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Drug interactions at the blood-brain barrier: fact or fantasy?

Sara Eyal, **Peng Hsiao**, and **Jashvant D. Unadkat**
Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, Washington

Abstract

There is considerable interest in the therapeutic and adverse outcomes of drug interactions at the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). These include altered efficacy of drugs used in the treatment of CNS disorders, such as AIDS dementia and malignant tumors, and enhanced neurotoxicity of drugs that normally penetrate poorly into the brain. BBB- and BCSFB-mediated interactions are possible because these interfaces are not only passive anatomical barriers, but are also dynamic in that they express a variety of influx and efflux transporters and drug metabolizing enzymes. Based on studies in rodents, it has been widely postulated that efflux transporters play an important role at the human BBB in terms of drug delivery. Furthermore, it is assumed that chemical inhibition of transporters or their genetic ablation in rodents is predictive of the magnitude of interaction to be expected at the human BBB. However, studies in humans challenge this well-established paradigm and claim that such drug interactions will be lesser in magnitude but yet may be clinically significant. This review focuses on current known mechanisms of drug interactions at the blood-brain and blood-CSF barriers and the potential impact of such interactions in humans. We also explore whether such drug interactions can be predicted from preclinical studies. Defining the mechanisms and the impact of drug-drug interactions at the BBB is important for improving efficacy of drugs used in the treatment of CNS disorders while minimizing their toxicity as well as minimizing neurotoxicity of non-CNS drugs.

Keywords

Blood-brain barrier; blood-cerebrospinal fluid barrier; drug interactions; P-glycoprotein; ABC transporters; *in vitro-in vivo* correlations

1. Introduction

Drug-drug interactions (DDIs) have long been recognized as an important cause of alteration in drug efficacy or adverse drug effects (or toxicity), particularly for drugs that have a narrow therapeutic window. Much of the work on DDIs has been focused on changes in absorption, bioavailability or systemic concentration of the drug (Levy et al., 2000). However, it has been increasingly recognized that DDIs can affect the distribution of drugs into a particular compartment (e.g CNS) with or without affecting their systemic plasma (or blood) concentration. Furthermore, DDIs can result in CNS effect of medications that normally are not targeted to the brain (Endres et al., 2006).

Address correspondence to: Dr. Jashvant D. Unadkat, Department of Pharmaceutics, University of Washington, Box 357610, Seattle, WA 98195, Telephone: 206-543-9434, Fax: 206-543-3204, jash@u.washington.edu.

DDIs that involve the CNS can result from 1) changes in plasma concentrations (unbound or total) of at least one of the interacting drugs (pharmacokinetic interactions), 2) changes in drug's effects at target sites or its disposition within the CNS (pharmacodynamic and pharmacokinetic interactions, respectively), or a combination of the two (Table 1). A third source for altered effects of drugs on the CNS resides in the interface between plasma and the CNS, namely the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). By modulating BBB or BCSFB function, a drug can affect the distribution of another drug into the brain, its removal from the brain, or both. In this case, the plasma concentration of the affected drug often remains unchanged, especially when only a small fraction of the dose distributes into the brain. To distinguish between barrier-mediated interactions and those caused by other mechanisms, the concentration of the affected drug should be measured in the CNS, in the presence and the absence of the precipitant drug. In the clinical setting, however, brain concentrations are normally not measured due to technical and ethical reasons. Thus, BBB-based interactions may be overlooked or confused with pharmacodynamic interactions. From the clinical point of view, DDIs that seem to be unexpected could potentially be prevented if their mechanisms are correctly identified.

