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Scientific and Regulatory Perspectives on Metabolizing Enzyme−Transporter Interplay and Its Role in Drug Interactions: Challenges in Predicting Drug Interactions†

Lei Zhang,* Yuanchao Zhang,‡ and Shiew-Mei Huang

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

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Abstract: Both metabolizing enzymes and drug transporters play important roles in modulating drug absorption, distribution, metabolism and elimination. Acting alone or in concert with each other they can affect the pharmacokinetics and pharmacodynamics of a drug. This paper will present cases from recent reviews of new drug application (NDA) and literature that exemplify the role of metabolizing enzyme−transporter interplay in a drug's disposition, and discuss challenges in predicting drug interactions. Finally, the discussion will focus on the need to leverage current knowledge to obtain more meaningful drug interaction information.

Keywords: Enzyme−transporter interplay; drug−drug interaction; regulatory; guidance; new drug application; drug development; labeling; risk management

Introduction

Our understanding of the correlation between *in vitro* and *in vivo* drug−drug interactions and our ability to predict drug interactions has improved over the years. Both qualitative and quantitative prediction have been discussed.[1−6] Decision trees for determining when clinical drug interaction studies are indicated based on *in vitro* evaluations of metabolic-based and P-glycoprotein (P-gp)-mediated interactions are included in the FDA draft drug interaction guidance.[7−9]

In spite of advances in predicting drug interactions based on *in vitro* evaluations, unexpected drug−drug interactions do occur that could be due to several variables that we do

* Corresponding author: Lei Zhang, PhD, Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, Food and Drug Administration, Building 51, Room 3106, 10903 New Hampshire Avenue, Silver Spring, MD 20993. Phone: 301-796-1635. Fax: 301-847-8720. Email: leik.zhang@fda.hhs.gov.

† Disclaimer: The opinions contained in this paper do not necessarily reflect the official views of the FDA.

‡ Yuanchao (Derek) Zhang. Current affiliation: Clinical Pharmacology and Regulatory Affairs, Frontage Laboratoires, Inc., 224 Valley Creek Boulevard, Suite 300, Exton, PA 19341.

(1) Ito, K.; Iwatsubo, T.; Kanamitsu, S.; Nakajima, Y.; Sugiyama, Y. Quantitative Prediction of in Vivo Drug Clearance and Drug Interactions From in Vitro Data on Metabolism, Together With Binding and Transport. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 461–499.

(2) Kato, M; Shitara, Y.; Sato, H.; Yoshisue, K.; Hirano, M.; Ikeda, T.; Sugiyama, Y. The Quantitative Prediction of CYP-Mediated Drug Interaction by Physiologically Based Pharmacokinetic Modeling. *Pharm. Res.* **2008**, *25*, 1891–1901.

(3) Obach, R. S. Predicting Drug-Drug Interactions From in Vitro Drug Metabolism Data: Challenges and Recent Advances. *Curr Opin. Drug Discov. Dev.* **2009**, *12*, 81–89.

(4) Zhang, L.; Zhang, Y. D.; Zhao, P.; Huang, S. M. Predicting Drug-Drug Interactions: An FDA Perspective. *AAPS J.* **2009**, *11*, 300–306.

(5) Benet, L. Z.; Cummins, C. L.; Wu, C. Y. Transporter-Enzyme Interactions: Implications for Predicting Drug-Drug Interactions From in Vitro Data. *Curr. Drug Metab.* **2003**, *4*, 393–398.

(6) Bjornsson, T. D.; Callaghan, J. T.; Einolf, H. J.; Fischer, V.; Gan, L.; Grimm, S.; Kao, J.; King, S. P.; Miwa, G.; Ni, L.; Kumar, G.; McLeod, J.; Obach, R. S.; Roberts, S.; Roe, A.; Shah, A.; Snikeris, F.; Sullivan, J. T.; Tweedie, D.; Vega, J. M.; Walsh, J.; Wrighton, S. A. The Conduct of in Vitro and in Vivo Drug-Drug Interaction Studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) Perspective. *Drug Metab. Dispos.* **2003**, *31*, 815–832.

(7) Draft Guidance for Industry: Drug Interaction Studies -Study Design, Data Analysis, and Implications for Dosing and Labeling. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf.

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not yet understand or cannot accurately measure. Collectively, these interactions may be defined as "complex drug interactions" including interactions involving multiple interacting drugs, concurrent inhibition and induction, inhibition of an enzyme or transporter in poor metabolizers of another pathway, drug interactions in patients with organ impairment, and the presence of inhibitory metabolite(s). The interplay between metabolizing enzymes and transporters represents one of the emerging confounding factors that have been recognized recently and contributes to the complex drug interactions. Dr. Benet's lab has postulated such enzyme−transporter interplay.[5,10] Drugs that are substrates, inhibitors or inducers of cytochrome P450 (CYP) or phase 2 enzymes can be substrates, inhibitors or inducers of transporters as well. Therefore, extrapolating interaction results from one substrate drug to another can be difficult or impossible.[11] Based on the location of transporters, the effect of concomitant drugs on transporters may either enhance or reduce access of drugs to the intracellular space where metabolism occurs. Both animal and human studies have been reported, which demonstrated the impact of interplay between enzymes and transporters.[12−16]

This paper will present several examples from recent reviews of new drug application (NDA) and literature to illustrate the emerging challenges in predicting drug interactions.

Challenges in Predicting *In Vivo* Drug Interactions

Pharmacokinetic drug interactions can occur via inhibition or induction of metabolic enzymes or transporters. While drug interactions can be evaluated via specific clinical studies in healthy subjects or patients, *in vitro* approaches are now becoming common as a critical first step in the assessment of drug interaction potential via specific pathways, and knowledge obtained from these studies may help focus on the needed clinical studies. The 2006 FDA draft drug interaction guidance has specific recommendations as to how to use *in vitro* models to address drug interaction potential and includes criteria for evaluating transporter-based drug interactions.[7] The guidance also includes tables for the classification of CYP substrates (sensitive substrates or substrates with narrow therapeutic range) and inhibitors (weak, moderate or strong) for selection of substrate or inhibitor drugs to be used in the drug interaction studies or for labeling purpose (e.g., to support class labeling without specific individual clinical studies).

