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Transporters and Drug Discovery: Why, When, and How

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Abstract: Drug transporters are now increasingly recognized as important determinants of variable drug disposition and response. In addition, transporter associated problems appear to be occurring with greater frequency during the drug discovery and development process. What has not been clear is whether drug transporter related issues are a truly new problem, or whether such issues had existed all along, but were previously unrecognized or ignored. In this review, a brief overview of key drug transporters will be outlined. In addition, a commentary on specific issues of relevance to pharmaceutical sciences in terms of the role and relevance of drug transporters to the drug discovery and development process is provided.

Keywords: Drug transport; P-glycoprotein; pharmacokinetics; drug discovery

1. Drug Transporters: Background of Key Players

Drug transporters could be broadly classified as uptake or efflux transporters. Numerous detailed reviews relating to the expression and function of drug transporters are available.[1−3] While a large array of genes encoding transporters is known to exist in the human genome, in reality, it now appears that only a handful of uptake and efflux transporters are involved in the disposition of a majority of compounds in discovery/development or clinical use (Figure 1). Outlined below is a brief description of transporters most likely to affect the disposition of a compound in development.

A. Efflux Transporters. (i) P-Glycoprotein (P-gp, MDR1, ABCB1). Human P-glycoprotein was initially identified because of its overexpression in cultured tumor cells associated with an acquired cross-resistance to multiple cytotoxic anticancer agents. It, therefore, provided a mechanistic explanation for the multidrug resistance phenomenon, which is observed clinically when cancer patients are treated with cancer chemotherapeutic drugs for a prolonged period. However, after the initial characterization of P-gp, the transporter was also recognized to be expressed in many normal tissues, suggestive of a physiological function.[4] For example, P-gp is located in the apical domain of the enterocyte of the gastrointestinal tract (jejunum, duodenum, ileum, and colon), suggesting that the transporter functions to facilitate excretion of substrates from the systemic circulation into the gastrointestinal tract. Similarly, P-gp in the canalicular domain of the hepatocyte and the brush border of the proximal renal tubule is consistent with a role for the transporter in the biliary and urinary excretion of xenobiotics and endogenous substrates. The localization of the transporter in other tissues suggests an additional physiological function, namely, that of a transport barrier. Experiments with *mdr1a* knockout mice revealed that, in addition to limiting the CNS entry of drugs (i.e., cyclosporin A, digoxin, vinblastine), P-gp also reduced oral absorption of drugs (i.e., paclitaxel) by

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(1) Ho, R. H.; Kim, R. B. Transporters and Drug Therapy: Implications for Drug Disposition and Disease. *Clin. Pharmacol. Ther.* **2005**, *78*, 260−277.

(2) Lee, W.; Kim, R. B. Transporters and Renal Drug Elimination. *Annu. Rev. Pharmacol. Toxicol.* **2004**, *44*, 137−166.

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

(4) Thiebaut, F.; Tsuruo, T.; Hamada, H.; Gottesman, M. M.; Pastan, I.; Willingham, M. C. Cellular Localization of the Multidrug-Resistance Gene Product P-Glycoprotein in Normal Human Tissues. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7735−7738.

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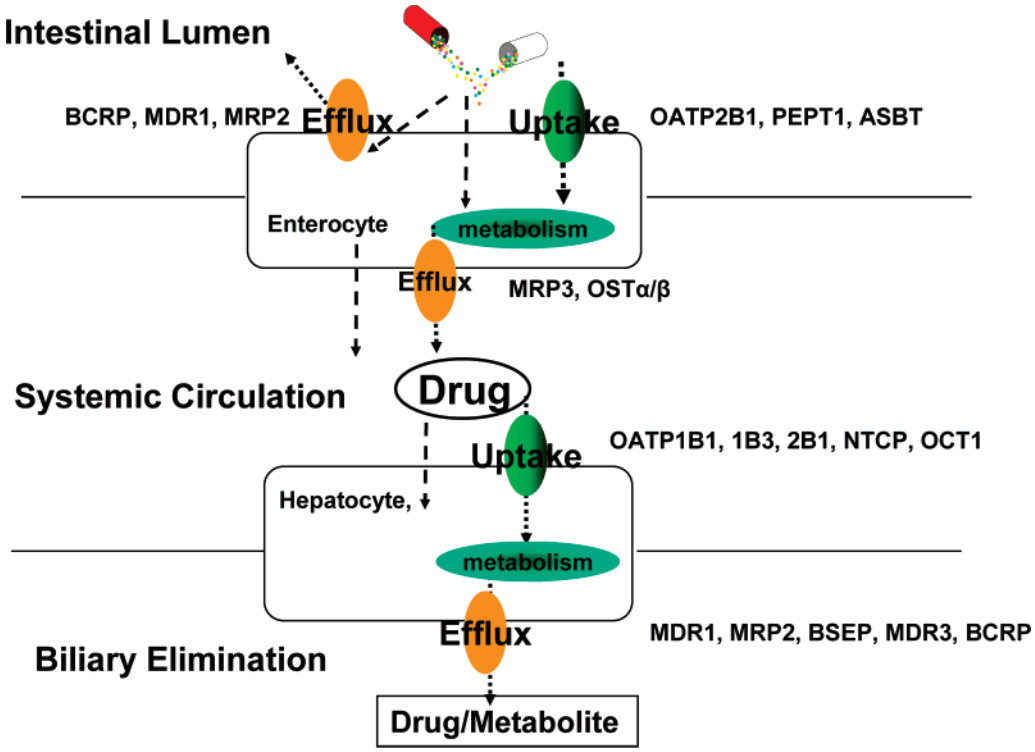

