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REVIEW

Drug-transporter interaction testing in drug discovery and development

Peter Krajcsi

Peter Krajcsi, Solvo Biotechnology, R and D, 2040 Budaors, Hungary
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Correspondence to: Peter Krajcsi, PhD, Solvo Biotechnology, R and D, 2040 Budaors, Hungary. krajcsi@solvo.com
Telephone: +36-23-503940 Fax: +36-23-503941
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Abstract

The human body consists of several physiological barriers that express a number of membrane transporters. For an orally absorbed drug the intestinal, hepatic, renal and blood-brain barriers are of the greatest importance. The ATP-binding cassette (ABC) transporters that mediate cellular efflux and the solute carrier transporters that mostly mediate cellular uptake are the two superfamilies responsible for membrane transport of vast majority of drugs and drug metabolites. The total number of human transporters in the two superfamilies exceeds 400, and about 40-50 transporters have been characterized for drug transport. The latest Food and Drug Administration guidance focuses on P-glycoprotein, breast cancer resistance protein, organic anion transporting polypeptide 1B1 (OATP1B1), OATP1B3, organic cation transporter 2 (OCT2), and organic anion transporters 1 (OAT1) and OAT3. The European Medicines Agency's shortlist additionally contains the bile salt export pump, OCT1, and the multidrug and toxin extrusion transporters, multidrug and toxin extrusion protein 1 (MATE1) and MATE2/MATE2K. A variety of transporter assays are available to test drug-transporter interactions, transporter-mediated drug-drug interactions, and transporter-mediated toxicity. The drug binding site of ABC transporters is accessible from the cytoplasm or the inner leaflet of the plasma membrane. Therefore, vesicular transport assays utilizing inside-out vesicles are commonly used assays, where the directionality of transport results in drugs being transported into the vesicle. Monolayer assays utilizing polarized cells expressing efflux transporters are the test systems suggested by regulatory agencies. However, in some monolayers, uptake transporters must be coexpressed with efflux transporters to assure detectable transport of low passive permeability drugs. For uptake transporters mediating cellular drug uptake, utilization of stable transfectants have been suggested. *In vivo* animal models complete the testing battery. Some issues, such as *in vivo* relevance, gender difference, age and ontogeny issues can only be addressed using *in vivo* models. Transporter specificity is provided by using knock-out or mutant models. Alternatively, chemical knock-outs can be employed. Compensatory changes are less likely when using chemical knock-outs. On the other hand, specific inhibitors for some uptake transporters are not available, limiting the options to genetic knock-outs.

Key words: ATP-binding cassette transporter; Solute carrier; Drug efflux; Drug uptake; Absorption-distribution-metabolism-excretion-toxicity; Regulatory guidance; ATPase; Vesicular transport; Monolayer assay; *In vivo*

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IMPORTANT BARRIERS AND TRANSPORTERS

The human body harbors a number of physiological barriers. From an oral drug administration point of view the intestinal, hepatic, renal and blood-brain barriers are considered pivotal.

The intestinal barrier is the site of absorption of orally administered drugs. The main cellular components of the intestinal barrier are the enterocytes. Generally, the small intestine is considered of utmost importance. The large surface area and the stomach-proximal position make the small intestine the site of absorption of many oral drugs. With the development of controlled release formulations, more and more studies are concerned with absorption through the colon. The activity of several metabolic enzymes is lower in the colon than in the small intestine[1,2] making the colon an attractive site for absorption. The regional transporter expression data from several papers are inconclusive. The only consensus is that there is significantly higher expression of P-glycoprotein (P-gp)/multidrug resistance protein 1 (ABCB1) in the colon compared to the small intestine, and higher expression of multidrug resistance associated protein 2 (MRP2, ABCC2) in the small intestine compared to the colon[1,3]. Transporters that are expressed in the enterocytes are depicted in Figure 1A. The only transporters that are highly expressed in the intestine and are on the shortlists of both the Food and Drug Administration (FDA)[4] and the European Medicines Agency (EMA)[5] are the apically located P-gp[6] and breast cancer resistance protein (BCRP, ABCG2). These transporters are known to transport many xenobiotics and, therefore, constitute a barrier for drug absorption *via* the intestines.