As of note, several of the substrates recommended for drug interaction studies are not necessarily specific in that they may be substrates for more than one CYP enzymes or may be substrates for transporters (e.g., simvastatin, omeprazole and repaglinide).[17−19] The prediction for drug interactions via specific enzyme pathways is confounded when both metabolizing enzymes and transporters are involved in a drug's disposition.[5,10] The outcome of simultaneous inhibition of a dominant CYP enzyme(s) and an uptake or efflux transporter that controls the availability of the drug to CYP enzymes can be just as profound as that of simultaneous inhibition from multiple CYP inhibitors. For example, repaglinide is a substrate for CYP2C8, CYP3A, and OATP1B1. Gemfibrozil and its glucuronide metabolite are inhibitors of both CYP2C8 and organic anion transport protein (OATP). Coadministration of gemfibrozil and repa-

(8) Huang, S. M.; Strong, J. M.; Zhang, L.; Reynolds, K. S.; Nallani, S.; Temple, R.; Abraham, S.; Al, H. S.; Baweja, R. K.; Burckart, G. J.; Chung, S.; Colangelo, P.; Frucht, D.; Green, M. D.; Hepp, P.; Karnaukhova, E.; Ko, H. S.; Lee, J. I.; Marroum, P. J.; Norden, J. M.; Qiu, W.; Rahman, A.; Sobel, S.; Stifano, T.; Thummel, K.; Wei, X. X.; Yasuda, S.; Zheng, J. H.; Zhao, H.; Lesko, L. J. New Era in Drug Interaction Evaluation: US Food and Drug Administration Update on CYP Enzymes, Transporters, and the Guidance Process. *J. Clin. Pharmacol.* **2008**, *48*, 662–670.

(9) Zhang, L; Zhang, Y. D.; Strong, J. M.; Reynolds, K. S. Huang SM A Regulatory Viewpoint on Transporter-Based Drug Interactions. *Xenobiotica* **2008**, *38*, 709–724.

(10) Benet, L. Z.; Cummins, C. L.; Wu, C. Y. Unmasking the Dynamic Interplay Between Efflux Transporters and Metabolic Enzymes. *Int. J. Pharm.* **2004**, *277*, 3–9.

(11) Benet, L. Z. A Holy Grail of Clinical Pharmacology: Prediction of Drug Pharmacokinetics and Pharmacodynamics in the Individual Patient. *Clin. Pharmacol. Ther.* **2009**, *86*, 133–134.

(12) Lam, J. L.; Shugarts, S. B.; Okochi, H. Benet LZ Elucidating the Effect of Final-Day Dosing of Rifampin in Induction Studies on Hepatic Drug Disposition and Metabolism. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 864–870.

(13) Lam, J. L.; Okochi, H; Huang, Y. Benet LZ In Vitro and in Vivo Correlation of Hepatic Transporter Effects on Erythromycin Metabolism: Characterizing the Importance of Transporter-Enzyme Interplay. *Drug Metab. Dispos.* **2006**, *34*, 1336–1344.

(14) Lau, Y. Y.; Huang, Y; Frassetto, L. Benet LZ Effect of OATP1B Transporter Inhibition on the Pharmacokinetics of Atorvastatin in Healthy Volunteers. *Clin. Pharmacol. Ther.* **2007**, *81*, 194–204.

(15) van Giersbergen, P. L.; Treiber, A.; Schneiter, R.; Dietrich, H. Dingemanse J Inhibitory and Inductive Effects o.f Rifampin on the Pharmacokinetics of Bosentan in Healthy Subjects. *Clin. Pharmacol. Ther.* **2007**, *81*, 414–419.

(16) Zheng, H. X.; Huang, Y; Frassetto, L. A. Benet LZ Elucidating Rifampin's Inducing and Inhibiting Effects on Glyburide Pharmacokinetics and Blood Glucose in Healthy Volunteers: Unmasking the Differential Effects of Enzyme Induction and Transporter Inhibition for a Drug and Its Primary Metabolite. *Clin. Pharmacol. Ther.* **2009**, *85*, 78–85.

(17) Neuvonen, P. J.; Backman, J. T.; Niemi, M. Pharmacokinetic Comparison of the Potential Over-the-Counter Statins Simvastatin, Lovastatin, Fluvastatin and Pravastatin. *Clin. Pharmacokinet.* **2008**, *47*, 463–474.

(18) Scheen, A. J. Drug-Drug and Food-Drug Pharmacokinetic Interactions With New Insulinotropic Agents Repaglinide and Nateglinide. *Clin. Pharmacokinet.* **2007**, *46*, 93–108.

(19) Li, X. Q.; Weidolf, L.; Simonsson, R.; Andersson, T. B. Enantiomer/Enantiomer Interactions Between the S- and R-Isomers of Omeprazole in Human Cytochrome P450 Enzymes: Major Role of CYP2C19 and CYP3A4. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 777–787.

glinid caused an 8.1-fold increase in repaglinide AUC.[20] Coadministration of repaglinid and itraconazole (a CYP3A inhibitor) caused a 1.4-fold increase in repaglinide AUC. When these three drugs were coadministered, the AUC of repaglinide increased to 19-fold, more than the multiple of individual effects. The larger than additive effect of simultaneous coadministration of itraconazole and gemfibrozil on the systemic exposure (AUC) of repaglinide may be attributed to collective inhibitory effects on both enzyme and transporters that may not be readily predicted from isolated *in vitro* experiments or separate *in vivo* study results.[20] Similarly, induction of a dominant CYP enzyme(s) and at the same time inhibition of an uptake or efflux transporter that controls the availability of the drug to CYP enzymes can lead to confounding interactions. For example, the *in vivo* consequence of coadministration of repaglinide, a substrate for CYP2C8, CYP3A, and OATP1B1, and rifampin, a CYP2C8 and CYP3A inducer and an OATP1B1 inhibitor, showed time-dependency that could not be predicted from *in vitro* studies or extrapolated from other CYP2C8 or OATP1B1 substrates.[21,22]

Therefore, the interplay between metabolizing enzymes and transporters presents an emerging challenge in drug interaction prediction and risk management. Progress has been made in using the *in silico* physiologically based pharmacokinetic modeling and simulation to assist in prediction of drug interactions; however, challenges remain because the lack of true physiological representation in the models limits the ability to predict *in vivo* situations such as enzyme and transporter interplay at various tissues, e.g., different interplay of CYP3A4 and P-gp in the intestine vs in the liver.