Figure 1. Schematic of key uptake and efflux transporters expressed in the intestine and liver. For many drugs an interplay between passive diffusion relative to transporter-mediated active uptake and efflux determines the extent of absorption and elimination after an oral dose. Note that in organs such as the intestine, both the uptake and efflux transporter(s) may be expressed on the same cell membrane domain, while in organs such as the liver and kidney, expression of uptake and efflux tends to be more specific to either the basolateral or the apical domain, thereby enhancing the directional movement of shared substrate drugs for excretion into bile or urine.

extruding them from enterocytes back into the intestinal lumen.[5]

(ii) MRP2 (ABCC2). The ability of the liver to secrete amphipathic anionic compounds into bile led to the identification of a carrier-mediated transporter at the canalicular membrane. Originally termed cMOAT (canalicular multiple organic anion transporter), this was found to be an ABC-transporter of the MDR1-related protein subfamily, now termed MRP2.[6] In humans, the transporter is a 1545 amino acid protein whose gene is located in chromosomal region 10q23−24.[7] This transporter has been shown to be selective for drug conjugates, especially those conjugated with GSH, as well as endogenous compounds such as hormone and bilirubin conjugates. Recent studies indicate that MRP2 expression, while highest in the liver, is not exclusive to this organ. In fact, the kidney,[8] as well as the intestinal entrocytes[8,9] has been shown to express this efflux transporter. In humans, a genetic deficiency of MRP2 results in a disease known as Dubin−Johnson syndrome.[10]

(iii) SPGP (BSEP, ABCB11). Sister of P-glycoprotein (Spgp) was originally cloned from pig liver as a closely related member of MDR1/P-gp.[11] Later, rat Spgp was isolated and shown to be localized on the canalicular membrane of hepatocytes[12] and was determined to be the hepatic bile salt export pump (bsep).[12] Shortly thereafter, the human *SPGP*

(5) Marzolini, C.; Paus, E.; Buclin, T.; Kim, R. B. Polymorphisms in Human MDR1 (P-Glycoprotein): Recent Advances and Clinical Relevance. *Clin. Pharmacol. Ther.* **2004**, *75*, 13−33.
(6) Suzuki, H.; Sugiyama, Y. Excretion of GSSG and Glutathione Conjugates Mediated by MRP1 and CMOAT/MRP2. *Semin. Liver Dis.* **1998**, *18*, 359−376.
(7) Tirona, R. G.; Kim, R. B. Pharmacogenomics of Drug Transporters. In *Pharmacogenomics: The Search of Individualized Therapeutics*; Licinio, J., Wong, M. L., Eds.; Wiley-VCH: New York, 2002.
(8) Sandusky, G. E.; Mintze, K. S.; Pratt, S. E.; Dantzig, A. H. Expression of Multidrug Resistance-Associated Protein 2 (MRP2) in Normal Human Tissues and Carcinomas Using Tissue Microarrays. *Histopathology* **2002**, *41*, 65−74.
(9) Hirohashi, T.; Suzuki, H.; Chu, X. Y.; Tamai, I.; Tsuji, A.; Sugiyama, Y. Function and Expression of Multidrug Resistance-Associated Protein Family in Human Colon Adenocarcinoma Cells (Caco-2). *J. Pharmacol. Exp. Ther.* **2000**, *292*, 265−270.
(10) Paulusma, C. C.; Kool, M.; Bosma, P. J.; Scheffer, G. L.; ter Borg, F.; Scheper, R. J.; Tytgat, G. N.; Borst, P.; Baas, F.; Oude Elferink, R. P. A Mutation in the Human Canalicular Multispecific Organic Anion Transporter Gene Causes the Dubin-Johnson Syndrome. *Hepatology* **1997**, *25*, 1539−1542.
(11) Childs, S.; Yeh, R. L.; Georges, E.; Ling, V. Identification of a Sister Gene to P-Glycoprotein. *Cancer Res.* **1995**, *55*, 2029−2034.
(12) Childs, S.; Yeh, R. L.; Hui, D.; Ling, V. Taxol Resistance Mediated by Transfection of the Liver-Specific Sister Gene of P-Glycoprotein. *Cancer Res.* **1998**, *58*, 4160−4167.