The aim of this review is to present an overview of currently known mechanisms of drug interactions at blood-brain interfaces and the potential impact of such interactions. Particularly, we will focus on transporter-mediated DDIs. Most of the existing knowledge on DDIs at the BBB is based on studies in animal models, but few clinical studies and case reports are also available. *In vitro* studies are beyond the scope of this review, but general principles for prediction of DDIs at the human BBB from *in vitro* studies as well as from studies in animal models are presented. Detailed discussion of BBB structure and function and methodologies for evaluation of brain penetration of drugs are available elsewhere (Cecchelli et al., 2007; Endres et al., 2006; Hawkins & Davis, 2005; Langer & Müller, 2004; Liu et al., 2008; Nicolazzo et al., 2006; Redzic & Segal, 2004; Shen et al., 2004; Upton, 2007).

2. Drug transfer across blood-brain barriers

The BBB and the BCSFB are formed by brain endothelial cells and choroid plexus (CP) epithelial cells, respectively. Over the past few years it has been demonstrated that the BBB and the BCSFB are not only anatomical barriers, but also dynamic tissues that express multiple transporters and drug-metabolizing enzymes. Furthermore, brain capillaries are closely associated with perivascular astrocytic end-feet, pericytes, microglia and neuronal processes that regulate BBB permeability and, together with brain endothelial cells, constitute a “neurovascular unit” (Abbott et al., 2006; Hawkins & Davis, 2005).

2.1. The structural barrier

About a century ago, Ehrlich and Goldman demonstrated the existence of a barrier to solute distribution between the circulation and the CNS (Ehrlich, 1885; Goldmann, 1913). The nature of the barrier remained a mystery for many decades and is still being refined (Hawkins & Davis, 2005). In the late 1960's, Reese, Karnovsky and Brightman demonstrated that the BBB is a diffusion barrier formed by tight junctions (TJs) between adjacent brain capillary endothelial cells (Brightman & Reese, 1969; Reese & Karnovsky, 1967). Under physiological conditions, the TJs limit the paracellular diffusion of polar molecules between the circulation and brain interstitial fluid (ISF). The transcellular transfer of molecules is restricted by the absence of fenestrations and low transcytosis (Hawkins & Davis, 2005). Consequently, passive diffusion of drugs across the BBB is limited to small, lipophilic compounds, such as benzodiazepines and barbiturates. In certain brain structures that are adjacent to the ventricles (circumventricular organs), including the area postrema (the vomiting center) and the neurohypophysis, the endothelium is leaky (Shen *et al.*, 2004). Neurons in these structures are therefore exposed to substances in the bloodstream that are inaccessible to other brain regions. In addition, newly

formed blood vessels within a brain tumor (the “blood-tumor barrier”), are heterogenous and often relatively permeable (Bellavance et al., 2008; Gerstner & Fine, 2007; Motl et al., 2006).

For drugs that diffuse rapidly across brain endothelial cells, distribution into the brain may be limited by cerebral blood flow and not by the rate of diffusion across the BBB (“flow limited” kinetics) (Shen et al., 2004; Upton, 2007). In the resting state, a nearly 4-fold difference in blood flow exists between gray and white matter and variations in regional blood flow have been described within the gray matter (Raichle *et al.*, 2001). This, in turn, may result in regional variations in drug exposure. Regional cerebral blood flow may be altered during disease states and by a variety of drugs, including anesthetic agents (Cole et al.,2007; Långsjö et al., 2005), anticonvulsants (Joo et al., 2006; Spanaki et al., 1999; Venneri, 2007), antidepressnts (Heideman et al., 1986) and antihypertensive drugs (Hanyu et al., 2007; Sare et al., 2008). Thus, a drug that affects regional cerebral blood flow may alter the regional distribution of itself, another drug, or related metabolites, that exhibit “flow limited” kinetics, such as desmethyl-loperamide (Liow et al., 2009).

The BSCFB is primarily located within the CP, a leaflike organ that projects into brain ventricles. At the BCSFB, CP epithelial cells and not endothelial cells are sealed by TJs and limit drug transfer between blood and CSF (Redzic & Segal, 2004). The CP produces the CSF and regulates the transfer of endogenous and exogenous compounds between ventricular CSF and blood. The CSF leaves the CNS by reabsorption across the arachnoid epithelium and has a total turnover rate of about 0.38% per minute. Thus, ventricular CSF is replaced approximately 5 times every 24 hours (Motl *et al.*, 2006). This high turnover rate forms a net diffusion gradient between brain ISF and CSF, thereby enhancing drug removal from the CNS back into the general circulation.

