Symposium Report

Role of (Drug) Transporters in Imaging in Health and Disease

Bruno Stieger, Jashvant D. Unadkat, Bhagwat Prasad, Oliver Langer, and Hariprasad Gali

Department of Clinical Pharmacology and Toxicology, University Hospital, Zurich, Switzerland (B.S.); Department of Pharmaceutics, University of Washington, Seattle, Washington (J.D.U., B.P.); Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria and Health and Environment Department, AIT Austrian Institute of Technology GmbH, Seibersdorf, Austria (O.L.); and the Department of Pharmaceutical Sciences, College of Pharmacy, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma (H.G.)

Received July 6, 2014; accepted September 23, 2014

ABSTRACT

This report is the summary of presentations at the symposium sponsored by the American Society for Pharmacology and Experimental Therapeutics, April 26–30, at Experimental Biology 2014 in San Diego, CA. The presentations focused on the role of transporters in imaging in health and disease and on assessing transporter function in vivo. Imaging is an important diagnostic tool in clinics and is a novel tool for in vivo visualization of transporter function. Many imaging substrates and endogenous markers for organ function are organic anions. In this symposium, the bile salt transporter sodium taurocholate cotransporting polypeptide and the liver organic anion transporting polypeptides (OATPs) as well as the renal organic anion transporters (OATs) were addressed in detail; e.g., OATPs mediate transport of contrast agents used for magnetic resonance imaging of the liver or transport agents used for hepatobiliary scintigraphy, and OATs transport substances used

in renography. In addition, the symposium also focused on the multidrug-resistance transporter 1 (MDR1 or P-gp), which is the most important gatekeeper in epithelial or endothelial barriers for preventing entry of potentially harmful substances into organs. Novel substrates suitable for positron emission tomography (PET) allow the study of such transporters at the blood-brain barrier or while they are mediating uptake of drugs into hepatocytes, and, importantly, PET tracers also now allow renography. Finally, quantitative data on transporter expression in human organs allow the development of improved physiologically based pharmacokinetic (PBPK) models for drug disposition. Hence, the combined efforts using novel substrates for in vivo visualization of transporters and quantification of transporters will lead to a deeper understanding of transporter function in disease and allow development of novel PBPK models for disease states.

Introduction

Transporters are key elements controlling many homeostatic processes in the body and they are essential elements in the processes involved in drug disposition. In addition, transporters are now recognized as very important to our understanding of the molecular basis of diagnostic tools,

because they mediate, for example, the transport of endogenous (Stieger et al., 2012; Hoekstra et al., 2013) or exogenous (Van Beers et al., 2012; Pastor et al., 2014) liver function markers.

Against this background, it is important to understand the changes in transporter functions in disease states and to develop new tools for studying individual transporters in vivo. In addition to working with in vitro model systems, transporter function can be addressed by quantifying the amount of transporters and their post-translational modifications in tissue specimens from patients. To study transporters in vivo, the development of substrates that are specific for a transporter and are amenable to detection by noninvasive procedures is an attractive option. One area in which a lot of effort is currently being invested is the development of radiolabeled transporter substrates detectable by positron emission tomography (PET). Another important aspect is the development of detailed physiologically based pharmacokinetic (PBPK) models that enable in vitro study of the disposition and the

This work is supported by the Swiss National Science Foundation (SNF) [310030_144195] and by the SNF National Center of Competence in Research TransCure (University of Berne, Berne, Switzerland); by UWRAPT and NIDA [P01DA032507]; by the Austrian Science Fund (FWF) [F 3513-B20 and P 24894-B24] and by the European Community's Seventh Framework program [201380]; and by a University of Oklahoma College of Pharmacy startup grant and by the Oklahoma Center for the Advancement of Science and Technology (OCAST) grant no. H-R13-210.

All authors contributed equally to this work.

dx.doi.org/10.1124/dmd.114.059873.

ABBREVIATIONS: ABC, adenosine triphosphate-binding cassette; BBB, Blood-brain barrier; BCRP, breast cancer-resistance protein; BSEP, bile salt export pump; BSP, bromosulphophthalein; DTPA, diethylenetriaminepentaacetic acid; L,L-EC, L,L-ethylenedicycysteine; ICG, indocyanine green; IVIVE, in vitro-to-in vivo extrapolation; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MAG3, mercaptoacetyltriglycine; MDR1, multidrug-resistance protein; MRI, magnetic resonance imaging; MRP, multidrug resistance-associated protein; NTCP, sodium taurocholate cotransporting polypeptide; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; P-gp, multidrug resistance protein; PBPK, physiologically based pharmacokinetic; PFH, fluorhippurate; PET, positron emission tomography; SE, status epilepticus; SPECT, single photon emission computed tomography; ^{99m}Tc, technetium-99m.

potential interaction of drugs with new chemical entities. Such models require accurate knowledge of the protein abundance of transporters and drug-metabolizing enzymes in humans.

This report is a summary of presentations at the symposium sponsored by the American Society for Pharmacology and Experimental Therapeutics, April 26–30, at Experimental Biology 2014 in San Diego, CA. The presentations focused on the role of transporters in imaging in health and disease and were given by B.S., J.D.U., O.L. and H.G. The overall aim of the symposium was to highlight the role of transporters in classic diagnostic procedures as well as to provide examples of novel probe substrates for transporters. In addition, data on transporter expression levels in humans in the context of developing *in silico* models, e.g., for drug disposition, were discussed.

The Role of Transporters for Diagnostic Probes (B.S.)

One of the key functions of the liver is bile formation, and disturbance of this process may lead to liver disease. Bile salts are major constituents of bile and are amphipathic organic anions (Esteller, 2008). The clearance of radioactively labeled bile salts from blood plasma is reduced in patients with liver disease as demonstrated by LaRusso et al. (1975), suggesting that bile salts could be used as liver function markers. Today, the relevant transporters for hepatocellular bile salt transport have been cloned and functionally characterized (Meier and Stieger, 2002; Stieger, 2011; Hagenbuch and Stieger, 2013): the sodium taurocholate cotransporting polypeptide (NTCP), the organic anion transporting polypeptides (OATPs), and the bile salt export pump (BSEP) are key bile salt transporters in hepatocytes under normal physiologic conditions.

OATPs constitute a family of truly multispecific transporters and mediate in addition to bile acids the transport of other endogenous substrates, like steroid metabolites (Hagenbuch and Stieger, 2013) (Stieger and Hagenbuch, 2014), a wide variety of drugs (Hagenbuch and Stieger, 2013; Stieger and Hagenbuch, 2014), and endogenous (e.g., bilirubin and its conjugates) and exogenous liver function markers, such as bromosulphophthalein (BSP) or indocyanine green (ICG) (Stieger et al., 2012; Van Beers et al., 2012). The first OATP cloned was identified by using an expression-cloning approach from rat liver mRNA with the liver function marker BSP as lead substrate (Jacquemin et al., 1994), and this OATP is strongly inhibited by ICG (Kullak-Ublick et al., 1994). All OATPs expressed in human liver mediate *in vitro* transport of BSP (Kullak-Ublick et al., 2001). While BSP is not in clinical use anymore, ICG is used in clinics as an exogenous liver function marker (Sakka, 2007; Hoekstra et al., 2013). Interestingly, only OATP1B3, but not OATP1B1 and OATP2B1, mediate *in vitro* transport of ICG (de Graaf et al., 2011). Of note, the latter study also demonstrated NTCP-mediated ICG transport. The *in vivo* relevance of these findings on ICG is supported by the positive association of ICG accumulation in hepatocellular carcinomas with the expression of NTCP and OATP1B3 (Ishizawa et al., 2014). ICG is excreted into bile by the ATP-binding cassette (ABC) transporter multidrug resistance-associated transporter (MRP) 2. (Sathirakul et al., 1993). Plasma bilirubin is routinely determined in the workup of patients with liver disease. Bilirubin and its conjugates can therefore be considered as endogenous liver function markers. They are transported by OATP1B1 and OATP1B3 into hepatocytes and by MRP2 into bile or in disease states by MRP3 back into the sinusoids (Keppler, 2011; Stieger et al., 2012). These above examples demonstrate that transport systems for organic anions play a major role in handling of endogenous and exogenous liver function markers. Organic anion transporters are therefore very important as mediators of cellular uptake of diagnostic probes, that are anions.