Enzyme−Transporter Interplay: Examples

Human Immunodeficiency Virus (HIV) Drugs. Antiretroviral agents are one of the therapeutic drug classes where complex drug interactions involving both metabolic enzymes and transporters have been observed.[23,24] Unexpected interaction results between Kaletra (lopinavir/ritonavir) and fosamprenavir/ritonavir and difficulties in predicting the effects of tipranavir/ritonavir on drugs that are dual CYP3A and P-gp substrates (as detailed below) further indicate the need to consider the interplay between enzymes and transporters in drug interaction prediction.

Kaletra. Kaletra is a coformulation of lopinavir and ritonavir. A low dose of ritonavir inhibits P-gp and inhibits the CYP3A-mediated metabolism of lopinavir, thereby providing substantially increased plasma levels of lopinavir. When lopinavir is combined with ritonavir, the net effect on CYP3A is inhibition, and that net CYP3A inhibition attributable to Kaletra is due almost entirely to ritonavir. First, the IC_{50} or K_i value of lopinavir for CYP3A is 10-fold or more higher than that of ritonavir.[25] Second, the CYP3A phenotype in HIV patients taking Kaletra is indistinguishable from that in patients taking ritonavir.[26] Third, ritonavir itself is so potent an inhibitor that any dose of ritonavir—boosting dose (100 or 200 mg) or lower dose (e.g., 50 mg)—has been shown to produce essentially complete CYP3A inhibition.[27] There is evidence to show that ritonavir is a moderate CYP3A inducer, however, the net effect of ritonavir on CYP3A substrates reflects the inhibition of the enzyme.[28] With regard to P-gp, it is evident that both ritonavir and lopinavir are moderate inhibitors with acute exposure. *In vitro* data suggest that both lopinavir and ritonavir are moderate P-gp inducers as well,[29−31] while *in vivo* studies suggest that lopinavir is likely an inducer with extended exposure[29−31] and the net effect of extended exposure of rinonavir on P-gp is inhibition.[28] Therefore, it is difficult to predict the relative contributions of ritonavir and lopinavir to net P-gp induction or inhibition during treatment with Kaletra. Kaletra thus is anticipated to interact with drugs that are CYP3A and/or P-gp substrates with varying degrees of interactions, acting as a

(20) Niemi, M.; Backman, J. T.; Neuvonen, M. Neuvonen PJ Effects of Gemfibrozil, Itraconazole, and Their Combination on the Pharmacokinetics and Pharmacodynamics of Repaglinide: Potentially Hazardous Interaction Between Gemfibrozil and Repaglinide. *Diabetologia* **2003**, *46*, 347–351.

(21) Niemi, M.; Backman, J. T.; Kajosaari, L. I.; Leathart, J. B.; Neuvonen, M.; Daly, A. K.; Eichelbaum, M.; Kivisto, K. T. Neuvonen PJ Polymorphic Organic Anion Transporting Polypeptide 1B1 Is a Major Determinant of Repaglinide Pharmacokinetics. *Clin. Pharmacol. Ther.* **2005**, *77*, 468–478.

(22) Bidstrup, T. B.; Stilling, N.; Damkier, P.; Scharling, B.; Thomsen, M. S. Brosen K Rifampicin Seems to Act As Both an Inducer and an Inhibitor of the Metabolism of Repaglinide. *Eur. J. Clin. Pharmacol.* **2004**, *60*, 109–114.

(23) Kashuba, A. D. Drug-Drug Interactions and the Pharmacotherapy of HIV Infection. *Top. HIV Med.* **2005**, *13*, 64–69.

(24) McNicholl, I. R. Drug Interactions Among the Antiretrovirals. *Curr. Infect. Dis. Rep.* **2004**, *6*, 159–162.

(25) Weemhoff, J. L.; von Moltke, L. L.; Richert, C.; Hesse, L. M.; Harmatz, J. S.; Greenblatt, D. J. Apparent Mechanism-Based Inhibition of Human CYP3A in-Vitro by Lopinavir. *J. Pharm. Pharmacol* **2003**, *55*, 381–386.

(26) Knox, T. A.; Oleson, L.; von Moltke, L. L.; Kaufman, R. C.; Wanke, C. A.; Greenblatt, D. J. Ritonavir Greatly Impairs CYP3A Activity in HIV Infection With Chronic Viral Hepatitis. *JAIDS, J. Acquired Immune Defic. Syndr.* **2008**, *49*, 358–368.

(27) Mathias, A. A.; West, S.; Hui, J.; Kearney, B. P. Dose-Response of Ritonavir on Hepatic CYP3A Activity and Elvitegravir Oral Exposure. *Clin. Pharmacol. Ther.* **2009**, *85*, 64–70.

(28) Kharasch, E. D.; Bedynek, P. S.; Walker, A.; Whittington, D.; Hoffer, C. Mechanism of Ritonavir Changes in Methadone Pharmacokinetics and Pharmacodynamics: II Ritonavir Effects on CYP3A and P-Glycoprotein Activities. *Clin. Pharmacol. Ther.* **2008**, *84*, 506–512.

(29) Perloff, M. D.; von Moltke, L. L.; Fahey, J. M.; Daily, J. P.; Greenblatt, D. J. Induction of P-Glycoprotein Expression by HIV Protease Inhibitors in Cell Culture. *AIDS* **2000**, *14*, 1287–1289.

(30) Perloff, M. D.; von Moltke, L. L.; Marchand, J. E.; Greenblatt, D. J. Ritonavir Induces P-Glycoprotein Expression, Multidrug Resistance-Associated Protein (MRP1) Expression, and Drug Transporter-Mediated Activity in a Human Intestinal Cell Line. *J. Pharm. Sci.* **2001**, *90*, 1829–1837.

(31) Vishnuvardhan, D.; Moltke, L. L.; Richert, C.; Greenblatt, D. J. Lopinavir: Acute Exposure Inhibits P-Glycoprotein; Extended Exposure Induces P-Glycoprotein. *AIDS* **2003**, *17*, 1092–1094.

CYP3A inhibitor or a P-gp inhibitor or P-gp inducer depending on the extent of exposure.