Two major interfaces connecting the blood and brain compartments are the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). The BBB is by far the more important barrier, as the surface area of the human BBB is approximately 100-fold larger than the surface area of the BCSFB[7,8]. In addition, the distance between neurons and brain capillaries is less than 20 nm in the BBB while the distance between the brain ventricles and circumventricular organs is in millimeter or centimeter range in the BCSFB[9]. The barrier function in the BBB is provided by the microcapillary endothelial cells that contain no fenestrations. Transporters that are expressed in the brain microcapillary endothelial cells are depicted in Figure 1B. Similar to the intestinal barrier, the two transporters on the list of regulatory agencies are the luminally located P-gp and BCRP, indicating that, from a drug development point of view, the BBB mainly functions as a barrier for drug absorption.

The hepatic barrier is the major site of excretion of drugs and drug metabolites. The transporters that are expressed in the parenchymal cells (hepatocytes) are depicted in Figure 1C. The hepatic transporters on the FDA short list are uptake transporters of the organic anion transporting polypeptide (OATP)/Solute Carrier OATP (SLCO) family members rganic anion transporting polypeptide 1B1 (OATP1B1)/SLCO1B1 and OATP1B3 (SLCO1B3), and efflux transporters P-gp and BCRP. The EMA short list adds three additional hepatic transporters: organic cation transporter 1 (OCT1, SLC22A1), bile salt export pump (BSEP, ABCB11) and multidrug and toxin extrusion protein 1 (MATE1, SLC47A1). BSEP transports bile salts and, therefore, has toxicological significance. Noticeably, missing from both lists is MRP2 (ABCC2), a transporter on the canalicular membrane, which transports many drugs and phase II drug metabolites into the bile. The vectorial summation of the activity of the sinusoidal/basolateral uptake transporters and canalicular/apical efflux transporters drives the secretory function of this barrier.

The renal barrier is the other major site of excretion. The main cellular components of the renal secretory transport are the proximal tubule epithelial cells (PTC). The transporters that are expressed in the PTC are shown in Figure 1D. The renal transporters on the FDA short list are basolateral uptake transporters OCT2 (SLC22A2), OAT1 (SLC22A6), OAT3 (SLC22A8) and apical efflux transporters P-gp and BCRP. The EMA guidance also refers to MATE1 and MATE2/MATE2K (SLC47A2) as transporters that should be considered. This arrangement is similar to the hepatocyte, suggesting that the PTC mainly work in a secretory fashion as well. It should be noted that although significant xenobiotic reuptake occurs through PTC, literature data mainly focus on reuptake of physiological substrates.

In general, the transporters listed above have been shown to play a role in ADMET (Absorption-Distribution-Metabolism-Excretion-Toxicity) of drugs. However, regulatory guidances[4] note that additional transporters (*e.g.*, MRPs) should be considered when relevant for the therapeutic class of drug being studied.

TRANSPORTER-MEDIATED PERMEATION *VS* PASSIVE PERMEABILITY/DIFFUSION

In the pharmaceutical industry transcellular permeation of drugs has been viewed as the combination of passive and/or transporter-mediated processes[10]. Sequencing of the human genome yielded 883 putative transporter genes[11]. The suggested number of two main superfamilies of human membrane transporters, the ATP-binding cassette (ABC) transporters, mediating mainly cellular efflux, and the solute carriers (SLC), mediating mainly cellular uptake of their substrates, is well over 400[12]. It is likely that any particular cell may express dozens of transporters. Because of the large number of transporters and the broad substrate specificity of many of transporters, as well as the energetically unfavorable transbilayer permeation of small charged molecules, it has been suggested that drug transport is essentially carrier mediated[13]. It has been hypothesized that lack of saturation of transcellular permeation of some drugs, which is considered by many as the proof of passive diffusion[14],