Drugs can be transferred between blood and CNS across brain capillaries or epithelial cells of the CP or be removed from the CNS by bulk flow of the CSF and reabsorption at the arachnoid villi. Nevertheless, individual neurons may be located millimeters or centimeters from brain ventricles or circumventricular organs, but only less than 20 nm from a brain capillary (Schlageter et al., 1999). Hence, the primary interface for the transfer of drugs between the circulation and the CNS is the BBB.

2.2. The metabolic barrier

The transfer of drugs across the BBB and BCSFB is further restricted by a “metabolic barrier”, formed by enzymes capable of metabolizing endogenous compounds and xenobiotics. Cytochrome P450 (CYP) 1A and 2B (Ghersi-Egea et al., 1994), monoamine oxidase, catechol-O-methyl transferase (Lindvall et al., 1980), epoxide hydrolase, UDP-glucuronosyltransferase (Ghersi-Egea et al., 1994), and glutathione S-transferase (GST) (Bauer et al., 2008; Strazielle & Ghersi-Egea, 1999) have been identified in blood-brain interfaces of rodents, in particular at the choroid epithelium. Furthermore, Bauer et al. demonstrated that dexamethasone induces the expression of GSTπ in isolated rat brain capillaries (Bauer et al., 2008). A more limited set of data indicates that monoamine oxidase (Kalaria et al., 1988), epoxide hydrolase (Sura et al., 2008), GST (Ghersi-Egea et al., 2006) and the sulfotransferase isoenzyme SULT1A1 (Richard *et al.*, 2001) are active at the human CP. More recently, Dauchy et al. reported that CYP1B1, which is involved in the metabolism of endogenous compounds, is the predominant CYP isoform in human brain microvessels (Dauchy et al., 2008). In the immortalized human cerebral microvascular endothelial cell line hCMEC/D3 CYP1B1 is inducible, although the predominant form in these cells is CYP2U1 (Dauchy et al., 2008). CYP3A4, CYP2C9 and CYP2D6 which are involved in the hepatic metabolism of about 50% of drugs, have not been not detected at the human BBB (Dauchy et al., 2009) and the impact of the enzymatic barrier on cerebral disposition of drugs is currently unknown.

2.3. Drug transporters at blood-brain interfaces

Various transport processes operate at the BBB and the BCSFB to transfer essential molecules into the brain and to efflux waste products and potential toxins out of the brain. Transporters are located at the luminal and abluminal membranes of endothelial cells and CP epithelial cells and transfer a variety of molecules, including amino acids, glucose and hormones, as well as many drugs, in both the blood-to-brain and brain-to-blood directions (Fig. 1). Uptake transporters facilitate substrate influx into brain capillary endothelial cells and CP epithelial cells, whereas efflux transporters export their substrates from the cells, although some transporters (e.g. facilitative) can mediate both substrate influx and efflux. Localization of efflux transporters on the blood-facing membrane of blood-brain barriers is generally associated with drug removal from brain ISF (or CSF) (Fig. 1). This is because decreased drug concentrations in the cell cytoplasm drives substrate passage from brain ISF into endothelial cells or CP epithelial cells and further efflux to blood. For many drugs, the net transfer across these barriers is determined by interplay between several transport systems which can operate in the same direction or opposite directions. Differences between the BBB and the BCSFB in expression and function of these transporters may contribute to the different pharmacokinetics of drugs in the ISF, compared to CSF. Many drug transporters have also been detected in the brain parenchyma (Lee et al., 2001). However, so far only endothelial (or epithelial, in the CP) transporters have been directly associated with pharmacokinetic DDIs.