For drugs whose pharmacokinetics are mainly affected by CYP3A and less by P-gp, Kaletra would act as an inhibitor and increase these drugs' exposure. For example, Kaletra markedly increases atorvastatin and saquinavir systemic exposures (AUC), by 6- and 10-fold, respectively.[32] The data may suggest that CYP3A plays a major role for atorvastatin and saquinavir clearance although both drugs are dual CYP3A and P-gp substrates. Atorvastatin is also an OATP1B1 substrate.[33,34] Recently, Kaletra was found to increase rosuvastatin exposure by 2-fold (AUC), likely via OATP1B1 inhibition,[35] therefore, the large inhibition effect on atorvastatin from Kaletra very likely in part be mediated by the OATP1B1 pathway because itraconazole, a dual inhibitor for P-gp and CY3A only increased atorvastatin exposure less than 3-fold.[33]

For CYP3A substrate drugs that also have significant P-gp component, the interaction with Kaletra is complicated. The interaction between fosamprenavir and Kaletra represents such a case.[36] Fosamprenavir, a prodrug of amprenavir, is almost completely converted to amprenavir during absorption. Amprenavir, the circulating active moiety following fosamprenavir administration, is a P-gp substrate and is metabolized by CYP3A with minimal unchanged drug excreted in urine. Low dose of ritonavir inhibits P-gp and inhibits the CYP3A-mediated metabolism of amprenavir, thereby providing substantially increased plasma levels of amprenavir when ritonavir is combined with fosamprenavir. As mentioned before, if Kaletra acts as a CYP3A/P-gp inhibitor, it was anticipated that amprenavir exposure would increase when fosamprenavir was coadministered with Kaletra. However, when fosamprenavir/ritonavir (700 mg/100 mg twice daily) and Kaletra (400 mg of lopinavir/100 mg of ritonavir twice daily) was coadministered, at the steady-state, instead of the anticipated increase of amprenavir concentrations, the amprenavir plasma concentrations were markedly decreased (approximately 60%) compared to the fosamprenavir/ritonavir (700 mg/100 mg twice daily) alone regimen.[36,37] It appears that an additional 100 mg of ritonavir from Kaletra did not impose more inhibition to amprenavir but instead showed a net "induction" effect. Furthermore, the interaction between fosamprenavir and Kaletra is neither a physicochemical incompatibility nor an acute effect on drug-metabolizing enzymes or transporters that can be overcome by separating or increasing drug doses.[38] Administration of amprenavir/ritonavir and Kaletra also had similar results: lower amprenavir levels with Kaletra as compared to those without Kaletra.[39] The exact underlying mechanism for the observed *in vivo* results is not clear, but likely in part due to P-gp induction effect from lopinavir as discussed earlier. In addition, ritonavir is also an inducer for several other enzymes such as CYP1A2, CYP2B6, CYP2C9, CYP2C19, and UGT;[40−44] therefore complicated interplay between enzyme and transporter inhibition and induction may play a role in this case. More data are needed to determine whether there are other metabolic or transport pathways for amprenavir that may be affected by ritonavir or lopinavir.

Tipranavir/Ritonavir. The effects of the HIV protease inhibitor, tipranavir, on drugs that are dual CYP3A and P-gp substrates are difficult to predict.[45−47]

Tipranavir coadministered with ritonavir at the recommended dose is a net inhibitor for CYP3A.[45,46] Thus tipranavir/ritonavir may increase plasma concentrations of

(32) KALETRA (Lopinavr;Ritonavir) Labeling. http://www.accessdata.fda.gov/drugsatfda_docs/label/2007/021226s022lbl.pdf. 2005.

(33) LIPITOR (Atorvastatin) Labeling. http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020702s056lbl.pdf. 2009.

(34) Lennernas, H. Clinical Pharmacokinetics of Atorvastatin. *Clin Pharmacokinet.* **2003**, *42*, 1141–1160.

(35) Kiser, J. J.; Gerber, J. G.; Predhomme, J. A.; Wolfe, P.; Flynn, D. M.; Hoody, D. W. Drug/Drug Interaction Between Lopinavir/Ritonavir and Rosuvastatin in Healthy Volunteers. *J Acquired Immune Defic. Syndr.* **2008**, *47*, 570–578.

(36) LEXIVA (Fosamprenavir) Labeling. http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021548s021,022116s005lbl.pdf. 2005.

(37) Kashuba, A. D.; Tierney, C.; Downey, G. F.; Acosta, E. P.; Vergis, E. N.; Klingman, K.; Mellors, J. W.; Eshleman, S. H.; Scott, T. R.; Collier, A. C. Combining Fosamprenavir With Lopinavir/Ritonavir Substantially Reduces Amprenavir and Lopinavir Exposure: ACTG Protocol A5143 Results. *AIDS* **2005**, *19*, 145–152.

(38) Corbett, A. H.; Patterson, K. B.; Tien, H. C.; Kalvass, L. A.; Eron, J. J.; Ngo, L. T.; Lim, M. L. Kashuba AD Dose Separation Does Not Overcome the Pharmacokinetic Interaction Between Fosamprenavir and Lopinavir/Ritonavir. *Antimicrob. Agents Chemother.* **2006**, *50*, 2756–2761.

(39) Cvetkovic, R. S.; Goa, K. L. Lopinavir/Ritonavir: a Review of Its Use in the Management of HIV Infection. *Drugs* **2003**, *63*, 769–802.

(40) Yeh, R. F.; Gaver, V. E.; Patterson, K. B.; Rezk, N. L.; Baxter-Meheux, F; Blake, M. J.; Eron, J. J., Jr; Klein, C. E.; Rublein, J. C. Kashuba AD Lopinavir/Ritonavir Induces the Hepatic Activity of Cytochrome P450 Enzymes CYP2C9, CYP2C19, and CYP1A2 but Inhibits the Hepatic and Intestinal Activity of CYP3A As Measured by a Phenotyping Drug Cocktail in Healthy Volunteers. *J. Acquired Immune Defic. Syndr.* **2006**, *42*, 52–60.

(41) Hogeland, G. W.; Swindells, S.; McNabb, J. C.; Kashuba, A. D.; Yee, G. C. Lindley CM Lopinavir/Ritonavir Reduces Bupropion Plasma Concentrations in Healthy Subjects. *Clin. Pharmacol. Ther.* **2007**, *81*, 69–75.

(42) Ouellet, D.; Hsu, A.; Qian, J.; Locke, C. S.; Eason, C. J.; Cavanaugh, J. H.; Leonard, J. M. Granneman GR Effect of Ritonavir on the Pharmacokinetics of Ethinyl Oestradiol in Healthy Female Volunteers. *Br. J. Clin. Pharmacol.* **1998**, *46*, 111–116.

(43) Foisy, M. M.; Yakiwchuk, E. M. Hughes CA Induction Effects of Ritonavir: Implications for Drug Interactions. *Ann. Pharmacother.* **2008**, *42*, 1048–1059.