gene was isolated, and mutations in the transporter were found to cause progressive familial intrahepatic cholestasis type 2 (PFIC2).[13] Other than bile acids, little is known about drug substrates for SPGP, although some data indicates that vinblastine[14] and the nonsteroidal antiinflammatory drug sulindac,[15] as well as the HMG-CoA reductase inhibitor pravastatin, may be substrates of the transporter.[16] Inhibition of BSEP has been implicated as a potential mechanism behind certain drug induced hepatotoxicity.[1]

(iv) BCRP (ABCG2). Breast cancer resistance protein (BCRP, also known as MXR or ABCP), first cloned from mitoxantrone and anthracycline-resistant breast and colon cancer cells,[17,18] is a half-transporter efflux pump believed to function as a homo- or heterodimer. In normal tissues, BCRP was detected in placental syncytiotrophoblasts, hepatocyte canalicular membrane, apical intestinal epithelia, and vascular endothelial cells.[19] These findings support the important role BCRP plays in modulating topotecan bioavailability, fetal exposure, and hepatic elimination.[20] There appears to be some overlap in the substrate and tissue distributions between BCRP and P-gp.[21]

B. Uptake Transporters. (i) Organic Anion Transporting Polypeptide (OATP) Family. Work carried out in a number of laboratories, including ours, has demonstrated a family of uptake transporters known as organic anion transporting polypeptides (OATP, gene symbol *SLCO*) expressed in organs such as the intestine, liver, and blood−brain barrier to be the key determinant in the cellular uptake of many endogenous and exogenous chemicals, including drugs in clinical use. The first human OATP, OATP1A2 (previously named OATP-A), was isolated from human liver.[22] Despite that, OATP1A2 has been reported to be expressed in various tissues such as liver, brain, lung, kidney, and testes by Northern blot analysis. Indeed OATP1A2 immunodetectable protein can be found in brain capillary endothelial cells,[23] thus indicating a role for this transporter in regulating blood−brain-barrier permeability of solutes. What is certain, however, is that OATP1A2 is truly multispecific and is capable of transporting diverse compounds including bromosulfophthalein (BSP), bile acids, steroid sulfates,[24] bulky organic cations,[25,26] fexofenadine, thyroid hormones, and opioid peptides.[23] However, in human liver, the best known member of the OATP family is OATP1B1**,** also referred to as OATP-C, SLC21A6, liver specific transporter 1 (LST-1), or OATP2. Immunohistochemical analysis proved OATP1B1 expression to be locoalized to the basolateral membrane of hepatocytes.[27] OATP1B1 trans-

(13) Strautnieks, S. S.; Bull, L. N.; Knisely, A. S.; Kocoshis, S. A.; Dahl, N.; Arnell, H.; Sokal, E.; Dahan, K.; Childs, S.; Ling, V.; Tanner, M. S.; Kagalwalla, A. F.; Nemeth, A.; Pawlowska, J.; Baker, A.; Mieli-Vergani, G.; Freimer, N. B.; Gardiner, R. M.; Thompson, R. J. A Gene Encoding a Liver-Specific ABC Transporter Is Mutated in Progressive Familial Intrahepatic Cholestasis. *Nat. Genet.* **1998**, *20*, 233−238.

(14) Lecureur, V.; Sun, D.; Hargrove, P.; Schuetz, E. G.; Kim, R. B.; Lan, L. B.; Schuetz, J. D. Cloning and Expression of Murine Sister of P-Glycoprotein Reveals a More Discriminating Transporter Than MDR1/P-Glycoprotein. *Mol. Pharmacol.* **2000**, *57*, 24−35.

(15) Bolder, U.; Trang, N. V.; Hagey, L. R.; Schteingart, C. D.; Ton-Nu, H. T.; Cerre, C.; Elferink, R. P.; Hofmann, A. F. Sulindac Is Excreted into Bile by a Canalicular Bile Salt Pump and Undergoes a Cholehepatic Circulation in Rats. *Gastroenterology* **1999**, *117*, 962−971.

(16) Hirano, M.; Maeda, K.; Hayashi, H.; Kusuhara, H.; Sugiyama, Y. Bile Salt Export Pump (BSEP/ABCB11) Can Transport a Nonbile Acid Substrate, Pravastatin. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 876−882.

(17) Doyle, L. A.; Yang, W.; Abruzzo, L. V.; Krogmann, T.; Gao, Y.; Rishi, A. K.; Ross, D. D. A Multidrug Resistance Transporter From Human MCF-7 Breast Cancer Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15665−15670.

(18) Miyake, K.; Mickley, L.; Litman, T.; Zhan, Z.; Robey, R.; Cristensen, B.; Brangi, M.; Greenberger, L.; Dean, M.; Fojo, T.; Bates, S. E. Molecular Cloning of CDNAs Which Are Highly Overexpressed in Mitoxantrone-Resistant Cells: Demonstration of Homology to ABC Transport Genes. *Cancer Res.* **1999**, *59*, 8−13.