Drug transporters belong to two major superfamilies, ABC (adenosine triphosphate binding cassette) and SLC (solute carrier) transporters. Another non-ABC, non-SLC protein, RLIP76, has been associated with drug resistance in patients with epilepsy (Awasthi et al., 2005), but its localization and function remain controversial (Soranzo *et al.*, 2007).

2.3.1. Transporters of the ABC superfamily—ABC transporters are primary active transporters, which couple ATP hydrolysis to active efflux of their substrates against concentration gradients. The 49 human ABC transporter genes are classified into seven subfamilies designated A through G (Dean & Annilo, 2005). The most extensively studied BBB transporter of the ABC family is P-glycoprotein (P-gp), but members of the MRP family (ABCC) and breast cancer resistance protein (BCRP; ABCG2) have also been identified in brain endothelial cells and CP epithelial cells.

P-glycoprotein: P-gp is encoded in humans by the multidrug resistance gene MDR1 (ABCB1). In mice and rats, two multidrug resistance proteins are encoded by the genes Mdr1a and Mdr1b (Gottesman & Pastan, 1993; Schinkel et al., 1997). P-gp was initially discovered in 1976 in multidrug resistant tumor cell lines (Juliano & Ling, 1976). Subsequent studies have shown that P-gp is expressed in healthy tissues, including those involved in drug absorption, distribution and elimination, namely the small intestine, the BBB, liver and kidney (Cordon-Cardo et al., 1990; Thiebaut et al., 1987). In brain capillaries, P-gp is predominantly expressed in the luminal membrane (Beaulieu et al., 1997; Lee et al., 2005; Roberts et al., 2008a; Stewart et al., 1996; Sugawara et al., 1990; Tsuji et al., 1992). There, it extrudes substrates back into the circulation after they initially diffuse into the endothelial cell membrane, thereby restricting their penetration into the brain. Bendayan et al. have suggested that endothelial P-gp is expressed abluminally and intracellularly as well (Babakhanian et al., 2007; Bendayan et al., 2006). P-gp has also been detected in blood vessels that supply human gliomas and metastatic brain tumors, but at reduced levels, compared to those at the BBB (Gerstner & Fine, 2007). Both Mdr1a and Mdr1b are found in rodent brain, but only Mdr1a is found in endothelial cells (Schinkel et al., 1997). Compared to the BBB, the localization of P-gp at the BCSFB is less well established. P-gp expression (by immunostaining) in the CP of human adults, neonates and in rats has been detected by some investigators (Daood et al., 2008; Rao et al., 1999), but others have reported it to be undetectable (Gazzin et al., 2008; Roberts et al., 2008a). When

detected in native CP and cultured CP epithelial cells, P-gp is mainly located at the apical (CSF-facing) membrane and in sub-apical cell compartments (Baehr et al., 2006; Rao et al., 1999). This apical membrane localization is thought to allow P-gp to transport substrates into the CSF (Fig. 1). Thus, the direction of substrate transport at the BCSFB is likely opposite to that at the BBB (de Lange, 2004), although direct evidence for such transport in humans is not available.

Because P-gp was initially discovered as a mediator of drug resistance in tumor cells, the first identified substrates were mainly agents used in cancer chemotherapy, such as vinca alkaloids, taxanes and anthracyclines. However, many commonly prescribed drugs from various chemical and pharmacological classes are now known to be P-gp substrates (Table 2). Typically, these substrates (ranging in size from less than 200 to almost 1900 Daltons) are organic amphipathic molecules (Endres et al., 2006;Lin & Yamazaki, 2003). The list includes the antiretroviral agents indinavir, nelfinavir and saquinavir (Kim et al., 1998), the immunosuppressants cyclosporine A (cyclosporine) and tacrolimus, the cardiac agents digoxin and verapamil and the opioid loperamide.