Magnetic resonance imaging (MRI) or liver scintigraphy are frequently used for diagnosing liver disease, e.g., for focal lesions. Diagnostic probes

used in these procedures are mostly negatively charged (Hoekstra et al., 2013; Pastor et al., 2014). Hence, MRI agents (Pastor et al., 2014) and mebrofenin (de Graaf et al., 2011) have been demonstrated to be substrates of organic anion transporters such as OATPs or MRP2. Mebrofenin has recently been recommended for use as a liver function marker for helping to improve the outcome of liver resections (Hoekstra et al., 2013).

Focal and diffuse liver disease may lead to an alteration of transporter expression (Geier et al., 2007; Pollheimer et al., 2014) in diseased and healthy tissue, which in turn will either influence the plasma clearance or the hepatocellular accumulation of a diagnostic probe. Parenthetically, expression of transporters for diagnostic probes may be affected by diseases in all organs. Therefore, alterations of results from liver function tests in disease can increasingly be understood at the molecular level. In addition to altered transporter expression in disease states, other confounding factors need to be considered when interpreting data from liver function markers: The impact of interindividual variability of transporter expression will be discussed in the section on the quantification of transporters. In addition, drugs may act as inhibitors of transport systems. For example, rat OATP1A1 and OATP1A4 are suspect to inhibition by the antibiotics rifampicin and rifamycin SV (Fattinger et al., 2000). In rats, the accumulation of the MRI contrast agent gadobenate dimeglumine (BOPTA) in the liver is inhibited in a dose-dependent manner by rifampicin (Daali et al., 2013). In contrast, in a human study, no evidence was found for a pharmacokinetic interaction between gadoxetic acid clearance and coadministration of erythromycin (Huppertz et al., 2011).

Next to drug interactions, disease-related changes in the local environment of a transporter may affect its transport properties and hence the disposition of diagnostic probes. Therefore, a detailed understanding of the transport mechanism(s) of transporters used for diagnostic probes is needed. With respect to OATPs, the transport mechanism is not known in full detail (Hagenbuch and Stieger, 2013; Stieger and Hagenbuch, 2014), impeding prediction of consequences for diagnostic probes resulting from alterations in OATP-mediated transport. For example, most, if not all, OATPs have more than one substrate binding site, which do not always overlap in substrate specificity (Hagenbuch and Stieger, 2013; Stieger and Hagenbuch, 2014). This may affect not only the binding of diagnostic probes, but puts limits on the prediction of drug interactions with diagnostic probes. Furthermore, endogenous substances or some drugs may stimulate rather than inhibit the transport activity of individual OATPs (Hagenbuch and Stieger, 2013; Stieger and Hagenbuch, 2014). And finally, most OATPs are in a substrate dependent manner stimulated by an acid extracellular pH (Leuthold et al., 2009; Hagenbuch and Stieger, 2013; Stieger and Hagenbuch, 2014). These issues clearly show that there will be no “one size fits all” concept for the characterization of transport systems with imaging tools. The full potential of transporters in the clinical use of diagnostic probes will not be fully realized without considerable efforts to fully understand the mechanisms of transporters as well as the impact of diseases on transporters at the levels of function and expression.

Nevertheless, first studies demonstrating the use of bile salts as an example for an endogenous imaging marker provide a proof of concept for the feasibility of visualizing transporters involved in bile formation *in vivo* (Frisch et al., 2012; Jia et al., 2014).

Quantification of Drug Transporters to Understand Interindividual Variability in Drug Disposition and Drug Response (B.P. and J.D.U.)

While great strides have been made in our understanding of pharmacokinetics of drugs, these advances have been primarily made

through measurement of plasma (and not tissue) concentration of drugs. However, from the perspective of drug efficacy or toxicity, in most instances it is the target tissue drug concentration (rather than plasma concentration) that is most relevant. For obvious reasons such concentrations are not routinely measured in humans. Therefore, when conducting pharmacokinetic or efficacy and toxicity studies, the typical approach has been to assume that the unbound concentration in the tissues can be approximated by the unbound concentration in the plasma. With the emerging knowledge about transporters and their localization in multiple tissues, it is clear that this assumption is no longer valid. For example, as described in the previous section, a drug may be transported into the liver by the sinusoidal OATPs where it may be metabolized or excreted into the bile by one of the canalicular efflux transporters [e.g., breast cancer–resistance protein (BCRP), multidrug-resistance protein (P-gp or MDR1)]. Depending on the relative magnitude of the influx and efflux (or metabolic) clearance of the drug, the unbound hepatic concentration of the drug can exceed, equal, or be lower than the unbound plasma concentration of the drug. Indeed we have recently demonstrated this phenomenon by imaging the hepatic distribution of [^{11}C]rosuvastatin in the rat (He et al., 2014). Similarly, as previously demonstrated by us and others (Sasongko et al., 2005; Hsiao et al., 2008; Eyal et al., 2009, 2010; Muzi et al., 2009; Bauer et al., 2012; Mullauer et al., 2012; Bankstahl et al., 2013; Ke et al., 2013; Deo et al., 2014), when a drug is effluxed out of the brain, its concentration in the brain can be much lower than that in the plasma. This disconnect between tissue and plasma drug concentration will be modulated in the presence of a drug–drug interaction (DDI). For example, when P-gp is inhibited at the human blood–brain barrier, the brain concentration of [^{11}C]verapamil, a P-gp substrate, almost doubles, but the plasma concentration profile of [^{11}C]verapamil is not affected (Sasongko et al., 2005; Eyal et al., 2010).

With advances in imaging such as PET or MRI, minimally invasive measurement of human tissue concentration of drugs is now possible. But these methods can be applied to drugs only under selective circumstances; for instance, when specific chemical characteristics are present (MRI) or specialized equipment, expertise, and facilities (PET) are available. Thus, there is a need to develop methods to routinely predict tissue concentrations of drugs, especially when transporters are involved. One such method, proposed by us (and others) (Ohtsuki et al.), is to extrapolate *in vitro* transport kinetics of a drug (e.g., in cell lines expressing the transporter of interest) to that *in vivo* using scaling factors (IVIVE; eqs. 1 and 2) and then, through PBPK modeling, predict the tissue concentration of the drug. To implement this method, the expression of the transporter protein *in vivo* (as measured in tissues) as well as *in vitro*, in cell lines or vesicles used to determine the transport kinetics of the drug, must be available. Where multiple transporters are involved (e.g., influx or efflux transporters), the clearance via these transporters will be the sum of the clearances via each transporter. However, we note here that clearance via the influx and efflux transporters are not additive.

$$CL_{in\ vitro} = \frac{V_{max}}{K_m + C}; \text{ where } V_{max} = [T] \cdot K_{cat} \quad (1)$$

$$CL_{in\ vivo} = SF \cdot CL_{in\ vitro} \text{ where } SF = \frac{[T]_{in\ vivo}}{[T]_{in\ vitro}} \quad (2)$$

In the above equations, K_{cat} is transport turnover rate, SF is scaling factors, [T] is transporter expression.

While transporter expression in tissues or cells can be quantified using antibodies, because purified standards of the majority of transporters are not available, such quantification is not absolute (e.g., pmol amounts per mg protein) and therefore cannot be used in PBPK model

predictions. To address this deficiency our laboratory (and others) now uses liquid chromatography–tandem mass spectrometry (LC-MS/MS) proteomics and surrogate peptides to quantify transporter–protein expression in tissues and cell lines (Li et al., 2009; Shawahna et al., 2011; Uchida et al., 2013; Prasad et al., 2014). Irrespective of the method used to quantify transporter expression, several methodological questions arise when utilizing such data for IVIVE and PBPK model predictions. These methodological questions are discussed below.

Methodological Issues. First, for IVIVE, the method used for quantification of transporters should ideally measure functional transporters present in the plasma membrane and not those present intracellularly. However, it is widely acknowledged that high-throughput methods to purify plasma membranes are currently not available. Therefore, for IVIVE to be successful, the expression of the transporters in plasma membrane versus total expression in the cell should be similar *in vitro* (i.e., in cell lines used for IVIVE) and *in vivo* (i.e., in human tissues). Secondly, the protein expression and activity of the transporter must correlate. That is, one should be able to extrapolate from transporter activity and protein expression measured in overexpressed cell lines (where transporter expression is typically high) to *in vivo*, where the expression is typically lower and varies across individuals. Below we discuss results obtained in our laboratory to address these questions.