(19) Maliepaard, M.; Scheffer, G. L.; Faneyte, I. F.; van Gastelen, M. A.; Pijnenborg, A. C.; Schinkel, A. H.; van De Vijver, M. J.; Scheper, R. J.; Schellens, J. H. Subcellular Localization and Distribution of the Breast Cancer Resistance Protein Transporter in Normal Human Tissues. *Cancer Res.* **2001**, *61*, 3458−3464.

(20) Jonker, J. W.; Smit, J. W.; Brinkhuis, R. F.; Maliepaard, M.; Beijnen, J. H.; Schellens, J. H.; Schinkel, A. H. Role of Breast Cancer Resistance Protein in the Bioavailability and Fetal Penetration of Topotecan. *J. Natl. Cancer Inst.* **2000**, *92*, 1651−1656.

(21) Litman, T.; Brangi, M.; Hudson, E.; Fetsch, P.; Abati, A.; Ross, D. D.; Miyake, K.; Resau, J. H.; Bates, S. E. The Multidrug-Resistant Phenotype Associated With Overexpression of the New ABC Half-Transporter, MXR (ABCG2). *J. Cell Sci.* **2000**, *113*, 2011−2021.

(22) Kullak-Ublick, G.-A.; Hagenbuch, B.; Stieger, B.; Schteingart, C.; Hoffmann, A. F.; Wolkoff, A. W.; Meier, P. J. Molecular and Functional Characterization of an Organic Anion Transporting Polypeptide Cloned From Human Liver. *Gastroenterology* **1995**, *109*, 1274−1284.

(23) Lee, W.; Glaeser, H.; Smith, L. H.; Roberts, R. L.; Moeckel, G. W.; Gervasini, G.; Leake, B. F.; Kim, R. B. Polymorphisms in Human Organic Anion-Transporting Polypeptide 1A2 (OATP1A2): Implications for Altered Drug Disposition and Central Nervous System Drug Entry. *J. Biol. Chem.* **2005**, *280*, 9610−9617.

(24) Kullak-Ublick, G. A.; Fisch, T.; Oswald, M.; Hagenbuch, B.; Meier, P. J.; Beuers, U.; Paumgartner, G. Dehydroepiandrosterone Sulfate (DHEAS): Identification of a Carrier Protein in Human Liver and Brain. *FEBS Lett.* **1998**, *424*, 173−176.

(25) van Montfoort, J. E.; Hagenbuch, B.; Fattinger, K. E.; Muller, M.; Groothuis, G. M.; Meijer, D. K.; Meier, P. J. Polyspecific Organic Anion Transporting Polypeptides Mediate Hepatic Uptake of Amphipathic Type II Organic Cations. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 147−152.

(26) van Montfoort, J. E.; Muller, M.; Groothuis, G. M.; Meijer, D. K.; Koepsell, H.; Meier, P. J. Comparison of "Type I" and "Type II" Organic Cation Transport by Organic Cation Transporters and Organic Anion-Transporting Polypeptides. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 110−115.

(27) Konig, J.; Cui, Y.; Nies, A. T.; Keppler, D. A Novel Human Organic Anion Transporting Polypeptide Localized to the Basolateral Hepatocyte Membrane. *Am. J. Physiol.* **2000**, *278*, G156−G164.

ports a broad range of compounds such as bile acids,[28] sulfate and glucuronide conjugates,[27] thyroid hormones,[28] peptides,[29] and drugs such as methotrexate[30] and pravastatin.[31,32] Other key OATPs include OATP1B3 (OATP8), which is similar to OATP1B1 with respect to amino acid composition (80% amino acid identity) and liver-specific tissue distribution.[33] In addition, OATP1B3 exhibits substrate overlap with OATP1B1 for compounds such as BSP, steroid sulfates and glucuronides, thyroid hormone, bile acids, peptide compounds and methotrexate,[29,30] albeit with some differences in affinity. Another OATP member with the potential to alter drug disposition in vivo is OATP2B1, initially cloned from human brain. OATP2B1 mRNA has been detected in a number of tissues including liver, lung, kidney, placenta, heart, and small intestine.[34] Within the liver, OATP2B1 protein is localized to the basolateral membrane of hepatocytes,[29] indicating a blood to liver uptake role for this transporter. Substrate specificity of OATP2B1 appears to be more limited when compared to OATP1B1 or 1B3. However, given its expression in a number of other tissues/organs, it is possible that OATP2B1 may be an important mediator not just of intestinal or hepatic uptake of a compound but also in facilitating tissue distribution.

