number of important drug transporters have been cloned, resulting in considerable progress toward our understanding of their molecular characteristics. The P-gp transporter has been the most intensely studied; therefore we will use this transporter to form the basis of our discussion.

1. Methods for Determining Whether an NME Is a P-gp Substrate or Inhibitor in Vitro. To determine whether an NME is a substrate or inhibitor of P-gp in vitro we suggest using a bidirectional transport assay as the definitive assay for identifying P-gp substrates and inhibitors. Other assays, for example, the ATPase activity assay and uptake/efflux assays, can screen compounds rapidly, but they are not designed to distinguish P-gp substrates from inhibitors.

Several bidirectional transport models are available, including Caco-2 cells, MDR1-transfected Madine-Darby canine kidney cells (MDR1-MDCK), LLC-PK1 pig kidney cells, and MDR1-transfected LLC-PK1 cells (L-MDR1). Known P-gp substrates and inhibitors should be used in this assay as positive controls that exhibit low to moderate passive membrane permeability ($2-30 \times 10^{-6}$ cm/s) and that are not significantly metabolized. It is recommended that an acceptable cell system produces net flux ratios for known probe substrates similar to values reported in the literature with a minimum net flux ratio of 2. Examples of substrates that have been successfully used in this assay include digoxin, loperamide, quinidine, vinblastine, and talinolol. P-gp inhibitors exhibiting low K_i or IC_{50} values (e.g., <10 uM) are preferred such as cyclosporin A, ketoconazole, zosuquidar (LY335979), valspodar (PSC833), verapamil, or elacridar (GF120918).

To strengthen the results from bidirectional transport studies designed to determine whether a drug is a P-gp substrate, it is recommended that additional experiments be conducted. Since multiple transporters can be expressed in the model cell systems and some inhibitors may inhibit multiple transporters, it is suggested that additional experiments with two or three known P-gp inhibitors be conducted. Experiments that compare efflux activity observed in over-expressed-MDR1 cells to that observed in their respective wild-type cells can also help to determine whether a drug is a P-gp substrate.

2. Criteria Used for Determining Whether an NME Is a Substrate for P-gp in Vitro and Suggested in Vivo Interaction Studies. Following validation of the in vitro method for evaluating drug transporter interactions, we suggest using the decision tree shown in Figure 3, which describes the process we propose for determining whether an NME is a P-gp substrate in vitro and whether in vivo interaction studies with P-gp inhibitors are warranted. If the drug's net flux ratio as defined in Figure 3 is greater than 2 and two or three known P-gp inhibitors significantly reduce the net flux ratio, the drug is likely a P-gp substrate. If a significant amount of efflux activity is not inhibited by the P-gp inhibitors studied, then other efflux transporters may contribute to the efflux activity. Further studies to determine which efflux transporters are involved may be warranted.

If an investigational drug is a P-gp substrate in vitro, evaluation of available in vivo data can help determine whether an in vivo drug interaction study with P-gp inhibitors is to be recommended. These studies to explore drug transporter interactions in vivo can be conducted by co-administering known P-gp inhibitors such as ritonavir, cyclosporine, or verapamil with the caveat that due to safety concerns certain restrictions may apply when administered to healthy subjects. In cases when the drug is also a CYP3A substrate, it may be appropriate to estimate the maximum inhibition by using a strong inhibitor of both P-gp and CYP3A, such as ritonavir.

3. Criteria Used for Determining Whether an NME Is a P-gp Inhibitor in Vitro and Suggested in Vivo Interaction Studies. Following validation of the in vitro method for evaluating drug transporter interactions, we suggest using the decision tree shown in Figure 4, which describes the process we propose for determining whether an NME is a P-gp inhibitor in vitro and whether in vivo interaction studies

(49) Antiviral Drugs Advisory Committee Meeting, May 19, 2005 (FDA Presentations, Drug Interactions). http://www.fda.gov/ohrms/dockets/ac/05/slides/2005-4139S1_10_FDA-Zhang.ppt, **2005**.