Virtually every paper in the field attributes P-gp's functional importance at the BBB to the findings obtained in Mdr1a$^{(-/-)}$ knockout (KO) mice and other P-gp-deficient animal models. In the next sections we will examine to what extant this statement is true for the human BBB. Upon their development in 1994, Mdr1a$^{(-/-)}$ KO mice showed complete absence of P-gp in brain endothelial cells and displayed almost 100-fold greater sensitivity to the neurotoxicity of the antiparasitic compound ivermectin (Schinkel et al., 1994). Subsequent studies demonstrated that mice with P-gp deficiency were more sensitive to loperamide, morphine, domperidone and vincristine (Choo et al., 2006; Schinkel et al., 1994; Schinkel et al., 1996; Thompson et al., 2000). Likewise, Collie and other dog breeds that naturally lack P-gp demonstrate neurological symptoms when they are exposed to loperamide, vincristine, vinblastine, doxorubicin or ivermectin (Hugnet et al., 1996; Mealey et al., 2001; Mealey et al., 2008; Mealey et al., 2003; Sartor et al., 2004).

In most studies in P-gp deficient mice, the effect of P-gp ablation on plasma concentrations of substrate drugs administered intravenously or orally is modest, but the impact on drug distribution into the brain is large. Because the drug concentration in the CNS is dependent on its plasma concentration, and genetic or chemical interventions may also affect drug absorption, distribution and elimination, the role of the BBB or the BCSFB in DDIs is better assessed by normalizing the drug CNS concentration by its plasma concentration (see Section 3.1). When compared with wild type (WT) mice, in the P-gp deficient mice the increase in P-gp substrate brain-to-plasma concentration ratio can be as large as 30-fold. For example, the increase in this ratio for anticancer drugs, antiretroviral protease inhibitors, opioids and the calcium channel blocker verapamil is up to 11-fold, 31-fold, 20-fold, and 9-fold, respectively (Table 3). These and additional studies in various models of P-gp$^{(-/-)}$ KO mice (reviewed by Chen et al., 2003b;Klaassen & Lu, 2008;Linnet & Ejsing, 2008) have contributed to the widespread view of P-gp as a major gatekeeper at the BBB in preventing entry of drugs into the CNS (Schinkel, 1999). Accordingly, the majority of approved CNS drugs tested in KO mice, with the exception of risperidone, show little or no recognition by P-gp (Table 3) (Doran et al., 2005;Schinkel et al., 1996). The impact of polymorphism in the human MDR1 gene on drug transport across the BBB has been investigated, but the data collectively are inconclusive (Basic et al., 2008;Brunner et al., 2005;Mosyagin et al., 2008;Pauli-Magnus et al., 2003;Skarke et al., 2003;Takano et al., 2006;Toornvliet et al., 2006).

Multidrug resistance-associated proteins: Members of the second ABC superfamily, the multidrug resistance-associated proteins (MRPs), are predominantly organic anion transporters but in addition transport neutral organic compounds (Deeley et al., 2006). While they are also ATP-dependent transporters, some require the presence of co-factors for transport (Deeley *et*

al., 2006). For most MRP isoforms, data on subcellular localization in humans, as well as level of expression and substrate recognition are inconsistent, but it seems that MRP4 and MRP5 (and possibly MRP2, in epileptogenic brain tissue from humans and rodents) are located on the luminal membrane of brain endothelial cells (Fig. 1) (Aronica et al., 2004; Dombrowski et al., 2001; Leggas et al., 2004; Nies et al., 2004; van Vliet et al., 2005). MRP1 (Rao et al., 1999; Wijnholds et al., 2000) and MRP4 (Leggas et al., 2004; Nies et al., 2004) are present in the blood-facing membrane of the human CP epithelial cells. MRP1, MRP 4 and MRP5 were also identified in endothelial cells from brain tumors (Bronger et al., 2005; Calatozzolo et al., 2005; Haga et al., 2001). MRP3 (ABCC3) has been detected in glioma capillaries (Calatozzolo et al., 2005; Haga et al., 2001), but not in normal human brain endothelial cells (Calatozzolo et al., 2005; Nies et al., 2004).