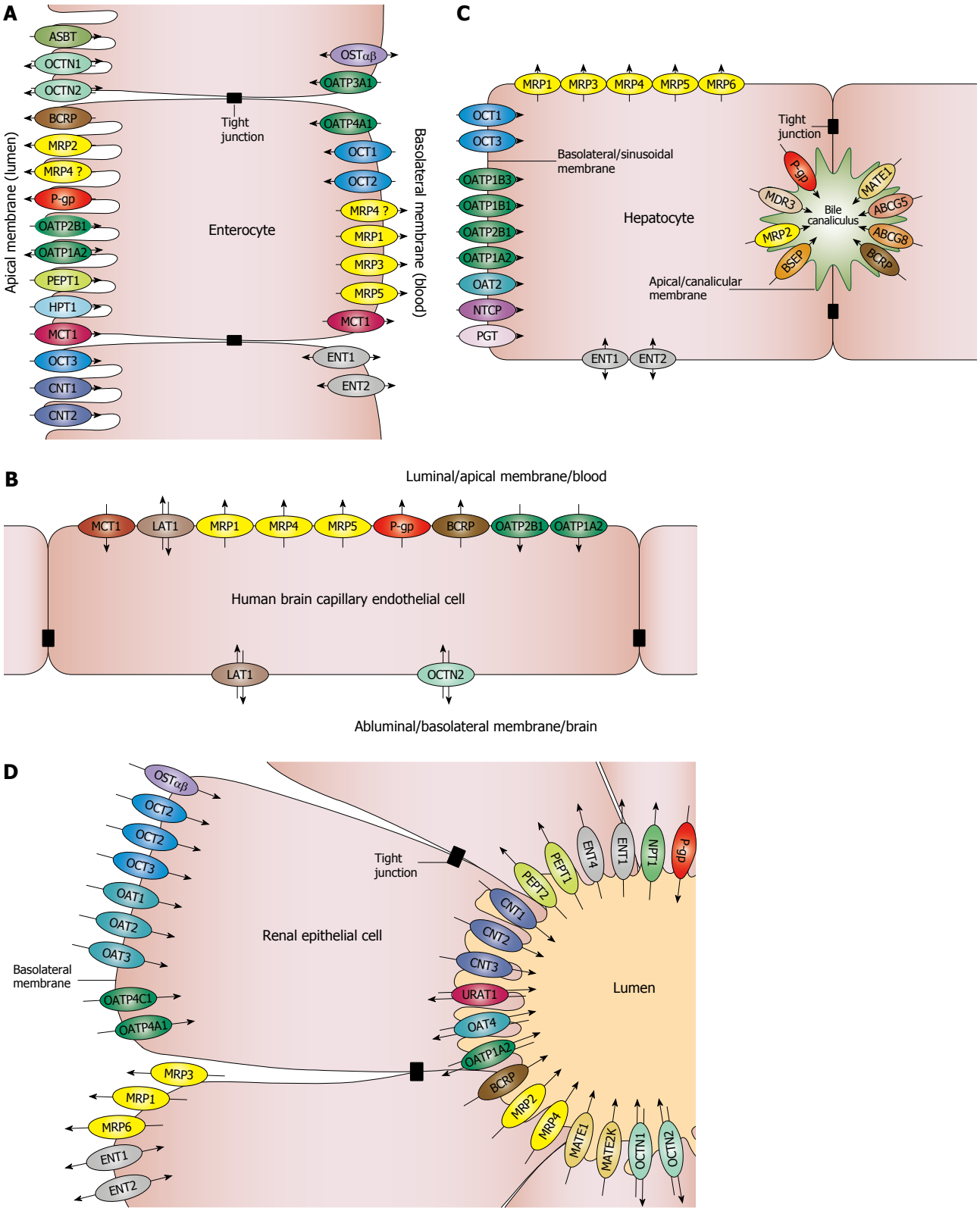

Figure 1 Expression of transporters in human enterocytes (A), brain microcapillary endothelial cells (B), hepatocytes (C) and renal proximal tubule epithelial cells (D). OCTN: Organic cation transporter novel; BCRP: Breast cancer resistance protein; MRP: Multidrug resistance associated protein; OATP: Organic anion transporting polypeptide; ENT: Equilibrative nucleoside transporter; PEPT: Peptide transporter; P-gp: P-glycoprotein; MATE: Multidrug and toxin extrusion protein; BSEP: Bile salt export pump.