(44) Kharasch, E. D.; Mitchell, D; Coles, R. Blanco R Rapid Clinical Induction of Hepatic Cytochrome P4502B6 Activity by Ritonavir. *Antimicrob. Agents Chemother.* **2008**, *52*, 1663–1669.

(45) 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.

(46) APTIVUS Clinical Pharmacology Biopharmaceutics Review. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/21814_000_AptivusTOC.cfm. 2005.

(47) APTIVUS (Tipranavir) Labeling. http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021814s006,022292s001lbl.pdf. 2007.

Table 1. Effect of Tipranavir/Ritonavir on Drugs That Are CYP3A and/or P-gp Substrates[a]

substrate type	effect of tipranavir/ritonavir on coadministered drug
CYP3A Only	
midazolam	AUC ↑
P-gp Only	
loperamide	AUC ↓ 51%
Dual CYP3A and P-gp	
atorvastatin	AUC ↑ 5−9-fold
clarithromycin	AUC ↑ 19%
amprenavir	AUC ↓ 50%
lopinavir	AUC ↓ 50−70%
saquinavir	AUC ↓ 80%

[a] The net effects on CYP3A substrates only, P-gp substrates only, and dual CYP3A and P-gp substrates are increase, decrease, increase or decrease, respectively [47].

agents that are primarily metabolized by CYP3A (e.g., midazolam) and could increase or prolong their therapeutic and adverse effects (Table 1).[45−47] On the other hand, the net effect of tipranavir/ritonavir on P-gp is induction over time.[45,46] Thus tipranavir/ritonavir may decrease plasma concentrations of agents that are primarily transported by P-gp, e.g., loperamide (Table 1). However the net effect of tipranavir/ritonavir on oral bioavailability and plasma concentrations of drugs that are dual substrates of CYP3A and P-gp will vary depending on the relative affinity of the coadministered drugs for CYP3A and P-gp, and the extent of intestinal first-pass metabolism/efflux (Table 1).[47] For example, clarithromycin is a P-gp and CYP3A dual substrate. Steady-state tipranavir/ritonavir administration (500/200 mg bid) increased clarithromycin AUC_{0-12h} only by 19%. However, the formation of the major metabolite, 14-OH-clarithromycin, was almost fully inhibited at the steady-state of tipranavir/ritonavir administration. The degree of clarithromycin exposure increase is less than expected based on the degree of reduction of 14-OH-clarithromycin formation. A possible explanation is that tipranavir is a P-gp inducer and the low dose of ritonavir can not compensate for the P-gp induction effect caused by tipranavir. Because clarithromycin is a P-gp substrate, it is pumped back to intestinal lumen essentially as unabsorbed drug by increased activity of intestinal P-gp. The net interplay between intestinal CYP3A and P-gp may have led to the similar systemic exposure of clarithromycin when coadministered with tipranavir/ritonavir at steady state compared to that of clarithromycin alone. In another case, additional coadministration of tipranavir/ritonavir at 500 mg/200 mg bid decreased amprenavir, lopinavir and saquinavir steady-state trough plasma concentrations by 52%, 80% and 56%, respectively, when these protease inhibitors were also administered with 200 mg ritonavir. All the protease inhibitors (PIs) studied in this trial are known dual substrates of CYP3A and P-gp and subject to high intestinal first-pass effect. The net interplay between intestinal CYP3A and P-gp caused lower systemic exposure of these PIs when coadministered with tipranavir/ritonavir at the steady state. On the other hand, coadministration of tipranavir/ritonavir caused a 5−9-fold increased in atorvastatin exposure,[47] similar to the results described earlier for atorvastatin and Kaletra coadministration. Again the data suggest that although atorvastatin is a substrate for CYP3A and P-gp, CYP3A appears to be a more important pathway than P-gp for atorvastatin exposure. In addition, as mentioned earlier atorvastatin is also an OATP1B1 substrate, and if tipranavir/ritonavir is an OATP1B1 inhibitor, then the inhibition may be partly due to the OATP1B1 pathway. Whether tipranavir/ritonavir inhibits OATP1B1 and contributes to the increase in atorvastatin exposure is not clear.

Rifampin. Rifampin is a well-known inducer for multiple P450 enzymes including CYP1A2, -2B6, -2C8, -2C9, -2C19 and -3A4. It is also known to be an inducer for transporters such as P-gp and MRP2 possibly via PXR-activation process. Recently, a number of studies have shown that rifampin is a substrate and potent inhibitor of different members of the OATP family of drug transporters including OATP1B1 and OATP1B3.[48,49] The IC_{50} values of rifampin for inhibition of OATP1B1 and OATP1B3 (in μM range) are lower or similar to the maximal plasma concentrations attained (i.e., about 19 μM) in humans after multiple-dose administration of 600 mg once a day. Consistent with projection from the *in vitro* data, inhibition of OATP by rifampin has been demonstrated with several OATP substrate drugs.[14−16,21,22,48,50] The extent and direction of change in systemic exposure for drugs that are substrates for metabolizing enzymes and/or transporters affected by rifampin can vary depending on the timing and length of rifampin coadministration (Table 2). For example, coadministration of rifampin and bosentan (a CYP2C9, CYP3A, OATP1B1, OATP1B3 substrate) resulted in a time-dependent drug interaction.[15] There was an initial increase in bosentan trough concentrations on day 2, and upon continued dosing for 5 days, there was a decrease in exposure. The inhibition of hepatic uptake of bosentan via OATP1B1 and OATP1B3 by rifampin is the most likely explanation for the initial increase in trough concentrations, whereas the CYP-inductive properties of rifampin after multiple dosing may explain the decrease in exposure to bosentan upon continued dosing. Similar findings were identified for atorvastatin (see more discussion below),[14] repaglinide,[11] atrasentan,[24] and, more recently, glyburide[16] (Table 2).

The above examples illustrate the importance to understand all the potential inductive and inhibitory effects that an interactive drug like rifampin may have on both

(48) Vavricka, S. R.; Van Montfoort, J.; Ha, H. R.; Meier, P. J. Fattinger K Interactions of Rifamycin SV and Rifampicin With Organic Anion Uptake Systems of Human Liver. *Hepatology* **2002**, *36*, 164–172.