(ii) Organic Anion Transporter Family (OAT). The classical basolateral organic anion transporter in kidney was first cloned from the rat (rOAT1),[35,36] leading to the subsequent identification of a human orthologue by several groups.[37] OAT1 (SLC22A6), expressed mainly in the proximal tubule, mediates the uptake of the prototypical organic anion, *p*-aminohippurate (PAH),[37−39] from blood through exchange with intracellular dicarboxylates.[38] Other OATs include OAT3 (SLC22A8)[40] with expression on the basolateral membrane of the proximal tubule and capable of transporting PAH, methotrexate, cimetidine, and estrone sulfate.[40] Within the OAT family, OAT4 (SLC22A11) is the only transporter expressed at appreciable levels in both the placenta and the kidney.[41] In the kidney, expression of OAT4 is thought to be apical and, thus, may be responsible for solute reabsorption. Steroid sulfates and ochratoxin A are efficient transport substrates of OAT4, whereas PAH is weakly transported.[41]

(iii) Organic Cation Transporter Family (OCT). Human OCT1 (SLC22A1) appears to be expressed predominantly in liver.[42,43] It is likely that OCT1 is localized to the basolateral membrane of human hepatocytes similar to the

(28) Abe, T.; kakyo, M.; Tokui, T.; Nakagomi, R.; Nishio, T.; Nakai, D.; Nomura, H.; Unno, M.; Suzuki, M.; Naitoh, T.; Matsuno, S.; Yawo, H. Identification of a Novel Gene Family Encoding Human Liver-Specific Organic Anion Transporter LST-1. *J. Biol. Chem.* **1999**, *274*, 17159−17163.

(29) Kullak-Ublick, G. A.; Ismair, M. G.; Stieger, B.; Landmann, L.; Huber, R.; Pizzagalli, F.; Fattinger, K.; Meier, P. J.; Hagenbuch, B. Organic Anion-Transporting Polypeptide B (OATP-B) and Its Functional Comparison With Three Other OATPs of Human Liver. *Gastroenterology* **2001**, *120*, 525−533.

(30) Abe, T.; Unno, M.; Onogawa, T.; Tokui, T.; Kondo, T. N.; Nakagomi, R.; Adachi, H.; Fujiwara, K.; Okabe, M.; Suzuki, T.; Nunoki, K.; Sato, E.; kakyo, M.; Nishio, T.; Sugita, J.; Asano, N.; Tanemoto, M.; Seki, M.; Date, F.; Ono, K.; Kondo, Y.; Shiiba, K.; Suzuki, M.; Ohtani, H.; Shimosegawa, T.; Iinuma, K.; Nagura, H.; Ito, S.; Matsuno, S. Lst-2, a Human Liver-Specific Organic Anion Transporter, Determines Methotrexate Sensitivity in Gastrointestinal Cancers. *Gastroenterology* **2001**, *120*, 1689−1699.

(31) Hsiang, B.; Zhu, Y.; Wang, Z.; Wu, Y.; Sasseville, V.; Yang, W. P.; Kirchgessner, T. G. A Novel Human Hepatic Organic Anion Transporting Polypeptide (OATP2). Identification of a Liver-Specific Human Organic Anion Transporting Polypeptide and Identification of Rat and Human Hydroxymethylglutaryl-CoA Reductase Inhibitor Transporters. *J. Biol. Chem.* **1999**, *274*, 37161−37168.

(32) Nakai, D.; Nakagomi, R.; Furuta, Y.; Tokui, T.; Abe, T.; Ikeda, T.; Nishimura, K. Human Liver-Specific Organic Anion Transporter, LST-1, Mediates Uptake of Pravastatin by Human Hepatocytes. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 861−867.

(33) Konig, J.; Cui, Y.; Nies, A. T.; Keppler, D. Localization and Genomic Organization of a New Hepatocellular Organic Anion Transporting Polypeptide. *J. Biol. Chem.* **2000**, *275*, 23161−23168.

(34) Tamai, I.; Nezu, J.; Uchino, H.; Sai, Y.; Oku, A.; Shimane, M.; Tsuji, A. Molecular Identification and Characterization of Novel Members of the Human Organic Anion Transporter (OATP) Family. *Biochem. Biophys. Res. Commun.* **2000**, *273*, 251−260.

(35) Sekine, T.; Watanabe, N.; Hosoyamada, M.; Kanai, Y.; Endou, H. Expression Cloning and Characterization of a Novel Multispecific Organic Anion Transporter. *J. Biol. Chem.* **1997**, *272*, 18526−18529.

(36) Sweet, D. H.; Wolff, N. A.; Pritchard, J. B. Expression Cloning and Characterization of ROAT1. The Basolateral Organic Anion Transporter in Rat Kidney. *J. Biol. Chem.* **1997**, *272*, 30088−30095.

(37) Hosoyamada, M.; Sekine, T.; Kanai, Y.; Endou, H. Molecular Cloning and Functional Expression of a Multispecific Organic Anion Transporter From Human Kidney. *Am. J. Physiol.* **1999**, *276*, F122−F128.

(38) Lu, R.; Chan, B. S.; Schuster, V. L. Cloning of the Human Kidney PAH Transporter: Narrow Substrate Specificity and Regulation by Protein Kinase C. *Am. J. Physiol*. **1999**, *276*, F295−F303.

(39) Race, J. E.; Grassl, S. M.; Williams, W. J.; Holtzman, E. J. Molecular Cloning and Characterization of Two Novel Human Renal Organic Anion Transporters (HOAT1 and HOAT3). *Biochem. Biophys. Res. Commun.* **1999**, *255*, 508−514.