The substrate and inhibitor selectivity of individual MRPs may partially overlap with that of other ABCC transporters, P-gp, ABCG2, and organic anion transporters. For example, an initial report demonstrated greater CSF concentrations of topotecan in Mrp4$^{(-/-)}$ KO mice than in the WT controls (Leggas et al., 2004). However, a subsequent study provided evidence that P-gp and BCRP and not Mrp4 are major contributors to the brain distribution of topotecan (de Vries et al., 2007). Similarly, BCRP and not only Mrp4 may restrict adefovir brain distribution (Belinsky et al., 2007; Takenaka et al., 2007). Nevertheless, a 12-fold increase in the CSF-to-plasma concentration ratio of etoposide has been reported in Mrp1$^{(-/-)}$ KO mice, compared with WT controls (Wijnholds *et al.*, 2000). In MRP2-deficient TR-rats with induced seizures, phenytoin extracellular concentrations and anticonvulsant activity were two-fold greater than in rats that do not lack Mrp2 (Potschka et al., 2003).

Breast cancer resistance protein: Breast cancer resistance protein, (BCRP, ABCG2, or MXR) is an ABC half transporter. BCRP is expressed at the luminal membrane of human microvessel endothelium (Cooray et al., 2002; Zhang et al., 2003) and on the CSF side of murine CP epithelial cells (Tachikawa *et al.*, 2005). Together with MDR1, BCRP is the main ABC transporter expressed in human brain microvessels (Dauchy et al., 2008). Unlike P-gp, BCRP seems to be upregulated in tumor capillaries relative to those of the normal brain (de Vries et al., 2007; Zhang et al., 2003).

The substrate specificity of BCRP partially overlaps with that of P-gp and includes zidovudine, lamivudine, prazosin, pantoprazole, and the chemotherapeutic agents methotrexate, doxorubicin, daunorubicin, mitoxantrone, topotecan, irinotecan, imatinib (Gleevec) (Mao & Unadkat, 2005) and gefitinib (Iressa) (Elkind et al., 2005). Recent studies in Bcrp $^{(-/-)}$ mice have shown that this transporter contributes only to a moderate extent to the brain distribution of dantrolene, prazosin and triamterene (Enokizono et al., 2008). By the use of mice with triple KO for Bcrp, Mdr1a and Mdr1b, de Vries et al (de Vries et al., 2007) demonstrated that Bcrp and P-gp work in concert to limit brain penetration of topotecan. The brain-to-plasma area under the concentration-time curve (AUC) ratio of topotecan was not different in Bcrp $^{(-/-)}$ mice and was two times higher in the Mdr1a/b$^{(-/-)}$ mice compared to WT controls. However, in Mdr1a/b$^{(-/-)}$/Bcrp $^{(-/-)}$ mice, where both P-gp and BCRP are absent, the ratio increased 3.2-fold. The brain-to-plasma concentration ratio of imatinib (Oostendorp et al., 2009) and dasatinib (Chen et al., 2008) increased 12-13-fold and 10-fold, respectively, in the triple KO mice.

2.3.2. Transporters of the SLC superfamily—Proteins of the SLC family include facilitated transporters and ion-coupled transporters and exchangers that do not require ATP. Over 360 human SLC transporters have been identified so far and more than 40 SLC transporter families are included in the Human Genome Organization (HUGO) Nomenclature Committee Database (Hediger *et al.*, 2004 and http://www.bioparadigms.org/slc/intro.htm). Among these, members of the organic anion transporting polypeptides (SLCO) and organic anion/cation/

zwitterions (SLC22) transporter families are of special interest in terms of drug transport across the BBB. Additional transporters which can potentially contribute to DDIs across the BBB include monocarboxylate transporters, system L, and nucleoside transporters.