is the result of transport by a series of transporters with different affinities[13]. It has also been argued that lack of stereospecificity in permeability of some drugs can be attributed to the broad substrate specificity of transporters[15]. Correlation of apparent permeability coefficients (P_{app}) for the same drug across different cell lines is a

focus of the debate[10,13]. However, multiple drugs show identical P_{app} values in A > B and B > A direction when the known transporters are blocked[16-18]. As in polarized monolayers the transporter expression and activity on the basolateral and apical membranes are likely different these observations require explanation. Even the simplest models used to extract kinetic parameters of transcellular transport of drugs require extensive computation[16,19-23]. Therefore, the consideration of multiple transporters may be a challenging concept to develop into a generally accepted model for use by the pharmaceutical industry.

IN VITRO TESTING

The vast majority of drugs are effluxed by ABC transporters. Other important transporters include members of the MATE/SLC47 and the equilibrative nucleoside transporter (SLC29) families. In addition, efflux action by SLCO[24,25] and OCT novel (SLC22A4-5) family members[26,27] has been suggested. From a pharmacological point of view the main function of MATE transporters is drug efflux. However, based on their classification as an SLC, as well as the predominant assay format (cellular uptake), these transporters will be discussed among uptake transporters. The list of the ABC and SLC transporters identified by the regulatory agencies as of special importance is shown in Table 1.

Efflux transporters

Both membrane-based assays and cellular assays are widely used to test drug transport and drug-drug interactions by ABC efflux transporters. Membrane assays include ATPase and vesicular transport (VT) assays[28]. ATPase assays are based on coupling of ATPase activity to transport and can be considered as surrogate transport assays. VT assays utilize inside-out vesicles and measure accumulation of substrates into the vesicles. Cell-based assays include monolayer efflux assays, cytotoxicity assays, cellular accumulation and efflux assays as well as dye efflux assays. Monolayer efflux assays monitor transcellular transport of substrates and measure the vectorial contribution of transporters. Monolayer efflux assays can be performed in a bidirectional mode or in a unidirectional mode in the presence and absence of an inhibitor. Cytotoxicity assays are mostly used to measure efflux transporter mediated drug resistance[29,30] which can be reversed by a transporter specific inhibitor. It is assumed that efflux transporters inhibit accumulation, hence, efficacy of substrate chemotherapeutics. Thus, the assay is a surrogate transport assay. Cell accumulation and efflux assays are performed in cells overexpressing the transporter. The most common setup involves accumulation in the presence and absence of a specific inhibitor. In cellular efflux assays, after the initial loading, substrate efflux is measured in the presence and absence of specific inhibitors and cell associated drug content is plotted as the percentage of drug remaining in the cells *vs* time[31]. With the exception of reversal agent development neither cellular accumulation nor cellular efflux assays are commonly performed in drug ADME studies. Dye efflux assays monitor efflux activity of transporters using fluorescent probe substrates or non-fluorescent precursor probes[29]. The Calcein assay is the prototype of dye efflux assays which use non-fluorescent dyes as probes[29]. The non-fluorescent calcein-AM, which is a substrate for both P-gp[32] and MRP1[33], is cleaved by intracellular esterases to yield the fluorescent calcein, which is a substrate for MRP1, but not for P-gp[34]. Calcein is hydrophilic and will not diffuse out of the cells, therefore it accumulates at a slower rate in P-gp or MRP1 overexpressing cells compared to control cells, unless the transporters are inhibited. The advantage of using a non-fluorescent substrate is that it can be conveniently performed in high throughput without the need of a fluorescence activated cell sorter or extensive washing. Dye efflux assays are commonly performed as inhibition assays[35] applicable to various cell types and, therefore, can be done in a tissue/cell type specific manner[36].

Two large studies correlated P-gp ATPase and P-gp monolayer efflux measurements[37,38]. Both studies found that a group of high passive permeability substrates that were efficacious ATPase activators did not appear to be P-gp substrates in the monolayer assay. The likely explanation is that the contribution of the transporter to the overall permeability of these compounds is negligible. These compounds were then termed as non-transported substrates[37]. However, several of these compounds such as verapamil[37], ketoconazole[37] and itraconazole (Fekete *et al*: manuscript in preparation) have shown P-gp dependent BBB permeability in humans[39] and mice[40,41]. Due to their high passive permeability, none of the cellular or other vesicular assays would work for these compounds. Therefore, for this group of ABC transporter substrates the ATPase assay is the only assay that predicts a P-gp limited penetration of the BBB.