(40) Cha, S. H.; Sekine, T.; Fukushima, J. I.; Kanai, Y.; Kobayashi, Y.; Goya, T.; Endou, H. Identification and Characterization of Human Organic Anion Transporter 3 Expressing Predominantly in the Kidney. *Mol. Pharmacol.* **2001**, *59*, 1277−1286.

(41) Cha, S. H.; Sekine, T.; Kusuhara, H.; Yu, E.; Kim, J. Y.; Kim, D. K.; Sugiyama, Y.; Kanai, Y.; Endou, H. Molecular Cloning and Characterization of Multispecific Organic Anion Transporter 4 Expressed in the Placenta. *J. Biol. Chem.* **2000**, *275*, 4507−4512.

(42) Zhang, L.; Dresser, M. J.; Gray, A. T.; Yost, S. C.; Terashita, S.; Giacomini, K. M. Cloning and Functional Expression of a Human Liver Organic Cation Transporter. *Mol. Pharmacol.* **1997**, *51*, 913−921.

(43) Gorboulev, V.; Ulzheimer, J. C.; Akhoundova, A.; Ulzheimer-Teuber, I.; Karbach, U.; Quester, S.; Baumann, C.; Lang, F.; Busch, A. E.; Koepsell, H. Cloning and Characterization of Two Human Polyspecific Organic Cation Transporters. *DNA Cell Biol.* **1997**, *16*, 871−881.

rat orthologue.[44] OCT1 has been shown capable of mediating the uptake of small protonated molecules such as 1-methyl 1,4-phenylpyridinium (MPP^+),[42] tetraethylammonium (TEA),[43] and *N*-1-methylnicotinamide (NMN)[43] as well as larger, bulkier (type II) cations including *N*-methyl-quinine, *N*-methyl-quinidine, and quinidine (at pH 6).[26] OCT2 (SCL22A2) mRNA has been detected in kidney and brain, and immunohistochemical methods indicated that OCT2 is localized to the apical membrane of the distal tubule.[43] The importance of OCT2 to the renal elimination of organic cations and drugs in humans remains to be clarified. In the brain, OCT2 was noted to be expressed in neurons when assessed using in situ hybridization and immunohistochemical methods.[45] Tissue-dependent expression of OCT2 may be clinically relevant since OCT transports monoamine neurotransmitters and the antiparkinsonian drug amantadine.[45] Similarly, OCT3 (SLC22A3) has been shown capable of transporting the neurotransmitters adrenaline, noradrenaline, and the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+).[46] OCT3 is thought to be highly expressed in placenta, intestine, and heart, with lower levels observed in kidney and brain.[47−49]

2. Drug Transporters: Transgenic Animal Models

The generation of mice with targeted disruption of *mdr1a/1b* has greatly enhanced our understanding of the in vivo relevance of P-gp to drug disposition. Interestingly, comparative studies in *mdr1a* (−/−) and *mdr1a/1b* (−/−) mice have shown that *mdr1a* is the major drug-transporting P-gp and the only form expressed in the brain capillaries, intestinal tract, and placenta, while *mdr1b* is expressed only in the liver and the kidney. Therefore the two P-gp isoforms in mice appear to fulfill the same function as the single *MDR1* encoded P-gp in humans.[50] There still remains some controversy with regard to potential species related differences in substrate specificity or affinity for compounds transported by P-gp. Nevertheless, these mice are now routinely and widely utilized to assess the potential in vivo relevance of P-gp to the absorption, distribution, and elimination of substrate compounds. Overall, P-gp deficient mice models have greatly enhanced our understanding of the clinical relevance of this transporter, particularly for limiting the CNS entry of substrate drugs.

In addition, naturally occurring mutations in Mrp2 in rats have allowed for selective breeding of rats homozygous for such mutations. Two strains, TR- and EHBR, are widely used for assessing the role of Mrp2 to drug disposition. These rats suffer from conjugated hyperbilirubinemia. Not surprisingly, rats lacking Mrp2 have shown a particular propensity for reduced capacity for efflux transport of drug conjugates.[51] In terms of other transporters, gene knockout mice have been created for Bcrp,[52] Mrp1,[53] Mrp3,[54] Mrp4,[55] Bsep,[56] Oct1,[57] and Oat3.[58] However, they have yet to be broadly utilized during drug discovery or development.

(44) Meyer-Wentrup, F.; Karbach, U.; Gorboulev, V.; Arndt, P.; Koepsell, H. Membrane Localization of the Electrogenic Cation Transporter ROCT1 in Rat Liver. *Biochem. Biophys. Res. Commun.* **1998**, *248*, 673−678.