Briefly, using commercially available kits (Qiagen, Valencia, CA, and Abcam, Cambridge, MA), we isolated various cellular fractions from cells overexpressing OATP1B1 (MDCKII cells), BCRP (MDCKII cells), OATP2B1 (HEK 293 cells), or human liver tissues ($n = 3$). OATP1B1, OATP2B1, or BCRP expression, measured by LC-MS/MS in different subcellular fractions, was expressed relative to the expression of a signature protein representing each organelle (e.g., Na^+/K^+ -ATPase for plasma membrane, GS28 for Golgi, calreticulin for endoplasmic reticulum, TIM23 for mitochondria, GAPDH for cytosol, and histone 3 for nucleus). While none of the methods yielded pure plasma membrane (manuscript in preparation), the expression of OATP1B1, OATP2B1, or BCRP in all subcellular fractions isolated from cells or liver tissue tracked the expression of Na^+/K^+ -ATPase. Therefore, we further evaluated the total membrane preparation kit (Calbiochem/EMD Millipore, Billerica, MA) that we routinely use for transporter quantification by LC-MS/MS. Using this kit, we found that the membrane expression of the above transporters, relative to that of Na^+/K^+ -ATPase, remained constant irrespective of whether the total membrane was isolated from overexpressing cells or liver tissue. Assuming that Na^+/K^+ -ATPase is predominately expressed on the plasma membrane, these data suggest that the expression of these transporters on the plasma versus total membrane is similar in cells versus tissues. That is, scaling transporter expression from cell lines to tissue is a reasonable approach.

To address the question of whether membrane expression and activity of transporters are correlated, we created, using lentiviral shRNA, clones of cells expressing varying levels of BCRP or OATP1B1 expression. We then quantified the total membrane expression (by LC-MS/MS) and transporter activity in each clone. We found that the expression was highly correlated with activity ($r^2 > 0.9$). These data suggest that even when transporter expression and activity is high, as in overexpressed cell lines, such cell lines can be used for IVIVE of transporter activity.

Finally, we have addressed a methodological problem specific to LC-MS/MS proteomics. Briefly, this method is based on LC-MS/MS quantification of unique surrogate peptides liberated after trypsin digestion of the protein. Therefore, as we have stressed before (Prasad and Unadkat, 2014), this method assumes that tryptic digestion of the protein is complete. This assumption can be tested only when the purified protein is available. We were able to test this assumption with purified P-gp. Our assumption of complete digestion of P-gp was found to be correct. We were able to recover

the concentration of P-gp in the purified sample using the LC-MS/MS peptide quantification approach (Prasad et al., 2013). Ideally, if the purified transport protein is available, this assumption should be tested for every transport protein quantified.

Transporter Expression in Human Tissues. Having addressed the above methodological issues, we used LC-MS/MS to quantify the expression of a number of drug transporters and the interindividual variability in their expression in human livers ($n = 64$), kidneys ($n = 14$), and intestines ($n = 17$), using the surrogate peptide approach. In human livers, we have quantified the expression of hepatic OATP1B1, OATP1B3, OATP2B1, P-gp, BCRP, and MRP2. Quantification of expression of other transport proteins important in drug transport and hepatic toxicity (e.g., BSEP, NTCP, MRP3) has been completed (manuscript in preparation). The key findings from our study were: 1) the mean $\pm$ S.D. (fold range in parentheses) protein expression (fmol/ μ g of membrane protein) in human liver tissue was: OATP1B1, 2.0 ± 0.9 (7); OATP1B3, 1.1 ± 0.5 (8); OATP2B1, 1.7 ± 0.6 (5); P-gp, 0.4 ± 0.2 (8); BCRP, 0.14 ± 0.04 (>3.4); and MRP2, 1.5 ± 0.6 (7); 2) the transporter expression was found not to be correlated with age (7–70 years) or sex; 3) consistent with literature data, OATP1B1 and BCRP expression correlates with their genotypes (Deo et al., 2012; Prasad et al., 2013, 2014). For example, livers with *SLCO1B1* (encoding OATP1B1) haplotypes *14/*14 and *14/*1a (i.e., representing SNPs c.388A>G and c.463C>A) had significantly higher ($P < 0.0001$) protein expression than the reference haplotype (*1a/*1a) (Prasad et al., 2014). Similarly, BCRP expression in livers with the variant C421A allele was significantly lower than that in the wild-type livers ($P < 0.002$) (Prasad et al., 2013). Furthermore, we used genotype-dependent OATP1B1 expression to predict drug disposition of its substrates repaglinide and rosuvastatin. Based on the genotype-dependent OATP1B1 protein expression data, we predicted (using Simcyp) up to $\sim 40\%$ decrease in mean area under the curve of rosuvastatin or repaglinide in those individuals harboring the *14/*14 and *14/*1a variant alleles compared with those harboring the wild-type alleles (Prasad et al., 2014). These predictions are in agreement with data observed in vivo (Kalliokoski et al., 2008) and demonstrate the utility of the proteomics approach to predicting the genotype-dependent disposition of drugs in the clinic.

Besides quantification of transporters expressed in human livers, we are quantifying the expression of relevant drug transporters in human kidneys, brain, placenta, pediatric livers, and, in collaboration with Dr. Stefan Oswald and others at University of Greifswald, intestines (Drozdzik et al., 2014).

In summary, the above data demonstrate that transporter proteomics using LC-MS/MS and surrogate peptides can quantify transporter protein expression in cells and tissues. The data obtained to date on genotype-expression relationship have been successfully incorporated into PBPK models to predict the disposition of drugs. However, using cell lines and transporter proteomics for IVIVE and therefore prediction of in vivo pharmacokinetics of drugs, including tissue distribution, needs to be validated. To validate such predictions, data on in vivo tissue distribution of drugs in humans and animals are needed. Such data are best obtained using noninvasive imaging methods (such as PET imaging) to determine drug transport concentrations in various tissues (e.g., brain, fetus, and liver). Such data are available from our laboratory (Hsiao et al., 2008; Eyal et al., 2009, 2010; Muzi et al., 2009; Ke et al., 2013; Deo et al., 2014; He et al., 2014) and others. Therefore, the accuracy of the above proposed IVIVE method can now be tested.

PET Imaging of ABC Efflux Transporters at the Blood-Brain Barrier in Humans and Animal Models (O.L.)

Drug delivery to the brain is partly controlled by ABC transporters, such as P-gp, BCRP, or MRP4, which are expressed at the luminal

membrane of brain capillary endothelial cells (Kalvass et al., 2013). These transporters are capable of effluxing a multitude of chemically diverse compounds from the endothelial intracellular space into the vascular compartment, thereby limiting their brain distribution. Disease-induced changes in transporter expression at the blood-brain barrier (BBB) may lead to changes in drug delivery to the brain, causing decreased efficacy or increased central nervous system side effects of drugs.

It has been shown that epileptic seizures cause upregulation of P-gp in epileptic brain tissue. This may hinder access of antiepileptic drugs that are P-gp substrates to their target structures in the brain and thereby possibly contribute to drug resistance in epilepsy (transporter hypothesis of drug resistance) (Löscher et al., 2011). An upregulation of P-gp in human epileptic brain tissue has been demonstrated by P-gp immunostaining of surgically removed brain tissue specimens (Löscher et al., 2011). However, it is currently poorly understood if this P-gp upregulation has functional consequences, i.e., is able to lower regional antiepileptic drug concentrations below therapeutically effective concentrations. Experimental studies in rat models of drug resistant epilepsy have shown that treatment with the potent third-generation P-gp inhibitor tariquidar (Fig. 1A) is able to restore sensitivity to phenobarbital or phenytoin and to enhance drug concentrations in epileptic brain tissue (Löscher et al., 2011). These findings suggest that inhibition of P-gp or prevention of seizure-induced P-gp upregulation may present a promising strategy to overcome drug resistance in epilepsy in the clinic. However, the key question that remains to be answered is if this P-gp-mediated resistance mechanism also occurs in humans.

PET is a nuclear imaging method that allows for measuring the function and expression of proteins in vivo by intravenous injection of protein-specific radiotracers and noninvasive measurement of their tissue distribution over time. Mathematical modeling of PET data can provide quantitative outcome parameters describing the target proteins of interest. For instance, in the case of transporters situated at the BBB these outcome parameters may be the rate constants describing exchange of radiotracer between the plasma and brain tissue compartments. Several radiotracers have been developed to measure cerebral P-gp function, such as [11 C]loperamide, [11 C]*N*-desmethyl-loperamide, racemic [11 C]verapamil, and (*R*)-[11 C]verapamil (Mairinger et al., 2011). Among these, (*R*)-[11 C]verapamil (Fig. 1A) is a promising radiotracer that has been used both in animals and humans. We have shown that (*R*)-[11 C]verapamil is selectively transported by P-gp among major efflux transporters expressed at the mouse BBB (Römermann et al., 2013).