Passive permeability is a key determinant in assay selection. For example, low passive permeability compounds may be false negatives in P-gp ATPase activation assays[37]. VT/uptake assays work best for low passive permeability compounds[28]. For low and intermediate passive permeability compounds monolayer assays work well, although, for some low passive permeability compounds, an uptake transporter is required for significant transcellular transport[42]. Passive permeability does not play a role in membrane assays when used in an inhibition format. However, monolayer assays will not necessarily work for low passive permeability inhibitors. The effect of passive permeability on assay selection is depicted in Figure 2.

Membrane lipid composition is also an important determinant of transporter activity. BCRP[43-45] and BSEP[46,47] activity is significantly greater in mammalian or cholesterol enriched insect cell membranes than in native insect cell membranes, which contain significantly lower amounts of cholesterol[44], and both BCRP[48] and BSEP[49] are localized in cholesterol rich microdomains. Interestingly, perhaps with the exception of the cyclosporin A-BSEP inter-

Table 1 Characteristics of transporters on the shortlists of regulatory

Transporter	Expression (tissue/cell type/ localization)	Physiological substrates	Select drug substrates	Guidance
P-gp	Brain/endothelial cell/apical Kidney/epithelial cell/apical Liver/hepatocyte/canalicular Small intestine/enterocyte/apical (colon)	Phospholipids, cytokines, steroids	Aliskiren, ambrisentan, colchicine, dabigatran etexilate, digoxin, everolimus, fexofenadine, imatinib, indinavir, itraconazole, lapatinib, maraviroc, nilotinib, paclitaxel, posaconazole, ranolazine, saxagliptin, sirolimus, sitagliptin, talinolol, tolvaptan, topotecan, vinca alkaloids	FDA/ EMA
BCRP	Brain/endothelial cell/apical Liver/hepatocyte/canalicular Small intestine/enterocyte/apical Kidney/epithelial cell/apical Placenta/syncytiotrophoblast/ apical (maternal)	Vitamins (riboflavin, biotin), porphyrins, estrogen sulfate conjugates	Methotrexate, mitoxantrone, daunorubicin, doxorubicin, imatinib, irrinotecan, lapatinib, rosuvastatin, pitavastatin, provastatin, sulfasalazine, topotecan	FDA/ EMA
BSEP	Liver/hepatocyte/canalicular	Taurocholate, glycocholate	Pravastatin, paclitaxel, vinblastine	EMA
OATP1B1	Liver/hepatocyte/basolateral	Bilirubin and its conjugates, thyroxin, triiodothyronine, bile acids, eicosanoids (thromboxane B2, prostaglandin E2, leukotriene C4), dehydroepiandrosterone sulfate, estradiol 17β-glucuronide, estrone 3-sulfate, glycocholate	Atrasentan, atorvastatin, bosentan, ezetimibe, fluvastatin, glyburide, methotrexate, olmesartan, pitavastatin, pravastatin, repaglinide, rifampin, rosuvastatin, simvastatin acid, SN-38 (active metabolite of irinotecan), valsartan	FDA/ EMA
OATP1B3	Liver/hepatocyte/basolateral	Estradiol 17β-glucuronide, taurocholate, estrone 3-sulfate, dehydroepiandrosterone sulfate, thyoxin	Atorvastatin, bosentan, digoxin, methotrexate, olmesartan, paclitaxel, pitavastatin, rosuvastatin, telmisartan, valsartan	FDA/ EMA
OAT1	Kidney/proximal tubular cell/ basolateral	Para-aminohippuric acid, homocysteine, Cysteine,dicarboxylates, prostaglandine E2, urate, estrone-3-sulfate	Adefovir, captopril, cidofovir, furosemide, lamivudine, methotrexate, oseltamivir, tenofovir, zalcitabine, zidovudine	FDA/ EMA
OAT3	Kidney/proximal tubular cell/ basolateral	Estrone 3-sulfate, estradiol 17β-glucoronide, cAMP, taurocholate, cortisol, dehydroepiandrosterone sulfate, prostaglandine E2, urate, succinate, para-aminohippuric acid	Acyclovir, bumetanide, ciprofloxacin, famotidine, furosemide, methotrexate, oseltamivir acid, (the active metabolite of oseltamivir), penicillin G, pravastatin, rosuvastatin, sitagliptin, valacyclovir, zidovudine	FDA/ EMA
1-Oct	Liver/hepatocyte/basolateral Small intestine/enterocyte/ basolateral	Corticosterone, β-oestradiol, progesterone, testosterone, choline, creatinine, guanidine, L-carnitine, thiamine, thyramine, acetylcholine, dopamine	Acyclovir, amantadine, gancyclovir, imatinib, lamivudin, metformin, oxaliplatin, quinidine, quinine, ranitidine, zalcitabine	EMA
2-Oct	Kidney/epithelial cell/ basolateral	β-oestradiol, progesterone, testosterone, choline, creatinine, guanidine, L-carnitine, acetylcholine, dopamine, epinephrine, norepinephrine, histamin, serotonin, choline, dopamine, prostagladine E2	Amantadine, amilorid, cimetidine, cisplatin, dofetilide, famotidine, lamivudin, metformin, oxaliplatin, pindolol, procainamide, ranitidine, zalcitabine	FDA/ EMA
MATE1	Kidney/epithelial cell/apical Liver/hepatocyte/canalicular	Choline, creatinine, guanidine, corticosterone, estrone 3-sulfate, thiamine	Acyclovir, cimetidine, fexofenadine, gancyclovir, metformin, procainamide, topotecan	EMA
MATE2/ MATE2K	Kidney/epithelial cell/apical	Choline, creatinine, guanidine, corticosterone, estrone 3-sulfate, thiamine	Acyclovir, cimetidine, gancyclovir, metformin, procainamide, topotecan	EMA