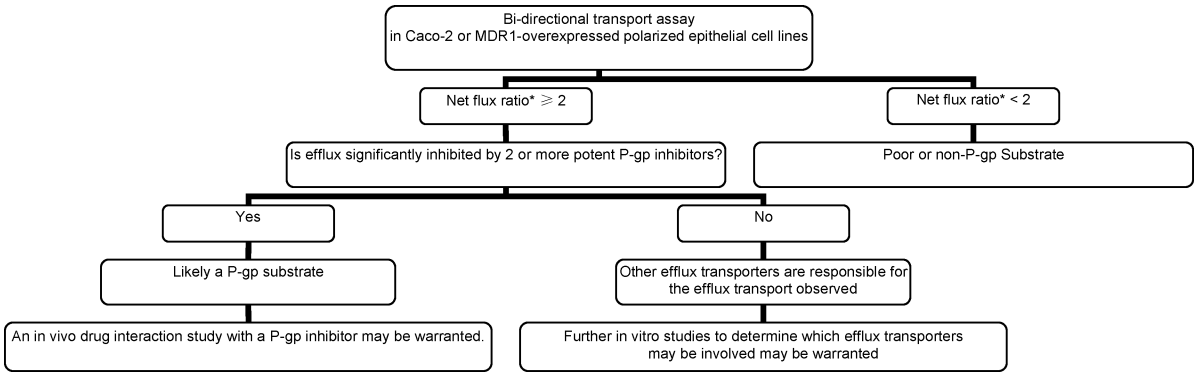

Figure 3. Decision tree to determine whether an NME is a substrate for P-gp and whether an in vivo drug interaction study with a P-gp inhibitor is needed. (*) For Caco-2 cells, net flux ratio is calculated as ($permeability_{app,B-A}$/$permeability_{app,A-B}$). For MDR1-overexpressed cell lines, net flux ratio is calculated as ratio of ($permeability_{app,B-A}$/$permeability_{app,A-B}$)$_{MDR1}$ to ($permeability_{app,B-A}$/$permeability_{app,A-B}$)$_{wild-type}$.

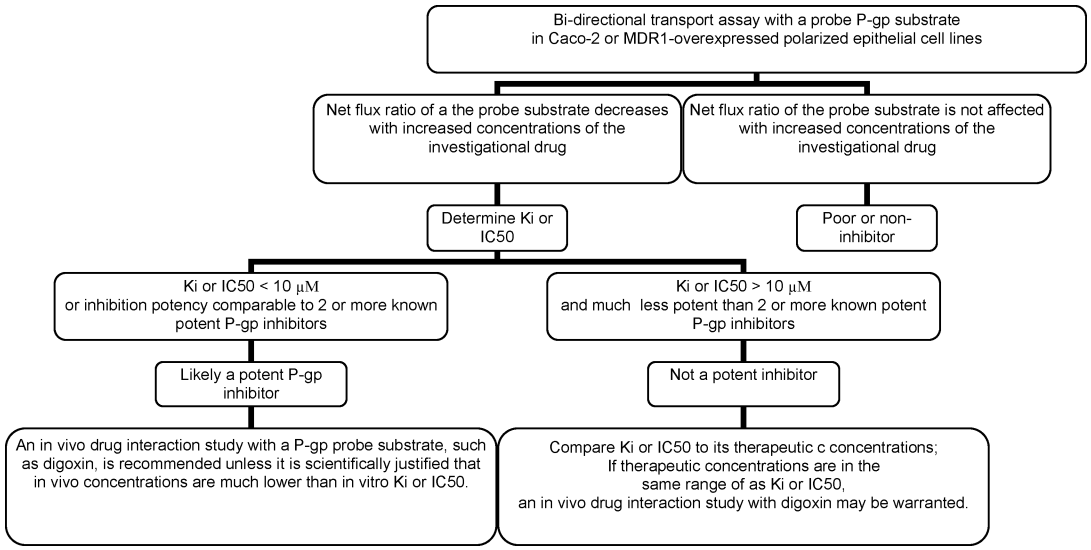

Figure 4. Decision tree to determine whether an NME is an inhibitor for P-gp and whether an in vivo drug interaction study with a P-gp probe substrate, such as digoxin, is needed. (*) For Caco-2 cells, net flux ratio is calculated as ($permeability_{app,B-A}$/$permeability_{app,A-B}$). For MDR1-overexpressed cell lines, net flux ratio is calculated as ratio of ($permeability_{app,B-A}$/$permeability_{app,A-B}$)$_{MDR1}$ to ($permeability_{app,B-A}$/$permeabilityy_{app,A-B}$)$_{wild-type}$.

with P-gp substsrates are warranted. If the efflux of the known probe substrates are inhibited by the investigational drug, with IC_{50} or K_i values $<10\ \mu$M, or are comparable to those obtained for two or three known potent inhibitors, the investigational drug is likely a P-gp inhibitor.