A limitation of (*R*)-[11 C]verapamil in studies of regional upregulation of P-gp in the brain has been its low brain uptake, which makes it difficult to detect a signal reduction caused by increased P-gp function. We have shown before that pretreatment of rats with tariquidar enhances brain uptake of (*R*)-[11 C]verapamil (Kuntner et al., 2010). We were interested in finding out if modulation of P-gp by tariquidar might make (*R*)-[11 C]verapamil more sensitive for detecting regional differences in P-gp function in the brain. For this approach it appears critical that P-gp is only partly inhibited as complete inhibition of P-gp would cause (*R*)-[11 C]verapamil brain distribution to be dependent on passive diffusion and not on P-gp function. We therefore performed a study in rats to identify a tariquidar dose that only partly inhibits P-gp (Kuntner et al., 2010). Rats underwent (*R*)-[11 C]verapamil PET scans after pretreatment with different tariquidar doses. A tariquidar dose-dependent increase in (*R*)-[11 C]verapamil brain distribution was observed with a half-maximum effective dose (ED₅₀) of 3.0 mg/kg (Kuntner et al., 2010).

We then validated our P-gp imaging protocol in a rat model of regionally heterogeneous cerebral P-gp expression (Bankstahl et al., 2011). We used rats at 48 hours after pilocarpine-induced status epilepticus (SE) that were shown to exhibit a regional upregulation of P-gp in the brain, most notably in hippocampal substructures

Fig. 1. (A) Chemical structures of (R)-[¹¹C]verapamil (left) and tariquidar (right). (B) PET average images (0–60 minutes) of (R)-[¹¹C]verapamil recorded at 2 hours after intravenous administration of tariquidar (3 mg/kg, over 1 minute) in a control rat (upper row) and a rat 48 hours post-status epilepticus (lower row). PET signal reduction in cerebellum is indicated by a white arrow. (C) P-gp immunopositive labeling in brain capillaries of the third cerebellar lobule showing P-gp upregulation in 48 hours post-status epilepticus rat (right) versus control rat (left). Adapted from Bankstahl et al. (2011).

(Bankstahl and Löscher, 2008). Post-SE and control rats underwent (R)-[¹¹C]verapamil PET scans after administration of either 15 mg/kg tariquidar (which completely inhibits P-gp at the BBB) or 3 mg/kg tariquidar (which corresponds to the ED₅₀). Both in control and post-SE animals regional brain distribution of (R)-[¹¹C]verapamil was uniform after administration of 15 mg/kg tariquidar. However, after treatment with 3 mg/kg tariquidar a nonuniform distribution pattern of (R)-[¹¹C]verapamil with clearly reduced uptake in cerebellum was observed in post-SE rats (Bankstahl et al., 2011) (Fig. 1B). PET data were analyzed with a two-tissue, four-rate-constant compartment model, which demonstrated that the efflux rate constant of (R)-[¹¹C]verapamil from brain (k_2) was significantly increased in certain brain regions of post-SE versus control rats (cerebellum, thalamus, hippocampus). In the same brain regions where k_2 was increased P-gp immunoreactivity was also found to be increased (Fig. 1C), suggesting that our PET protocol was capable of measuring regionally increased P-gp function in the rat brain.

We then translated this imaging protocol to healthy human volunteers, who underwent (R)-[¹¹C]verapamil PET scans after administration of different tariquidar doses to identify a tariquidar dose that partly inhibits P-gp at the human BBB (Bauer et al., 2012). A tariquidar dose-dependent increase in (R)-[¹¹C]verapamil brain distribution was also observed in humans. The concentration-response curve of tariquidar reached a plateau in (R)-[¹¹C]verapamil brain distribution at plasma concentrations >1000 ng/ml, suggesting that P-gp was completely inhibited at the human BBB (Bauer et al., 2012). When comparing concentration-response curves of tariquidar in humans and rats, it was noted that half-maximum effective concentrations (EC₅₀) of tariquidar to

enhance brain uptake of (R)-[¹¹C]verapamil were similar (EC₅₀ human: 561 ± 24 ng/ml, rat: 544 ± 32 ng/ml). However, the maximum effect of tariquidar was larger in rats than in humans, with an 11-fold increase in (R)-[¹¹C]verapamil brain distribution over baseline scans (without tariquidar administration) observed in rats and a 2.7-fold increase observed in humans (Bauer et al., 2012).

Our imaging protocol was used at the University of Manchester (UK) and the University College London (UK) within the EU project EURIPIDES in a study in drug-resistant temporal lobe epilepsy patients (Feldmann et al., 2013). Patients and control subjects underwent (R)-[¹¹C]verapamil PET scans before and after administration of tariquidar (either 2 or 3 mg/kg). PET data were analyzed in terms of percent increase in (R)-[¹¹C]verapamil brain uptake (measured as the (R)-[¹¹C]verapamil influx rate constant from plasma into brain K_1 derived from a one-tissue, two-rate-constant compartment model) after tariquidar administration. It was found that the tariquidar-induced increase in (R)-[¹¹C]verapamil K_1 was lower in the hippocampus of drug resistant temporal lobe epilepsy patients (+20–30%) compared with controls (+60–70%) (Feldmann et al., 2013). This effect was not caused by differences in tariquidar plasma concentrations between the two groups (Feldmann et al., 2013) and suggested that P-gp expression was higher in the hippocampus of drug-resistant patients compared with control subjects. Five patients underwent epilepsy surgery, which allowed for correlation of PET results with P-gp immunostaining data in surgically removed brain tissue. A negative correlation between tariquidar-induced increase in (R)-[¹¹C]verapamil K_1 in temporal lobe and P-gp immunostaining in surgically resected brain tissue was found, which confirmed that our approach for analyzing the

PET data was valid, i.e., a high tariquidar-induced increase in (R)-[¹¹C]verapamil K_1 indicated low P-gp expression and a low increase in (R)-[¹¹C]verapamil K_1 indicated high P-gp expression (Feldmann et al., 2013). This study provided first in vivo evidence for regionally increased P-gp function in temporal lobe of drug-resistant epilepsy patients and suggested that (R)-[¹¹C]verapamil PET may become a useful imaging biomarker in epilepsy.

As alternative to (R)-[¹¹C]verapamil for measurement of P-gp function at the BBB, we developed [¹¹C]tariquidar as a potential radiotracer to measure P-gp density at the BBB (Bauer et al., 2010). In vitro autoradiography showed higher binding of [¹¹C]tariquidar in brain slices of FVB wild-type mice compared with *Mdr1a*^{b(-/-)}, *Bcrp1*^(-/-), and *Mdr1a*^{b(-/-)}*Bcrp1*^(-/-) mice, which was displaceable with an excess of unlabeled elacridar (1 μ M) (Bauer et al., 2010) and which pointed to P-gp-specific binding of [¹¹C]tariquidar. However, in vivo [¹¹C]tariquidar showed low brain uptake in wild-type, *Mdr1a*^{b(-/-)}, and *Bcrp1*^(-/-) mice and substantially higher brain uptake in *Mdr1a*^{b(-/-)}*Bcrp1*^(-/-) mice (14.5-fold higher than wild-type), which suggested that [¹¹C]tariquidar behaves as a dual P-gp/Bcrp substrate at the murine BBB (Bankstahl et al., 2013). When mice were pretreated with unlabeled tariquidar (15 mg/kg), brain uptake of [¹¹C]tariquidar was low in animals expressing Bcrp (wild-type and *Mdr1a*^{b(-/-)}) and high in animals lacking Bcrp (*Bcrp1*^(-/-) and *Mdr1a*^{b(-/-)}*Bcrp1*^(-/-)), which suggested that [¹¹C]tariquidar is only transported by Bcrp at therapeutic plasma concentrations (Bankstahl et al., 2013). In vitro uptake experiments with [³H]tariquidar in cell lines overexpressing murine P-gp (*Mdr1a*) or Bcrp supported the assumption that saturation of P-gp transport of tariquidar is reached at lower concentrations than saturation of Bcrp transport (Bankstahl et al., 2013).