(49) Tirona, R. G.; Leake, B. F.; Wolkoff, A. W. Kim RB Human Organic Anion Transporting Polypeptide-C (SLC21A6) Is a Major Determinant of Rifampin-Mediated Pregnane X Receptor Activation. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 223–228.

(50) Xiong, H; Carr, R. A.; Locke, C. S.; Katz, D. A.; Achari, R.; Doan, T. T.; Wang, P.; Jankowski, J. R. Sleep DJ Dual Effects of Rifampin on the Pharmacokinetics of Atrasentan. *J. Clin. Pharmacol.* **2007**, *47*, 423–429.

Table 2. Examples of Dual Effect of Rifampin on CYP and OATP Substrate Drugs

drugs	major metabolism/ transport pathways	study design	results summary	refs
repaglinide	CYP2C8, CYP3A, OATP1B1	rif (600 mg QD) for 7 days,		21, 22
		(a) repaglinide (4 mg) on day 7	(a) AUC ↓ 29% relative to baseline	
		(b) repaglinide (4 mg) on day 8	(b) AUC ↓ 46% relative to baseline	
atrasentan	glucoronidation, CYP3A, OATP1B1, OATP1B3	single doses of atrasentan administered on days 1 and 12; rifampin (600 mg) once daily on days 4−14; on day 12, atrasentan and rifampin were administered simultaneously	day 12 vs day 1: Cmax ↑ 150%, AUC no change	50
bosentan	CYP2C9, CYP3A, OARP1B1, OATP1B3	bosentan 125 mg bid for 6.5 days ± rifampin 600 mg once a day for 6 days	+rif, C_{trough} ↑ on day 2 (↑ 1.1−15-fold); +rif, 58% ↓ in AUCτ at steady state	15
atorvastatin (ATV)	CY3A, P-gp, OATP1B1	one oral dose (40 mg) ATV vs one oral dose ATV preceded by a 30 min iv infusion of rif (600 mg)	ATV-acid AUC and Cmax ↑ 7-fold and 12-fold, respectively	14
glyburide	CYP2C9, CYP3A, OATP1B1, OATP2B1	(a) on day 15: one oral dose (1.25 mg) of glyburide vs one oral dose of glyburide preceded by a 30 min iv infusion of rif (600 mg);	(a) glyburide AUC and Cmax ↑ 81% and 125% after single IV dose of RIF;	16
		(b) on days 22−27, 600 mg of oral rifampin once daily;	(b) glyburide AUC and Cmax ↓ 22% and 9% compared to control;	
		on day 28, 600 mg iv rifampin over 300 min followed by 1.25 mg of oral glyburide;		
		(c) on day 30: 1.25 mg of oral glyburide	(c) AUC and Cmax ↑ 120% and 90% (+rif on day 28 vs −rif on day 30)	

enzymatic and transporter-mediated processes for drugs that are substrates for both hepatic transporters and enzymes. For example, atorvastatin is a substrate for CYP3A, and transporters P-gp and OATP1B1. When atorvastatin was dosed on day 6 following 5 days of rifampin administration 600 mg, the area under the curve decreased by 80%, showing a strong induction effect (CYP3A and P-gp) from rifampin.[51] However, following single dose administration of rifampin, the exposure of atorvastatin increased 12-fold, showing an inhibition effect (OATP1B1).[14] The inhibition of OATP from rifampin can offset the induction effect of rifampin on CYP3A and P-gp following chronic coadministration of both atorvastatin and rifampin. Indeed, when atorvastatin and rifampin were coadministered for seven days, the net effect on atorvastatin AUC was an increase of 30% and on Cmax was an increase of 2.7-fold.[33] To distinguish the dual effect of rifampin from other inducers that are not inhibitors for transporters such as efavirenz, the LIPITOR (atorvastatin) labeling (Section 7.4) states that "Concomitant administration of LIPITOR with inducers of cytochrome P450 3A4 (e.g., efavirenz, rifampin) can lead to variable reductions in plasma concentrations of atorvastatin. Due to the dual interaction mechanism of rifampin, simultaneous coadministration of LIPITOR with rifampin is recommended, as delayed administration of LIPITOR after administration of rifampin has been associated with a significant reduction in atorvastatin plasma concentrations."[33]

The findings of a dual effect of rifampin have implications on the interpretation of the induction data for a new molecular entity when using rifampin as an inducer. If the new molecular entity is a CYP and OATP substrate, then single-dose rifampin can increase an NME's exposure via inhibition of OATP, while multiple doses of rifampin can decrease an NME's exposure via induction of CYP activities. If the extent of individual increase and decrease is similar, then steady-state exposure will be comparable to the exposure at baseline. Literature data involving rifampin as an inducer may need to be re-examined to determine whether rifampin's induction effect may be masked by coadministration with an OATP substrate. The net steady-state effect may vary depending on the relative size of the individual effect on transporter and enzyme activities.

(51) Backman, J. T.; Luurila, H.; Neuvonen, M.; Neuvonen, P. J. Rifampin Markedly Decreases and Gemfibrozil Increases the Plasma Concentrations of Atorvastatin and Its Metabolites. *Clin. Pharmacol. Ther.* **2005**, *78*, 154–167.