FDA: Food and Drug Administration; P-gp: P-glycoprotein; EMA: European Medicines Agency; BCRP: Breast cancer resistance protein; OAT: Organic anion transporter; OCT: Organic cation transporter; MATE1: Multidrug and toxin extrusion protein 1.

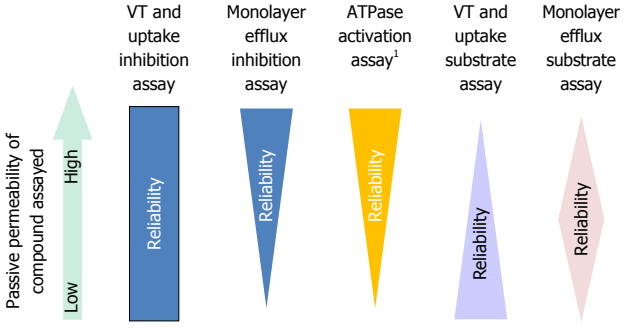

Figure 2 Application of different types of assays for low-high passive permeability compounds. [1]Shown for P-glycoprotein ATPase. VT: Vesicular transport.

action, cholesterol loading did not affect IC_{50} data[43,44,46]. All in all, these data show that the best and certainly the most relevant expression systems are the mammalian/human cells.

Transporter expression levels may affect apparent ADME parameters. Apparent K_M values generated in monolayer assays displayed a linear correlation with P-gp expression[50]. In contrast, the intrinsic K_M values that were based on intracellular concentrations showed independence from transporter expression[19,22]. Some IC_{50} values were also shown to depend on transporter expression with increasing values in higher expressers[36,51]. The phenomenon was predicted by simulations[21] and appears

to have *in vivo* relevance[52,53]. The simulation study also predicted that in a VT system steady-state is established in seconds, as no permeability barriers exist[21].

The monolayer assay system is the suggested assay format for efflux transporter substrate and inhibition assays[4,5]. The advantage of the system is that it shows if the contribution of an efflux transporter is comparable in magnitude to passive permeability and, thus, modulates transcellular permeability of substrate drugs. However, in some aspects VT substrate and inhibition assays offer advantages over the monolayer assays, as data obtained in VT assays are not confounded by permeability barriers. Along this line, in earlier publications VT inhibition assays have been suggested as drug-drug interaction assays for low passive permeability drugs[54].