We attempted to exploit the observed concentration dependence of tariquidar transport by P-gp to visualize Bcrp function at the murine BBB, which is of interest as recent quantitative proteomics data suggest that BCRP is the most highly expressed ABC transporter at the human BBB (Uchida et al., 2011). We could show that [¹¹C]tariquidar brain distribution in *Mdr1a*^{b(-/-)} mice increased dose dependently when animals were pretreated with increasing doses of the Bcrp inhibitor Ko143 (Wanek et al., 2012). We then pretreated FVB wild-type mice with a dose of tariquidar (8 mg/kg) that completely inhibits P-gp, and subjected these animals to [¹¹C]tariquidar PET scans after administration of different doses of Ko143. This resulted again in a Ko143 dose-dependent increase in [¹¹C]tariquidar brain uptake, which suggested that our PET protocol can be used to visualize Bcrp function at the murine BBB (Wanek et al., 2012).

A first study with [¹¹C]tariquidar in healthy human volunteers revealed lower uptake of [¹¹C]tariquidar in human brain than for (R)-[¹¹C]verapamil (Bauer et al., 2013) (Fig. 2A). In a previous study in healthy human volunteers we observed a pronounced rise in brain concentration-time curves of (R)-[¹¹C]verapamil (+74%) in response to an infusion of unlabeled tariquidar administered during the (R)-[¹¹C]verapamil PET scan (Wagner et al., 2009) (Fig. 2B). This was attributed to inhibition of P-gp at the BBB by tariquidar and influx of unmetabolized (R)-[¹¹C]verapamil from blood into brain. When the same protocol was employed for [¹¹C]tariquidar, only a modest increase in [¹¹C]tariquidar brain distribution was observed in response to tariquidar infusion (+27%) (Bauer et al., 2013) (Fig. 2B). This difference in response of (R)-[¹¹C]verapamil and [¹¹C]tariquidar to administration of unlabeled tariquidar was attributed to the fact that [¹¹C]tariquidar is additionally transported by BCRP at the human BBB, which attenuates the increase in radiotracer distribution to the brain when P-gp is inhibited with tariquidar. Taken together these data suggest that [¹¹C]tariquidar may be used as a PET tracer to study P-gp and BCRP function at the human BBB, which is of interest as recent data suggest that many

therapeutic drugs (such as tyrosine kinase inhibitors) are prevented from reaching therapeutically effective concentrations by combined P-gp and BCRP efflux.

New Radiopharmaceuticals for PET Renography Utilizing Organic Anion Transporter 1 (H.G.)

Radionuclide renography is an important diagnostic study clinically used to evaluate renal function. It is conducted by a dynamic planar imaging technique using technetium-99m (half-life: 6 hours)-labeled radiopharmaceuticals: technetium-99m diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA), technetium-99m mercaptoacetyl triglycine (^{99m}Tc-MAG3, Fig. 3A), and technetium-99m L,L-ethylenedicysteine (^{99m}Tc-L,L-EC, Fig. 3A) (Taylor, 2014). ^{99m}Tc-DTPA is considered a glomerular filtration agent because it is freely filtered by the glomerulus owing to its low plasma protein binding of ~4% and it is neither secreted nor reabsorbed (Klopper et al., 1972). Thus, the renal excretion efficiency of ^{99m}Tc-DTPA is considered to be ~20%, equal to the rate at which it is filtered. ^{99m}Tc-MAG3 and ^{99m}Tc-L,L-EC are considered tubular agents because they are cleared almost exclusively by tubular secretion (Eshima and Taylor, 1992; Van Nerom et al., 1993).

Fig. 2. (A) PET average images (0–60 minutes) of (R)-[¹¹C]verapamil and [¹¹C]tariquidar in the same healthy volunteer. Radiation scale is expressed as standardized uptake value (SUV). (B) Mean ($\pm$ standard deviation) concentration-time curves of (R)-[¹¹C]verapamil and [¹¹C]tariquidar in whole brain of healthy volunteers ($n = 5$). During the PET scan an intravenous infusion of unlabeled tariquidar was given over 30 minutes: (R)-[¹¹C]verapamil: 2 mg/kg tariquidar at 40 minutes after tracer injection; [¹¹C]tariquidar: 3 mg/kg tariquidar at 60 minutes after tracer injection. The time point of the start of tariquidar infusion is indicated by an arrow. Adapted from Wagner et al. (2009) and (Bauer et al., 2013).

The plasma protein binding of ^{99m}Tc -MAG3 was found to be $\sim 90\%$ and is not filtered by glomerulus or reabsorbed (Bubeck et al., 1990). Similarly, the plasma protein-bound fraction of ^{99m}Tc -L,L-EC was found to be $\sim 30\%$ (Van Nerom et al., 1993). The clearance of both ^{99m}Tc -MAG3 and ^{99m}Tc -L,L-EC was found to be ~ 60 and $\sim 75\%$, respectively, of *ortho*-[^{131}I]iodohippurate (^{131}I -OIH, Hippuran, a gold standard tubular agent, Fig. 3A) (Eshima and Taylor, 1992; Van Nerom et al., 1993). ^{131}I -OIH was discontinued from the market after the introduction of ^{99m}Tc -MAG3 because of iodine-131 (half-life: 8 day), which is a β -particle emitter with suboptimal nuclear properties for imaging. Low clearance of ^{99m}Tc -DTPA results in images with high background radioactivity especially in the patients with poor renal function. In this respect, higher clearance of ^{99m}Tc -MAG3 and ^{99m}Tc -L,L-EC enables them to provide better quality images and, thus, are preferred radiopharmaceuticals for renography (Taylor, 2014). In addition, tubular agents are best suited to evaluation of patients with impaired tubular function.

^{99m}Tc -MAG3 is extracted from the plasma present in the peritubular capillaries into the tubular cells via the basolateral membrane governed by the organic anion transporter (OAT) system expressed in the proximal convoluted tubules, e.g., OAT1 and OAT3 (Shikano et al., 2004; Takahara et al., 2013). Results from a recent clinical study suggested a larger contribution of OAT1 than OAT3 for the renal uptake of ^{99m}Tc -MAG3 (Takahara et al., 2013). These transporters are substrate selective, saturable, and mediate the uptake of several drugs. Thus, the treatment with the drugs secreted via those transporters could affect ^{99m}Tc -MAG3 renal uptake and excretion. Although, the carbonylglycine ($-\text{CO}-\text{NH}-\text{CH}_2-\text{COOH}$) moiety is generally believed to be essential for an efficacious fit with the receptor proteins of the OAT system according to Despopoulos' theory (Despopoulos, 1965), in the case of ^{99m}Tc -MAG3 and ^{99m}Tc -L,L-EC, it is assumed that the oxotechnetium glycine ($\text{O}=\text{Tc}-\text{N}-\text{C}-\text{COOH}$) substructure mimics carbonylglycine and fits with the OAT-receptor proteins (Mang'era et al., 1996; Taylor et al., 1997). In addition, the $\text{TcO}-\text{N}_3\text{S}$ and TcON_2S_2 cores of ^{99m}Tc -MAG3 and ^{99m}Tc -L,L-EC, respectively, provide the necessary hydrophobicity required for binding to the hydrophobic pocket of the OAT system (Fritzsche et al., 1989). Transport of ^{99m}Tc -MAG3 from the tubular cell into the urine via the luminal membrane is thought to be governed by MRP2 (Sekine et al., 2006).

Although renography provides important information about the renal function that helps in the diagnosis and management of patients with suspected renal and urinary tract problems; there are drawbacks associated with the use of the current imaging technique. Planar

imaging provides limited structural information, low-quality images, and poor quantitative data. These drawbacks could be overcome by using a tomographic imaging technique such as single photon emission computed tomography (SPECT) or PET. However, conducting a clinical dynamic SPECT study is technically challenging. The clinical SPECT camera depends on rotational movement (step-and-shoot) of detector heads, which cannot be used for renography because of the fast pharmacokinetics exhibited by the renal tubular agents. PET overcomes these technical challenges and enables the use of tomographic imaging for renography. PET uses a series of detector blocks arranged in a cylindrical geometry around the subject, which allows acquisition of imaging data with high temporal and spatial resolution. In addition, PET provides substantially superior quantitative data and also better structural information owing to high sensitivity, spatial resolution, and signal-to-noise ratios (Townsend, 2008). For example, spatial resolutions of a clinical PET camera and a clinical SPECT camera are 4–6 mm and about 10 mm, respectively.