(45) Busch, A. E.; Karbach, U.; Miska, D.; Gorboulev, V.; Akhoundova, A.; Volk, C.; Arndt, P.; Ulzheimer, J. C.; Sonders, M. S.; Baumann, C.; Waldegger, S.; Lang, F.; Koepsell, H. Human Neurons Express the Polyspecific Cation Transporter HOCT2, Which Translocates Monoamine Neurotransmitters, Amantadine, and Memantine. *Mol. Pharmacol.* **1998**, *54*, 342−352.

(46) Grundemann, D.; Schechinger, B.; Rappold, G. A.; Schomig, E. Molecular Identification of the Corticosterone-Sensitive Extraneuronal Catecholamine Transporter. *Nat. Neurosci.* **1998**, *1*, 349−351.

(47) Verhaagh, S.; Schweifer, N.; Barlow, D. P.; Zwart, R. Cloning of the Mouse and Human Solute Carrier 22a3 (Slc22a3/SLC22A3) Identifies a Conserved Cluster of Three Organic Cation Transporters on Mouse Chromosome 17 and Human 6q26-Q27. *Genomics* **1999**, *55*, 209−218.

(48) Wu, X.; Huang, W.; Ganapathy, M. E.; Wang, H.; Kekuda, R.; Conway, S. J.; Leibach, F. H.; Ganapathy, V. Structure, Function, and Regional Distribution of the Organic Cation Transporter OCT3 in the Kidney. *Am. J. Physiol.* **2000**, *279*, F449−F458.

(49) Kekuda, R.; Prasad, P. D.; Wu, X.; Wang, H.; Fei, Y. J.; Leibach, F. H.; Ganapathy, V. Cloning and Functional Characterization of a Potential-Sensitive, Polyspecific Organic Cation Transporter (OCT3) Most Abundantly Expressed in Placenta. *J. Biol. Chem.* **1998**, *273*, 15971−15979.

(50) Schinkel, A. H.; Mayer, U.; Wagenaar, E.; Mol, C. A.; van Deemter, L.; Smit, J. J.; van der Valk, M. A.; Voordouw, A. C.; Spits, H.; van Tellingen, O.; Zijlmans, J. M.; Fibbe, W. E.; Borst, P. Normal Viability and Altered Pharmacokinetics in Mice Lacking Mdr1-Type (Drug-Transporting) P-Glycoproteins. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4028−4033.

(51) Suzuki, H.; Sugiyama, Y. Single Nucleotide Polymorphisms in Multidrug Resistance Associated Protein 2 (MRP2/ABCC2): Its Impact on Drug Disposition. *Adv. Drug Delivery Rev.* **2002**, *54*, 1311−1331.

(52) Jonker, J. W.; Buitelaar, M.; Wagenaar, E.; van der Valk, M. A.; Scheffer, G. L.; Scheper, R. J.; Plosch, T.; Kuipers, F.; Elferink, R. P.; Rosing, H.; Beijnen, J. H.; Schinkel, A. H. The Breast Cancer Resistance Protein Protects Against a Major Chlorophyll-Derived Dietary Phototoxin and Protoporphyria. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15649−15654.

(53) Wijnholds, J.; Scheffer, G. L.; van, d., V.; van, d., V.; Beijnen, J. H.; Scheper, R. J.; Borst, P. Multidrug Resistance Protein 1 Protects the Oropharyngeal Mucosal Layer and the Testicular Tubules Against Drug-Induced Damage. *J. Exp. Med.* **1998**, *188*, 797−808.

(54) Belinsky, M. G.; Dawson, P. A.; Shchaveleva, I.; Bain, L. J.; Wang, R.; Ling, V.; Chen, Z. S.; Grinberg, A.; Westphal, H.; Klein-Szanto, A.; Lerro, A.; Kruh, G. D. Analysis of the in Vivo Functions of Mrp3. *Mol. Pharmacol.* **2005**, *68*, 160−168.

(55) Assem, M.; Schuetz, E. G.; Leggas, M.; Sun, D.; Yasuda, K.; Reid, G.; Zelcer, N.; Adachi, M.; Strom, S.; Evans, R. M.; Moore, D. D.; Borst, P.; Schuetz, J. D. Interactions Between Hepatic Mrp4 and Sult2a As Revealed by the Constitutive Androstane Receptor and Mrp4 Knockout Mice. *J. Biol. Chem.* **2004**, *279*, 22250−22257.

(56) Wang, R.; Salem, M.; Yousef, I. M.; Tuchweber, B.; Lam, P.; Childs, S. J.; Helgason, C. D.; Ackerley, C.; Phillips, M. J.; Ling, V. Targeted Inactivation of Sister of P-Glycoprotein Gene (Spgp) in Mice Results in Nonprogressive but Persistent Intrahepatic Cholestasis. *Proc. Natl. Acad. Sci. U.S.A* **2001**, *98*, 2011−2016.