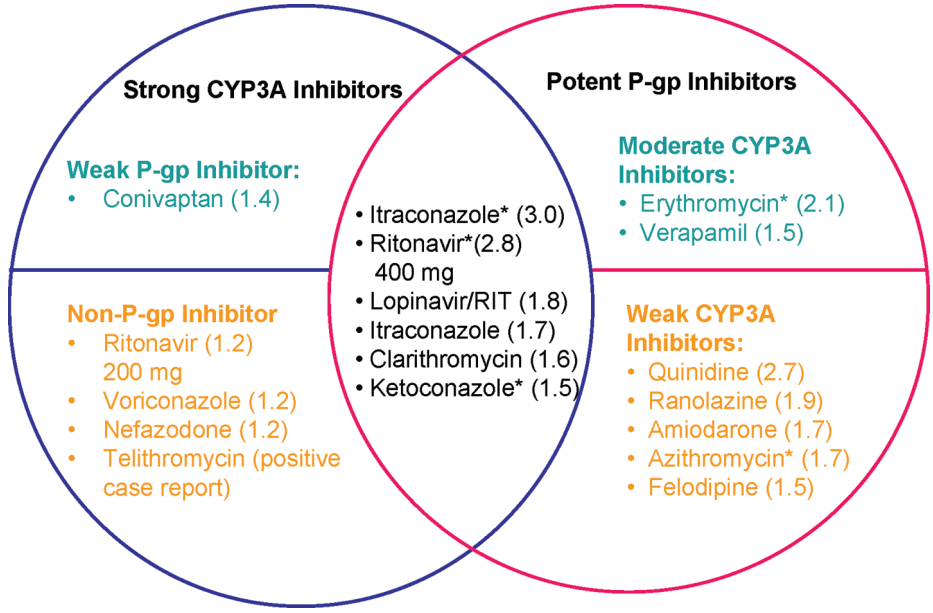

Figure 1. Examples of CYP3A and P-gp Inhibitors and their relative potency: the fold changes in the AUC of P-gp substrates (all data are from digoxin except where noted *, they are from fexofenadine) in the presence of the interacting drugs are listed in parentheses after the drug names. Strong, moderate, or weak CYP3A inhibitors are defined as those drugs that increase the AUC of oral midazolam or other CYP3A substrates ≥5-fold, 2−5-fold, and 1.25−2-fold. Potent, weak or non-P-gp inhibitors are defined as those drugs that increase the AUC of digoxin or fexofenadine by ≥1.5-fold, 1.25−1.5-fold, or <1.25-fold. University of Washington Drug Interaction Database was used to search the data that defined the *in vivo* potency of various inhibitors for CYP3A (midazolam was searched as a substrate) and P-gp (digoxin or fexofenadine was searched as a substrate).

How To Leverage Current Knowledge To Obtain Useful Drug Interaction Information

The current draft drug interaction guidance suggests that *in vitro* studies be carried out to determine whether a new molecular entity is a substrate, inhibitor or inducer for major CYP enzymes (e.g., CYP1A2, -2B6, -2C8, -2C9, -2C19, -2D6 and -3A) or P-gp.[7] In addition, it recommends the evaluation of an NME as a substrate or inhibitor for other transporters such as OATP on a case by case basis.

In situations where interplay between enzymes and transporters may be anticipated, e.g., NMEs are dual substrates of enzymes and transporters, or inhibitor and inducers for different enzymes and transporters, the following factors need to be taken into considerations for drug interaction study design (e.g., selection of interacting drugs and timing of co-administration of interacting drugs) and labeling.

Overlapping CYP3A and P-gp Inhibition. The FDA draft drug interaction guidance includes tables that classify CYP inhibitors into strong (≥5-fold increase in AUC of substrates for specific CYP enzymes), moderate (2- to 5-fold increase in AUC), or weak (1.25−2-fold increase in AUC) inhibitors. Substrates that are sensitive for a particular enzyme (≥5-fold increase in AUC in the presence of specific CYP inhibitors) or substrates with a narrow therapeutic range (NTR) are also defined. NTR drugs are drugs whose exposure−response indicates that small increases in their exposure levels may lead to serious safety concerns (e.g., Torsades de Pointes). The classification can be used for selecting appropriate inhibitors or substrates for drug interaction evaluation. In addition, this classification system can be used in the drug labeling. For example, if an NME is a "sensitive CYP3A substrate" or a "CYP3A substrate with a narrow therapeutic range", there may be a warning in the labeling regarding a potential drug interaction with a list of strong or moderate CYP3A inhibitors without specific studies with all individual inhibitors.[52,53]

In the draft guidance,[7] there are also separate tables for CYP3A and P-gp inhibitors to be selected for drug interaction studies for drugs that are mainly CYP3A or P-gp substrates. In the cases where a new molecular entity (NME) is a dual substrate of P-gp and CYP3A, specific attribution of an AUC change due to P-gp or CYP3A may not be possible, and the selection of inhibitors would need to take into consideration of the relative potency of inhibitors on CYP3A or P-gp.

It is noted that there is a considerable overlap between CYP3A and P-gp inhibitors; however, a strong CYP3A inhibitor does not necessary cause a large increase in exposure of a P-gp substrate, such as digoxin or fexofenadine. Figure 1 shows that some strong CYP3A inhibitors (causing AUC change of CYP3A substrates ≥5-fold) such as itra-

(52) CDER Drug Development and Drug Interactions Web site. http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm. 2009.

(53) Huang, S. M.; Temple, R.; Throckmorton, D. C.; Lesko, L. J. Drug Interaction Studies: Study Design, Data Analysis, and Implications for Dosing and Labeling. *Clin. Pharmacol. Ther.* **2007**, *81*, 298–304.

Table 3. CYP3A and P-gp Inhibitors with Unknown Inhibition Potency on P-gp or CYP3A

strong CYP3A inhibitors with unknown P-gp inhibition potency	potent P-gp inhibitors with unknown CYP3A inhibition potency
indinavir	carvedilol
troleandomycin	cyclosporine
nelfinavir	
saquinavir	

conazole, clarithromycin, ketoconazole, and lopinavir/ritonavir cause ≥1.5-fold change in AUC of either digoxin or fexofenadine (P-gp substrates) whereas other strong CYP3A inhibitors such as voriconazole do not. In addition, some P-gp inhibitors such as amiodarone and quinidine that showed a potent P-gp inhibition (causing ≥1.5-fold change in digoxin or fexofenadine AUC) are weak CYP3A inhibitors (causing AUC change of CYP3A substrates between 1.25- and 2-fold). Finally, the potency for some strong CYP3A inhibitors to inhibit P-gp is not known and vice versa for some potent P-gp inhibitors (Table 3). The differential inhibiting effects on CYP3A and P-gp need to be recognized when selecting appropriate inhibitors for studying interaction with an NME that is a CYP3A, P-gp or dual CYP3A and P-gp substrate. For a dual CYP3A and P-gp substrate, inhibition may be studied using an inhibitor that shows strong inhibition for both P-gp and CYP3A, such as itraconazole. The interaction may represent the worst case scenario where both CYP3A and P-gp processes are inhibited. If the goal of the study is to tease out the CYP3A or P-gp effect, then a strong inhibitor for CYP3A only and a potent inhibitor for P-gp only should be selected from those listed in Figure 1.

Overlapping CYP/P-gp Induction and OATP Inhibition. As stated earlier, rifampin's inhibition on OATP can decrease the impact of its CYP induction effect for drugs that are substrates for both CYP and OATP (e.g., repaglinide, atrasentan, bosentan, atorvastatin). Therefore, if active hepatic uptake of a NME is suspected (i.e., OATP may be involved in a drug's disposition), a CYP induction study with rifampin needs to be designed and interpreted carefully. Timing of co-administration may become critical in situations when both enzymes and transporters can be affected.