In this regard, we recently reported a fluorine-18 (half-life: 110 minutes)-labeled hippurate analog, *para*-[^{18}F]fluorohippurate (^{18}F -PFH, Fig. 3B) as a potential renal tubular PET agent (Awasthi et al., 2011). ^{18}F -PFH is a PET analog of ^{131}I -OIH (Fig. 3B). ^{18}F -PFH was found to be rapidly excreted exclusively into the urine without undergoing any further metabolism when injected into healthy Sprague-Dawley rats. The *in vivo* plasma protein binding of ^{18}F -PFH was found to be 45.3%. The plasma clearance of ^{18}F -PFH was found to be comparable to ^{131}I -OIH and reduced by $\sim 50\%$ in probenecid-treated rats, which demonstrated that renal excretion of ^{18}F -PFH is principally by active tubular transport. The quality of renograms and images obtained from ^{18}F -PFH PET renography were remarkably better than that obtained from ^{99m}Tc -MAG3 renography (Pathuri et al., 2011). A key advantage of PET renography is that it provides temporal tomographic and slice-specific images of renal parenchyma and quantitative data without additional studies. For example, PET images shown in Fig. 4 could be used as an alternative to renal cortical scintigraphy. Thus, we anticipate that by using PET the clinical value of renography could be significantly increased.

Since ^{18}F -PFH is synthesized by a four-step procedure we evaluated another fluorine-18-labeled hippurate analog, *meta*-cyano-*para*-[^{18}F]fluorohippurate (^{18}F -CNPFH, Fig. 3B), which can be produced by a single-step reaction (Pathuri et al., 2012). ^{18}F -CNPFH was also demonstrated high stability with no metabolic degradation in both *in vitro* and *in vivo* conditions similar to ^{18}F -PFH. The *in vivo* plasma protein binding and erythrocytic uptake of ^{18}F -CNPFH was found to be comparable to that of ^{18}F -PFH. Initial biodistribution and PET renography studies in healthy Sprague Dawley rats revealed a rapid clearance of ^{18}F -CNPFH primarily through the renal-urinary pathway but to a minor extent also through the hepatobiliary pathway. The unexpected hepatobiliary elimination is adding undesirable background signal in the PET images and also could limit its use when the renal function is low. To our knowledge, ^{18}F -PFH and ^{18}F -CNPFH are the only two PET renal tubular agents reported so far in the literature that are suitable for renography. Currently, we are evaluating new renal agents based on a generator-produced PET radionuclide, gallium-68 (half-life: 68 minutes). These agents can be prepared in a kit form, which allows them to be distributed by the traditional nuclear pharmacies in a fashion similar to the Tc-99m radiopharmaceuticals.

In conclusion, our results obtained so far collectively indicate ^{18}F -PFH as a promising lead PET renal tubular agent. ^{18}F -PFH could be an ideal radiopharmaceutical for PET renography since it combines both desirable biologic properties of hippurate and nuclear properties of fluorine-18. The real value of using PET for renography will only be known when appropriate clinical studies are conducted in patients with renal disorders.

Fig. 3. Chemical structures of the renal tubular agents. (A) Agents used clinically and (B) PET agents.

Fig. 4. Abdominal PET/CT images: (A) maximum intensity projection and (B) transverse slice obtained at 1.5 minutes after injection of ^{18}F -PFH in a healthy Sprague-Dawley rats (C). ^{18}F -PFH PET renogram of a healthy female Sprague-Dawley rat. For renography, dynamic PET imaging data in list-mode was acquired over a period of 20 minutes after injection followed by CT imaging for 2 minutes. The PET scan time was divided into 30-second frames, and the data were reconstructed using a two-dimensional filtered back-projection algorithm. Regions of interest (ROIs) were drawn over kidneys guided by CT images using Amira 3.1 image visualization and analysis software. Radioactivity within ROIs of each frame was calculated and decay-corrected. The ROI activities were converted into percentage of injected dose (%ID). The time-activity curve of both kidneys (renogram) was plotted from the kidney %ID in each frame.

Summary

The role of transport systems is now well established in diagnosing diseases with endogenous and exogenous diagnostic markers. The development and the application of novel probes, especially for imaging, is rapidly progressing and has made possible the study of transport processes in vivo. In conjunction, rapid progress in quantifying the amount of individual transporters will lead to the development of better PBPK models. Although many new opportunities for research are at hand, clearly more work is needed before an understanding of the mechanistic details of the transporters allows an understanding of changes in transporter function in disease states. This should ultimately lead to the improvement in the use of imaging as a predictive tool for individual patients.

Authorship Contributions

Participated in research design: Unadkat, Prasad, Gali.

Conducted experiments: Prasad, Gali.

Contributed new reagents or analytic tools: Unadkat, Prasad, Gali.

Performed data analysis: Prasad, Gali.

Wrote or contributed to the writing of the manuscript: Stieger, Unadkat, Prasad, Langer, Gali.