3. Drug Transporters and Drug Discovery

Are They a Problem? It is difficult to know to what extent problems facing drug discovery relate to issues arising from drug transporters. It is clear that a majority of the compounds in development ultimately fail to reach clinical use, often related to suboptimal pharmacokinetic or safety profiles. Not surprisingly, companies spend a significant portion of their research portfolio trying to assess or predict the in vivo disposition profile of a candidate drug prior to first dose in human. Systematic and standardized assays available to assess the metabolic stability of a compound have allowed pharmaceutical scientists to avoid certain metabolic pathways or chemical series. Indeed, in many cases, targeted structural changes that enhance metabolic stability of a compound often result in more favorable pharmacokinetic profiles. However, it is also becoming evident that structural choices that confer metabolic stability can also result in the sometimes unpredictable involvement of drug transporters. In some cases, in vivo clearance of drugs devoid of metabolic biotransformation can turn out to be greater than that of related compounds which undergo significant metabolism. So in essence, it now seems that avoidance of metabolism can lead to the sometimes unexpected involvement of drug transporters.

Why Should We Care? A better understanding of drug transporters earlier in the drug discovery/development process may lead to better insights governing variable oral bioavailability or efficacy. For example, variation in transporter expression and function may be the reason for variable pharmacokinetics in vivo. Also, since we now know that there may be significant species-dependent differences in the expression or activity of many drug uptake or efflux transporters, a drug that shows poor oral bioavailability in rodents may turn out to have far greater bioavailability in humans and vice versa.

Second, it is now also clear that tissue distribution or organ-specific entry of drugs is often facilitated or hindered by the expression of drug uptake or efflux transporters. Again, understanding the extent and relevance of transporter involvement earlier in the discovery process may either aid in using a transporter to target an organ or systematically introduce structural changes to compounds that allow for the avoidance of transporters that limit the entry to the desired site of action.

The third, and perhaps the most relevant, point relates to unexpected toxicities or drug−drug interactions. As the key transporter-dependent pathways become better recognized earlier in the discovery process, it may also be possible to carry out a risk−benefit assessment in terms of liabilities associated with a certain transporter(s) in terms of drug−drug interactions and organ toxicities. Such an analysis may turn out to be cost efficient to the overall development program, since unintended toxicities may not surface until late in the development process or even after the drug is approved for general use.

When Should We Test for Transporters? The exact point in the drug discovery process that may warrant transporter assessment is dependent on the indication, target, and known properties of structurally related drugs already on the market. For example, if another HMG-CoA reductase inhibitor is being developed, it would make sense to carefully assess the impact of liver-enriched drug uptake transporters such as OATP1B1, since other statins in clinical use have been shown to utilize this transporter to gain high liver/plasma ratios. Since the target is that of hepatic HMG-CoA reductase, it makes sense to take advantage of such transporters. For drugs that target the CNS, issues relating to CNS drug transporters should be clarified early in the discovery process, as the expressed activity of P-gp, BCRP, and other efflux transporters may lead to lower than predicted CNS levels or efficacy of the candidate compound ultimately selected for further development.

How and Who Should Assess Transporters? In contrast to the field of drug metabolism, where a number of validated and broadly utilized in vitro assays exist for systematically determining the metabolic fate of a compound, such is not the case for drug transporters. Numerous expression systems ranging from *Xenopus laevis* oocytes, single and double transfected cell lines, and membrane vesicles, to various animal models exist. Thus in addition to the multiplicity of model systems that could be applied to the drug discovery process, perhaps a greater issue facing the pharmaceutical industry is the sometime obvious failure to recognize the value and need for in-house scientific expertise in relation to transporter biology. While there is no doubt that more and more useful transporter model systems will become available, lack of scientists with the expertise to systematically evaluate a given model system for suitability for discovery support or development may lead to inappropriate application of transporter technologies, or worse yet, misinterpretation of the derived data. It is clear that long-term support for the development of local expertise will be essential.

In conclusion, drug transporters are becoming a real issue for the drug discovery and development process. While a variety of uptake and efflux transporters are expressed in humans, only a handful appear to be broadly relevant to the disposition of structurally divergent compounds being developed by the pharmaceutical industry. A systematic application of transporter technologies has the potential to identify drug candidates with better safety, as well as in vivo disposition profiles. While the field of drug transport

(57) Jonker, J. W.; Wagenaar, E.; Mol, C. A.; Buitelaar, M.; Koepsell, H.; Smit, J. W.; Schinkel, A. H. Reduced Hepatic Uptake and Intestinal Excretion of Organic Cations in Mice With a Targeted Disruption of the Organic Cation Transporter 1 (Oct1). *Mol. Cell. Biol.* **2001**, *21*, 5471−5477.

(58) Sweet, D. H.; Miller, D. S.; Pritchard, J. B.; Fujiwara, Y.; Beier, D. R.; Nigam, S. K. Impaired Organic Anion Transport in Kidney and Choroid Plexus of Organic Anion Transporter 3 (Oat3 (Slc22a8)) Knockout Mice. *J. Biol. Chem.* **2002**, *277*, 26934−26943.

has yet to attain the level of maturity relative to drug metabolism, there seems little doubt that the extent of transporter involvement in drug discovery will only increase over time.

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