In vivo drug interaction study with digoxin or other known P-gp substrates is suggested unless it is determined that in vivo concentrations of the investigational drug are much lower than IC_{50} or K_i values determined in vitro. If $IC_{50} \geq 10\ \mu$M, or if the drug is a much less potent inhibitor than the positive control inhibitors, then the investigational drug is likely a weak P-gp inhibitor. In this case, an in vivo drug interaction study with a probe P-gp substrate, such as digoxin, may be warranted if therapeutic concentrations of the investigational drug are similar to the IC_{50} or K_i determined in vitro.

4. Evaluation of an NME as a Potential P-gp Inducer. Drugs have been reported to induce P-gp protein including rifampin and St. John's wort. Human pregnane X receptor (hPXR), an orphan nuclear receptor, was found to be a key regulator for P-gp and other enzymes such as CYP3A.[50,51] Structurally, the ligand-binding domains among animal species are remarkably divergent (rodent and rabbit <85% identical with hPXR), which may account for species difference in marked differences in PXR activation by certain drugs.[52] Therefore, animals may not be a useful model to study P-gp induction due to potential species differences in inductive response to P-gp inducers. The Caco-2 cell line is

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(51) Lehmann, J. M.; McKee, D. D.; Watson, M. A.; Willson, T. M.; Moore, J. T.; Kliewer, S. A. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* **1998**, *102* (5), 1016−1023.

not a suitable model for the in vitro evaluation of induction for P-gp, possibly due to lack of hPXR activity in this cell line.[53] In the literature, human colon adenocarcinoma cell LS180/WT and its adriamycin-resistant (LS 180/AD 50) or vinblastine-resistant (LS 180/V) sublines have been used to study induction for both P-gp and CYP3A.[54]

Methods for in vitro evaluation for P-gp induction are not well established; however, when needed the P-gp induction potential of an investigational drug may best be evaluated in vivo. For example, multiple dosing with digoxin as a probe substrate may provide evidence of the drug's potential to induce P-gp protein.

5. Other Transporters. Routine in vitro studies for other transporter-based interactions cannot be recommended at this time because no standardized methods or probe substrates and inhibitors have been established. Until additional knowledge and technologies are available, recommendations for evaluation of transporter-based drug interactions other than P-gp may be drug- or therapeutic-class specific. In special cases, it might be appropriate to use cyclosporine, an inhibitor of multiple transporters, to rule out transporter-based drug interactions. Similarly, it may be appropriate to perform a renal transporter inhibition study with a drug that is extensively secreted into renal tubular fluid and whose renal clearance is high. Drug classes such as β-lactam antibiotics and nucleoside analogue antiviral agents are likely candidates for renal transporter interactions.

Conclusion

Considerable progress has been made in the development of tools and techniques for studying transporter-based drug−drug interactions. These advances have provided a means to identify selective substrates and inhibitors for individual drug transporters and provide probes to evaluate potential drug−drug interactions mediated by transporters in vitro.

Many drugs are likely substrates and/or inhibitors for some transporters. Interplay between transporters and metabolizing enzymes may differ depending on the characteristics of the drugs.[55] Many challenges remain in understanding the net effect of drugs that interact with both drug transporters and metabolizing enzymes. More research is needed. Nevertheless, conducting transporter-based drug interactions during drug development will provide information on the involvement of transporters in an NME's disposition and help identify the potential for drug−drug transporter interactions. In recent years, understanding the metabolic disposition and identifying the potential of metabolic drug−drug interactions such as inhibition and induction of enzymes has become an integral part of the drug development process. Similar progress is anticipated in the transporter area to understand the effect of transporters on a drug's absorption, disposition, elimination, and metabolism.

Abbreviations Used

FDA, U.S. Food and Drug Administration; NDA, new drug application; NME, new molecular entity; P-gp, P-glycoprotein; MRP, multidrug resistance protein; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OAT, organic anion transporter; PEPT, peptide transporter; ABC, ATP-binding cassette transporter superfamily; SLC, solute-linked carrier transporter family; SLCO, solute-linked carrier organic anion transporter family; MDR, multidrug resistance; BSEP, bile salt export pump; BCRP, breast cancer resistance protein; AUC, area under the concentration−time curve.

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