References

- Awasthi V, Pathuri G, Agashe HB, and Gali H (2011) Synthesis and in vivo evaluation of p-18F-Fluorohippurate as a new radiopharmaceutical for assessment of renal function by PET. *J Nucl Med* **52**:147–153.
- Bankstahl JP and Löscher W (2008) Resistance to antiepileptic drugs and expression of P-glycoprotein in two rat models of status epilepticus. *Epilepsy Res* **82**:70–85.
- Bankstahl JP, Bankstahl M, Kuntner C, Stanek J, Wanek T, Meier M, Ding XQ, Müller M, Langer O, and Löscher W (2011) A novel positron emission tomography imaging protocol identifies seizure-induced regional overactivity of P-glycoprotein at the blood-brain barrier. *J Neurosci* **31**:8803–8811.
- Bankstahl JP, Bankstahl M, Römermann K, Wanek T, Stanek J, Windhorst AD, Fedorowicz M, Erker T, Müller M, and Löscher W, et al. (2013) Tariquidar and elacridar are dose-dependently transported by P-glycoprotein and Bcrp at the blood-brain barrier: a small-animal positron emission tomography and in vitro study. *Drug Metab Dispos* **41**:754–762.
- Bauer F, Kuntner C, Bankstahl JP, Wanek T, Bankstahl M, Stanek J, Mairinger S, Dörner B, Löscher W, and Müller M, et al. (2010) Synthesis and in vivo evaluation of [^{11}C]tariquidar, a positron emission tomography radiotracer based on a third-generation P-glycoprotein inhibitor. *Bioorg Med Chem* **18**:5489–5497.
- Bauer M, Zeitlinger M, Karch R, Matzner P, Stanek J, Jäger W, Böhmendorfer M, Wadsak W, Mitterhauser M, and Bankstahl JP, et al. (2012) Pgp-mediated interaction between (R)-[^{11}C]verapamil and tariquidar at the human blood-brain barrier: a comparison with rat data. *Clin Pharmacol Ther* **91**:227–233.
- Bauer M, Karch R, Zeitlinger M, Stanek J, Philippe C, Wadsak W, Mitterhauser M, Jäger W, Haslacher H, and Müller M, et al. (2013) Interaction of 11C-tariquidar and 11C-elacridar with P-glycoprotein and breast cancer resistance protein at the human blood-brain barrier. *J Nucl Med* **54**:1181–1187.
- Bubeck B, Brandau W, Weber E, Kälble T, Parekh N, and Georgi P (1990) Pharmacokinetics of technetium-99m-MAG3 in humans. *J Nucl Med* **31**:1285–1293.
- Daali Y, Millet P, Dayer P, and Pastor CM (2013) Evidence of drug-drug interactions through uptake and efflux transport systems in rat hepatocytes: implications for cellular concentrations of competing drugs. *Drug Metab Dispos* **41**:1548–1556.
- de Graaf W, Häusler S, Heger M, van Ginneven TM, van Cappellen G, Binnink RJ, Kullak-Ublick GA, Hesselmann R, van Gulik TM, and Stieger B (2011) Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green. *J Hepatol* **54**:738–745.
- Deo AK, Prasad B, Balogh L, Lai Y, and Unadkat JD (2012) Interindividual variability in hepatic expression of the multidrug resistance-associated protein 2 (MRP2/ABCC2): quantification by liquid chromatography/tandem mass spectrometry. *Drug Metab Dispos* **40**:852–855.
- Deo AK, Borson S, Link JM, Domino K, Eary JF, Ke B, Richards TL, Mankoff DA, Minoshima S, and O'Sullivan F, et al. (2014) Activity of P-Glycoprotein, a β -Amyloid Transporter at the Blood-Brain Barrier, Is Compromised in Patients with Mild Alzheimer Disease. *J Nucl Med* **55**:1106–1111.
- Despopoulos A (1965) A definition of substrate specificity in renal transport of organic anions. *J Theor Biol* **8**:163–192.
- Drozdzik M, Gröer C, Penski J, Lapczuk J, Ostrowski M, Lai Y, Prasad B, Unadkat JD, Siegmund W, and Oswald S (2014) Protein abundance of clinically relevant multidrug transporters along the entire length of the human intestine. *Mol Pharm* [Epub ahead of print].
- Eshima D and Taylor A, Jr (1992) Technetium-99m (99mTc) mercaptoacetyltriglycine: update on the new 99mTc renal tubular function agent. *Semin Nucl Med* **22**:61–73.
- Esteller A (2008) Physiology of bile secretion. *World J Gastroenterol* **14**:5641–5649.
- Eyal S, Chung FS, Muzi M, Link JM, Mankoff DA, Kaddoumi A, O'Sullivan F, Hebert MF, and Unadkat JD (2009) Simultaneous PET imaging of P-glycoprotein inhibition in multiple tissues in the pregnant nonhuman primate. *J Nucl Med* **50**:798–806.
- Eyal S, Ke B, Muzi M, Link JM, Mankoff DA, Collier AC, and Unadkat JD (2010) Regional P-glycoprotein activity and inhibition at the human blood-brain barrier as imaged by positron emission tomography. *Clin Pharmacol Ther* **87**:579–585.
- Fattinger K, Cattori V, Hagenbuch B, Meier PJ, and Stieger B (2000) Rifampicin SV and rifampicin exhibit differential inhibition of the hepatic rat organic anion transporting polypeptides, Oatp1 and Oatp2. *Hepatology* **32**:82–86.
- Feldmann M, Asselin MC, Liu J, Wang S, McMahon A, Anton-Rodriguez J, Walker M, Symms M, Brown G, and Hinz R, et al. (2013) P-glycoprotein expression and function in patients with temporal lobe epilepsy: a case-control study. *Lancet Neurol* **12**:777–785.
- Frisch K, Jakobsen S, Sørensen M, Munk OL, Alstrup AK, Ott P, Hofmann AF, and Keiding S (2012) [N-methyl- ^{11}C]cholylsarcosine, a novel bile acid tracer for PET/CT of hepatic excretory function: radiosynthesis and proof-of-concept studies in pigs. *J Nucl Med* **53**:772–778.
- Fritzsche G, Rummich G, and Ullrich KJ (1989) Anion transport through the contraluminal cell membrane of renal proximal tubule. The influence of hydrophobicity and molecular charge distribution on the inhibitory activity of organic anions. *Biochim Biophys Acta* **978**:249–256.
- Geier A, Wagner M, Dietrich CG, and Trauner M (2007) Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. *Biochim Biophys Acta* **1773**:283–308.
- Hagenbuch B and Stieger B (2013) The SLCO (former SLC21) superfamily of transporters. *Mol Aspects Med* **34**:396–412.
- He J, Yu Y, Prasad B, Link J, Miyaoka RS, Chen X, and Unadkat JD (2014) PET imaging of Oatp-mediated hepatobiliary transport of [^{11}C] rosuvastatin in the rat. *Mol Pharm* **11**:2745–2754.
- Hoekstra LT, de Graaf W, Nibourg GA, Heger M, Binnink RJ, Stieger B, and van Gulik TM (2013) Physiological and biochemical basis of clinical liver function tests: a review. *Ann Surg* **257**:27–36.
- Hsiao P, Bui T, Ho RJ, and Unadkat JD (2008) In vitro-to-in vivo prediction of P-glycoprotein-based drug interactions at the human and rodent blood-brain barrier. *Drug Metab Dispos* **36**:481–484.
- Huppertz A, Breuer J, Fels LM, Schultze-Mosgau M, Sutter G, Klein S, Frericks B, Hamm B, and Wagner M (2011) Evaluation of possible drug-drug interaction between gadoxetic acid and erythromycin as an inhibitor of organic anion transporting peptides (OATP). *J Magn Reson Imaging* **33**:409–416.
- Ishizawa T, Masuda K, Urano Y, Kawaguchi Y, Satou S, Kaneko J, Hasegawa K, Shibahara J, Fukayama M, and Tsuji S, et al. (2014) Mechanistic background and clinical applications of indocyanine green fluorescence imaging of hepatocellular carcinoma. *Ann Surg Oncol* **21**:440–448.