Digoxin is the consensus substrate for P-gp[4] and PSC833 is a commercially available P-gp specific inhibitor[55]. Dabigatran etexilate[56-58] or fexofenadine[58-60] could also be considered as probes as these are lower bioavailability substrates and are potential probes for clinical drug-drug interaction studies. However, only fexofenadine has been extensively studied *in vitro*[59,60]. Quinidine is an acceptable alternative to digoxin in microdialysis experiments where application of digoxin is not feasible due to non-specific adherence to tubing as well as toxicity[55,60]. No consensus has been reached on the probe substrates and inhibitors for BCRP. Topotecan[4,61], rosuvastatin[4], prazosin[44,62] and sulfasalazine[63,64] have all been suggested. However, these compounds are substrates of multiple efflux transporters that are co-expressed with BCRP on apical membranes. On the contrary, chlorothiazide, a non-metabolized[65], low bioavailability drug[66] is a specific BCRP substrate[67] and a potential probe. Ko134 and Ko143 have been extensively used in preclinical studies as BCRP-specific inhibitors. For BSEP, taurocholate is the consensus probe[46,68,69] and cyclosporin A, a cholestatic drug[70] is the reference inhibitor used most often[46,68,69,71,72]. Potential ABC transporter probe substrates are listed in Table 1.

Uptake transporters

Cellular uptake of drugs and endobiotics is mediated *via* uptake transporters of the SLC superfamily. The list of the uptake transporters identified by the regulatory agencies as important is shown in Table 1. Mechanistically these transporters are uniporters (*e.g.*, OCT1), symporters [*e.g.*, sodium taurocholate cotransporting polypeptide (NTCP, SLC10A1), peptide transporter 1 (PEPT1, SLC15A1)] or antiporters (*e.g.*, OATP1B1, OAT1, MATE1).

Membrane assays are applicable to symporters where the driving force of the transport is known and the assay set-up is straightforward. Na^+-taurocholate cotransporting polypeptide (NTCP)-mediated taurocholate transport into right-side out (ROV) rat sinusoidal membrane vesicles has been shown[73]. Proton gradient driven dipeptide transport into ROV prepared from intestinal brush-border membranes has also been published[74]. For exchangers (*e.g.*, OATPs, OATs) a vesicular uptake assay would be cumbersome to perform even if the identity of the exchange ion was known.

The most common assay system for uptake transporters are primary cells [*e.g.*, hepatocytes, brain microcapillary endothelial cells (BME), proximal tubule cells (PTC) of the kidney], cancer cell lines (*e.g.*, Caco-2), immortalized cell lines (*e.g.*, human brain endothelial cell line, hCMEC/D3) or transfectants. Transfectants are the test systems recommended by regulatory agencies[4].

Oocytes microinjected with the mRNA or cDNA of the respective transporter have been used early on. Oocytes offer the option of electrophysiological measurements as the transport of many substrates is electrogenic. However, the system is transient, the quality of the oocytes display seasonal variations, the lipid composition of the plasma membrane is different from physiological and the throughput is low-to-intermediate[75,76].

For uptake transporters brain slices[77], liver slices[78,79] and kidney slices[80,81] are commonly used to compute clearance values.