Other Enzymes and Transporters. Besides P-gp and OATP transporters discussed in this paper, other transporters such as breast cancer resistant protein (BCRP), organic anion transporters (OATs) and organic cation transporters (OCTs) have also been identified as playing an important role in determining a drug's pharmacokinetics and safety and efficacy.[54] Many drugs have been identified as substrates or inhibitors for transporters including P-gp, OATP, BCRP, OAT and OCT. The examples include colchicine, eltrombopag, lapatinab, topotecan, sitagliptin, metformin, and propranolol.[55] Potential interplay between enzymes and transporters needs to be considered for interaction study design, data interpretation, and labeling.

Impact on Labeling. The interplay between enzymes and transporters presents emerging challenges in designing of studies to evaluate drug interactions. *In vitro* data can be used in the labeling to state the interaction potential. *In vivo* interaction data may be extrapolated to other drugs in some cases. However as discussed earlier, in drugs that are substrates, inhibitors or inducers of cytochrome P450 (CYP) and/or phase 2 enzymes as well as substrates, inhibitors or inducers of transporters, the extrapolation of drug interaction results from one substrate drug to another can be difficult or impossible. Therefore, the drug labeling may describe the drug interaction potential with a statement that the extent of specific drug−drug interaction may vary and that definitive recommendations could not be provided for all interacting drugs. For example, variable changes in exposure were observed when dual CYP3A/P-gp substrates were coadministered with tipranavir/ritonavir (Table 1). When specific drug interaction data are available, the labeling describes clinical recommendations based on exposure−response of the affected drug. For example, tipranavir/ritonavir had a minimal effect on clarithromycin exposure (a 19% increase in AUC).[47] For dual CYP3A and P-gp substrates that did not have a clinical study, e.g., calcium channel blockers, the labeling states, "Cannot predict effect of TPV/ritonavir on calcium channel blockers that are dual substrates of CYP 3A and P-gp due to conflicting effect of TPV/ritonavir on CYP 3A and P-gp".[47]

Summary

Considerable progress has been made in the development of tools and techniques for studying enzyme- and transporter-based drug−drug interactions.[1,2,8,53,56−59] These advances make it possible to identify selective substrates and inhibitors for individual metabolizing enzymes and drug transporters and to evaluate potential drug−drug interactions *in vitro*. Enzymes and transporters work in concert in determining a drug's absorption, distribution, metabolism, and elimination.[5,10] As many drugs are likely substrates and/or inhibitors for both enzymes and transporters, the interplay between metabolizing

(54) The International Transporter Consortium. Membrane Transporters: ITC Report from the FDA Critical Path Workshop. Submitted to *Nat. Rev. Drug Discovery*.

(55) Huang S. M.; Zhang L.; Giacomini K. M. The International Transporter Consortium (ITC): A collaborative group of scientists from academia, industry and FDA. *Clin. Pharmacol. Ther.* (In Press).

(56) Mizuno, N.; Niwa, T.; Yotsumoto, Y.; Sugiyama, Y. Impact of Drug Transporter Studies on Drug Discovery and Development. *Pharmacol. Rev.* **2003**, *55*, 425–461.

(57) Obach, R. S.; Walsky, R. L.; Venkatakrishnan, K; Gaman, E. A.; Houston, J. B. Tremaine LM The Utility of in Vitro Cytochrome P450 Inhibition Data in the Prediction of Drug-Drug Interactions. *J. Pharmacol. Exp.Ther.* **2006**, *316*, 336–348.

(58) Shitara, Y.; Sato, H.; Sugiyama, Y. Evaluation of Drug-Drug Interaction in the Hepatobiliary and Renal Transport of Drugs. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 689–723.

(59) Tsuji, A. Transporter-Mediated Drug Interactions. *Drug Metab. Pharmacokinet.* **2002**, *17*, 253–274.

enzymes and transporters needs to be considered in assessing a drug's net effect on the exposure and effect of other drug or other drug's effect on itself.[60] New *in vitro* models are being developed when multiple transporters can be evaluated simultaneously in multiple-transfected cell lines.[61,62] Other new developments in the transporter area including data from pharmacogenetic evaluations can shed light on their role in drug disposition and drug−drug interactions. In addition, modeling and simulation tools can be useful in designing drug interaction studies and in interpreting complex drug interactions involving both enzymes and transporters.[63,64] Drug labeling will continue to improve and be informative as the mechanistic understanding of the drug−drug interactions continues to develop.

Abbreviations Used

NDA, new drug application; NME, new molecular entity; P-gp, P-glycoprotein; OATP, organic anion transporting polypeptide; BCRP, breast cancer resistance protein; OAT, organic anion transporter; OCT, organic cation transporter; AUC, area under the curve of plasma concentration−time profile; HIV, human immunodeficiency virus; TPV, tipranavir.

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(60) Wu, C. Y.; Benet, L. Z. Predicting Drug Disposition Via Application of BCS: Transport/Absorption/Elimination Interplay and Development of a Biopharmaceutics Drug Disposition Classification System. *Pharm. Res.* **2005**, *22*, 11–23.

(61) Nies, A. T.; Schwab, M. Keppler D Interplay of Conjugating Enzymes With OATP Uptake Transporters and ABCC/MRP Efflux Pumps in the Elimination of Drugs. *Expert. Opin. Drug Metab. Toxicol.* **2008**, *4*, 545–568.

(62) Shitara, Y; Horie, T; Sugiyama, Y. Transporters As a Determinant of Drug Clearance and Tissue Distribution. *Eur. J. Pharm. Sci.* **2006**, *27*, 425–446.

(63) Zhao, P.; Ragueneau-Majlessi, I.; Zhang, L.; Strong, J. M.; Reynolds, K. S.; Levy, R. H.; Thummel, K. E.; Huang, S. M. Quantitative Evaluation of Pharmacokinetic Inhibition of CYP3A Substrates by Ketoconazole: a Simulation Study. *J. Clin. Pharmacol.* **2009**, *49*, 351–359.

(64) Watanabe, T.; Kusuhara, H.; Maeda, K.; Shitara, Y.; Sugiyama, Y. Physiologically Based Pharmacokinetic Modeling to Predict Transporter-Mediated Clearance and Distribution of Pravastatin in Humans. *J. Pharmacol. Exp. Ther.* **2009**, *328*, 652–662.