- Jacquemin E, Hagenbuch B, Stieger B, Wolkoff AW, and Meier PJ (1994) Expression cloning of a rat liver Na(+)-independent organic anion transporter. *Proc Natl Acad Sci USA* **91**:133–137.
- Jia L, Jiang D, Hu P, Li X, Shi H, Cheng D, and Zhang L (2014) Synthesis and evaluation of (18) F-labeled bile acid compound: a potential PET imaging agent for FXR-related diseases. *Nucl Med Biol* **41**:495–500.
- Kalliokoski A, Backman JT, Neuvonen PJ, and Niemi M (2008) Effects of the SLCO1B1*1B haplotype on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide. *Pharmacogenet Genomics* **18**:937–942.
- Kalvass JC, Polli JW, Bourdet DL, Feng B, Huang SM, Liu X, Smith QR, Zhang LK, and Zamek-Gliszczynski MJ; International Transporter Consortium (2013) Why clinical modulation of efflux transport at the human blood-brain barrier is unlikely: the ITC evidence-based position. *Clin Pharmacol Ther* **94**:80–94.
- Ke AB, Eyal S, Chung FS, Link JM, Mankoff DA, Muzi M, and Unadkat JD (2013) Modeling cyclosporine A inhibition of the distribution of a P-glycoprotein PET ligand, 11C-verapamil, into the maternal brain and fetal liver of the pregnant nonhuman primate: impact of tissue blood flow and site of inhibition. *J Nucl Med* **54**:437–446.
- Kepler D (2011) Multidrug resistance proteins (MRPs, ABCs): importance for pathophysiology and drug therapy. *Handb Exp Pharmacol* (201):299–323.
- Klopper JF, Hauser W, Atkins HL, Eckelman WC, and Richards P (1972) Evaluation of 99m Tc-DTPA for the measurement of glomerular filtration rate. *J Nucl Med* **13**:107–110.
- Kullak-Ublick GA, Hagenbuch B, Stieger B, Wolkoff AW, and Meier PJ (1994) Functional characterization of the basolateral rat liver organic anion transporting polypeptide. *Hepatology* **20**:411–416.
- Kullak-Ublick GA, Ismail MG, Stieger B, Landmann L, Huber R, Pizzagalli F, Fattinger K, Meier PJ, and Hagenbuch B (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* **120**:525–533.
- Kuntner C, Bankstahl JP, Bankstahl M, Stanek J, Wanek T, Stundner G, Karch R, Brauner R, Meier M, and Ding X, et al. (2010) Dose-response assessment of tariquidar and elacridar and regional quantification of P-glycoprotein inhibition at the rat blood-brain barrier using (R)-[(11) C]verapamil PET. *Eur J Nucl Med Mol Imaging* **37**:942–953.
- LaRusso NF, Hoffman NE, Hofmann AF, and Korman MG (1975) Validity and sensitivity of an intravenous bile acid tolerance test in patients with liver disease. *N Engl J Med* **292**:1209–1214.
- Leuthold S, Hagenbuch B, Mohebbi N, Wagner CA, Meier PJ, and Stieger B (2009) Mechanisms of pH-gradient driven transport mediated by organic anion polypeptide transporters. *Am J Physiol Cell Physiol* **296**:C570–C582.
- Li N, Bi YA, Duignan DB, and Lai Y (2009) Quantitative expression profile of hepatobiliary transporters in sandwich cultured rat and human hepatocytes. *Mol Pharm* **6**:1180–1189.
- Löscher W, Luna-Tortós C, Römermann K, and Fedrowitz M (2011) Do ATP-binding cassette transporters cause pharmacoresistance in epilepsy? Problems and approaches in determining which antiepileptic drugs are affected. *Curr Pharm Des* **17**:2808–2828.
- Mairinger S, Erker T, Muller M, and Langer O (2011) PET and SPECT radiotracers to assess function and expression of ABC transporters in vivo. *Curr Drug Metab* **12**:774–792.
- Mang'era KO, Vanbilloen HP, Bellande E, Pasqualini R, and Verbruggen AM (1996) Influence of a 99mTcN core on the biological and physicochemical behavior of 99mTc complexes of L, L-EC and L,L-ECD. *Nucl Med Biol* **23**:987–993.
- Meier PJ and Stieger B (2002) Bile salt transporters. *Annu Rev Physiol* **64**:635–661.
- Müllauer J, Kuntner C, Bauer M, Bankstahl JP, Müller M, Voskuyl RA, Langer O, and Sävänen S (2012) Pharmacokinetic modeling of P-glycoprotein function at the rat and human blood-brain barriers studied with (R)-[(11)C]verapamil positron emission tomography. *EJNMMI Res* **2**:58.
- Muzi M, Mankoff DA, Link JM, Shoner S, Collier AC, Sasongko L, and Unadkat JD (2009) Imaging of cyclosporine inhibition of P-glycoprotein activity using 11C-verapamil in the brain: studies of healthy humans. *J Nucl Med* **50**:1267–1275.
- Ohtsui S, Uchida Y, Kubo Y, and Terasaki T (2011) Quantitative targeted absolute proteomics-based ADME research as a new path to drug discovery and development: methodology, advantages, strategy and prospects. *J. Pharm. Sci.* **100**: 3547–3559.
- Pastor CM, Müllhaupt B, and Stieger B (2014) The role of organic anion transporters in diagnosing liver diseases by magnetic resonance imaging. *Drug Metab Dispos* **42**:675–684.
- Pathuri G, Sahoo K, Awasthi V, and Gali H (2011) Renogram comparison of p-[(18)F] fluorohippurate with o-[(125)I]iodohippurate and [(99m)Tc]MAG3 in normal rats. *Nucl Med Commun* **32**:908–912.
- Pathuri G, Hedrick AF, Awasthi V, and Gali H (2012) Single-step radiosynthesis and in vivo evaluation of a novel fluorine-18 labeled hippurate for use as a PET renal agent. *Nucl Med Biol* **39**:1195–1201.
- Pollheimer MJ, Fickert P, and Stieger B (2014) Chronic cholestatic liver diseases: clues from histopathology for pathogenesis. *Mol Aspects Med* **37**:35–56.
- Prasad B, Lai Y, Lin Y, and Unadkat JD (2013) Interindividual variability in the hepatic expression of the human breast cancer resistance protein (BCRP/ABCG2): effect of age, sex, and genotype. *J Pharm Sci* **102**:787–793.
- Prasad B and Unadkat JD (2014) Optimized approaches for quantification of drug transporters in tissues and cells by MRM proteomics. *AAPS J* **16**:634–648.
- Prasad B, Evers R, Gupta A, Hop CE, Salphati L, Shukla S, Ambudkar SV, and Unadkat JD (2014) Interindividual variability in hepatic organic anion-transporting polypeptides and P-glycoprotein (ABCB1) protein expression: quantification by liquid chromatography tandem mass spectroscopy and influence of genotype, age, and sex. *Drug Metab Dispos* **42**:78–88.
- Römermann K, Wanek T, Bankstahl M, Bankstahl JP, Fedrowitz M, Müller M, Löscher W, Kuntner C, and Langer O (2013) (R)-[(11)C]verapamil is selectively transported by murine and human P-glycoprotein at the blood-brain barrier, and not by MRP1 and BCRP. *Nucl Med Biol* **40**:873–878.
- Sakka SG (2007) Assessing liver function. *Curr Opin Crit Care* **13**:207–214.
- Sasongko L, Link JM, Muzi M, Mankoff DA, Yang X, Collier AC, Shoner SC, and Unadkat JD (2005) Imaging P-glycoprotein transport activity at the human blood-brain barrier with positron emission tomography. *Clin Pharmacol Ther* **77**:503–514.
- Sathirakul K, Suzuki H, Yasuda K, Hanano M, Tagaya O, Horie T, and Sugiyama Y (1993) Kinetic analysis of hepatobiliary transport of organic anions in Eisai hyperbilirubinemic mutant rats. *J Pharmacol Exp Ther* **265**:1301–1312.
- Sekine T, Miyazaki H, and Endou H (2006) Molecular physiology of renal organic anion transporters. *Am J Physiol Renal Physiol* **290**:F251–F261.
- Shawahna R, Uchida Y, Declèves X, Ohtsuki S, Yousif S, Dauchy S, Jacob A, Chassoux F, Daumas-Duport C, and Couraud PO, et al. (2011) Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels. *Mol Pharm* **8**:1332–1341.
- Shikano N, Kanai Y, Kawai K, Ishikawa N, and Endou H (2004) Transport of 99mTc-MAG3 via rat renal organic anion transporter 1. *J Nucl Med* **45**:80–85.
- Stieger B (2011) The role of the sodium-taurocholate cotransporting polypeptide (NTCP) and of the bile salt export pump (BSEP) in physiology and pathophysiology of bile formation. *Handb Exp Pharmacol* (201):205–259.
- Stieger B and Hagenbuch B (2014) Organic anion-transporting polypeptides. *Curr Top Membr* **73**:205–232.
- Stieger B, Heger M, de Graaf W, Paumgartner G, and van Gulik T (2012) The emerging role of transport systems in liver function tests. *Eur J Pharmacol* **675**:1–5.
- Takahara N, Saga T, Inubushi M, Kusuhara H, Seki C, Ito S, Oyama N, Yokoyama O, Sugiyama Y, and Fujibayashi Y (2013) Drugs interacting with organic anion transporter-1 affect uptake of Tc-99m-mercaptoacetyl-triglycine (MAG3) in the human kidney: therapeutic drug interaction in Tc-99m-MAG3 diagnosis of renal function and possible application of Tc-99m-MAG3 for drug development. *Nucl Med Biol* **40**:643–650.
- Taylor A, Hansen L, Eshima D, Malveaux E, Folks R, Shattuck L, Lipowska M, and Marzilli LG (1997) Comparison of technetium-99m-II-EC isomers in rats and humans. *J Nucl Med* **38**: 821–826.
- Taylor AT (2014) Radionuclides in nephrourology, part 1: Radiopharmaceuticals, quality control, and quantitative indices. *J Nucl Med* **55**:608–615.
- Townsend DW (2008) Positron emission tomography/computed tomography. *Semin Nucl Med* **38**:152–166.
- Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J, and Terasaki T (2011) Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. *J Neurochem* **117**:333–345.
- Uchida Y, Tachikawa M, Obuchi W, Hoshi Y, Tomioka Y, Ohtsuki S, and Terasaki T (2013) A study protocol for quantitative targeted absolute proteomics (QTAP) by LC-MS/MS: application for inter-strain differences in protein expression levels of transporters, receptors, claudin-5, and marker proteins at the blood-brain barrier in ddY, FVB, and C57BL/6J mice. *Fluids Barriers CNS* **10**:21.
- Van Beers BE, Pastor CM, and Hussain HK (2012) Primovist, Eovist: what to expect? *J Hepatol* **57**:421–429.
- Van Nerom CG, Bormans GM, De Roo MJ, and Verbruggen AM (1993) First experience in healthy volunteers with technetium-99m L,L-ethylenediacysteine, a new renal imaging agent. *Eur J Nucl Med* **20**:738–746.
- Wagner CC, Bauer M, Karch R, Feurstein T, Kopp S, Chiba P, Kletter K, Löscher W, Müller M, and Zeitlinger M, et al. (2009) A pilot study to assess the efficacy of tariquidar to inhibit P-glycoprotein at the human blood-brain barrier with (R)-11C-verapamil and PET. *J Nucl Med* **50**:1954–1961.
- Wanek T, Kuntner C, Bankstahl JP, Mairinger S, Bankstahl M, Stanek J, Sauberer M, Filip T, Erker T, and Müller M, et al. (2012) A novel PET protocol for visualization of breast cancer resistance protein function at the blood-brain barrier. *J Cereb Blood Flow Metab* **32**: 2002–2011.

Address correspondence to: Dr. Bruno Stieger, University Hospital Zurich, Department of Clinical Pharmacology and Toxicology, Raemistrasse 100, 8091 Zurich, Switzerland. E-mail: bruno.stieger@uzh.ch