Uptake transporters have highly overlapping substrate specificities and multiple family members are expressed in the same cell type. Quantification of contribution of the different transporters is a challenge. OATP1B1 and OATP1B3 have very similar substrate specificities and are both expressed in hepatocytes. Estrone-3 sulfate and cholecystokinine octapeptide (CCK-8) are selective substrates of OATP1B1 and OATP1B3, respectively, and can be used as reference substrates to determine activities of these transporters in a hepatocyte preparation[82]. The most notable non-statin drugs are bosentan, a substrate of OATP1B1[83] and OATP1B3[83], valsartan[84] or repaglinide[85], substrates of OATP1B1, and telmisartan[86] or nafcilin[87], substrates of OATP1B3. Fluo-3 is a highly sensitive fluorescent probe of OATP1B3[88]. Rifampin and cyclosporin A are the recommended reference inhibitors[4] however various statins are also commonly used[89]. For clinical drug-drug interaction studies the use of statins as victims/probes has been suggested[4]. OAT1 and OAT3 are co-expressed in the basolateral membrane of PTC. These transporters have overlapping substrate specificities, with OAT3 having a bias for amphiphilic, larger molecular weight compounds[90]. Adefovir can be used as a reference substrate for OAT1 and benzylpenicillin for OAT3[80]. Tenofovir[91], azydothimidine/zidovudine[92], para-aminohippurate[4] for OAT1 and methotrexate[93], cimetidine[94], furosemide[95], estrone-3-sulfate[4] for OAT3 are also applicable. Probenecid inhibits both transporters[4] but benzylpenicilline is considered an OAT3-specific inhibitor[80,96]. P-aminohippurate has been used as a specific OAT1 inhibitor[80] and also as inhibitor of both transporters[96]. For OCT1[97,98] and OCT2[98-100] metformin is an accepted drug substrate. Alternatively, 1-methyl-4-phenylpyridinium (MPP^+) and cimetidine can be used for both OCT1[101,102] and OCT2[4,103]. Cimetidine or verapamil can be used as an OCT1[98,104] and OCT2 inhibitor[4,98,104], although, clinical relevance of cimetidine mediated inhibition of OCT2

has been questioned lately[105]. Metformin is a relevant substrate for both MATE1[106] and MATE2/MATE2K[106] and cimetidine[105] and verapamil[104] are potent inhibitors. Importantly, pyrimethamine has been shown to selectively inhibit MATE1 and MATE2/MATE2K[105,107].

Uptake transporters play a major role in pharmakokinetics of substrate drugs. Inhibition of hepatic[108] and/or renal[109] clearance by co-administered drugs can lead to clinically significant drug-drug interactions. Interactions of the hepatic uptake transporters often result in > 5-fold increase in C_{max} values of victim drugs[110]. Nevertheless, most *in vitro* assays commonly employ either physiological substrates such as estrone-3-sulfate or estradiol-17β-glucuronide for anion transporters or synthetic non-drug substrates, such as tetraethyl-ammonium for cation transporters[89]. Broad-scale application of LC/MS/MS methodology in drug quantification will facilitate revalidation of uptake transporter assays using drug probes.

IN VIVO TESTING

In vivo studies using knock-out and mutant animals shows the paramount importance of transporters[61,76,111]. Obviously, *in vivo* significance of a transporter in clearance of a drug can only be addressed by *in vivo* studies[61]. Other important applications, such as gender difference, as well as age and ontogeny are also preferably studied *in vivo*[112-114]. With the availability of double and triple knockouts, transporter complementation[115] and transporter-enzyme interplay[116] can now be addressed. Nevertheless, utilization of knockouts is perhaps not as extensive as originally envisioned. Compensatory changes may mask the effect of transporter deletion. P-gp is upregulated in Bsep knockout mice and the metabolism of bile acids is altered as well[117,118]. Cytochrome P450 enzymes which share substrate specificity with P-gp are dramatically up-regulated in P-gp knockout mice in a gender specific manner[119]. Species specificity issues also limit utilization of these models by the pharmaceutical industry. In addition to differences in substrate specificities[120], significant differences have been observed in transporter expression between species. Canalicular expression of MRP2/Mrp2 is about 10-fold greater in rodents than in humans[121] and the ratio of BCRP/P-gp expression in the BBB is about 4-fold greater in humans than it is mice[122]. Chemical knockouts can circumvent the problems stemming from compensatory changes. However, species specificity issues can only be overcome by utilization of humanized models. As the availability of humanized models increases, the relevance of *in vivo* studies will certainly increase as well[123-125].

CONCLUSION

In the past decade utilization of transporter assays by the pharmaceutical industry has been rapidly growing. Lower activity pharmacogenomic variants such as BCRP 412G>A[126] and OATP1B1 521 >C[85] make it possible to show the impact of the wild type transporters on human pharmacokinetics of substrate drugs and clearly demonstrate clinical relevance of drug-transporter interactions.

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