Organic anion transporting polypeptides: Organic anion transporting polypeptides (OATPs) are sodium-independent, multispecific anion exchangers, i.e., they exchange a drug for another ion or molecule. OATP-mediated transport can be bidirectional and depends on local substrate gradients. Among OATP family members, four transporters have been identified at human blood-brain interfaces. OATP1A2 and OATP2B1 are localized at the luminal membrane of brain endothelial cells (Bronger et al., 2005), whereas OATP3A1 is expressed in the CP (Huber et al., 2006; Roberts et al., 2008b). The thyroid hormone transporter, OATP1C1 has also been identified in human brain endothelial cells, but its precise localization is currently unknown (Roberts et al., 2008b). OATP1A2 and 2B1 have been detected in the blood-tumor barrier in gliomas and may affect the availability of chemotherapeutic drugs to tumor cells (Bronger et al., 2005). Rodent orthologs of human OATPs that are expressed at blood-brain interfaces include Oatp1a4, Oatp1a5 and Oatp1c1 (Bronger et al., 2005; Gao et al., 1999; Hagenbuch & Meier, 2004; Nies, 2007; Ohtsuki et al., 2004; Westholm et al., 2008).

OATP substrates are anionic amphipathic molecules with a molecular weight greater than 450 Daltons and a high degree of albumin binding (Hagenbuch & Meier, 2004). They include a broad range of drugs, such as fexofenadine (Cvetkovic et al., 1999), digoxin (Noé et al., 1997) and methotrexate (Badagnani *et al.*, 2006).

Organic anion transporters: The organic anion transporters (OATs) of the SLC22 gene family, in common with OATPs, are anion exchangers. The localization of most OATs in the brain is unclear, although OAT3 and OAT1 are found in epithelial cells of the human CP (Alebouyeh et al., 2003). The rodent Oat3 is predominantly localized at the abluminal membrane of brain endothelial cells and the luminal membrane of the CP epithelial cells (Mori et al., 2004; Nagata et al., 2004; Sweet et al., 2002).

OATs transport endogenous and exogenous compounds, including benzylpenicillin, valacyclovir, zidovudine, mercaptopurine, methotrexate and valproic acid (Rizwan & Burckhardt, 2007). The contribution of individual OATs to the brain disposition of their substrates is currently unknown. The substrate and inhibitor specificity of members of the SLCO and SLC22A partially overlaps with that of MRPs (Kusuhara & Sugiyama, 2005).

Organic cation transporters: Organic cation transporters (OCTs), like OATs, belong to the SLC22 family. They include the potential-sensitive OCTs and the proton gradient-driven OCTNs. OCTs are expressed in rodent and human brains, but so far have been localized in humans mainly to neurons and glial cells and not to endothelial cells (Koepsell et al., 2007). OCTs mediate the bidirectional transport of small, hydrophilic, positively charged compounds, such as cimetidine, desipramine, metformin, amantadine, memantine, (Jonker & Schinkel, 2004), cisplatin (Yonezawa et al., 2006) and quinine (Sweet et al., 2001). OCTN2 (SLC22A5) is expressed in brain endothelial cells of various species, including humans, and has been recently localized to the abluminal membrane in bovine brain capillary endothelial cells (Miecz *et al.*, 2008). OCTN2 mediates carnitine uptake into the brain (Inano et al., 2003; Kido et al., 2001; Miecz et al., 2008) and recognizes several cationic drugs, but its involvement in drug uptake into the CNS has yet to be assessed (Koepsell & Endou, 2004; Koepsell et al., 2007).

System L: System L transporters are heterodimers composed of a catalytic subunit (LAT1 or LAT2) covalently linked with the glycoprotein 4F2hc. System L transports bidirectionally large neutral amino acids with branched or aromatic side chains, such as L-phenylalanine, L-

tyrosine, L-tryptophan and L-leucine and amino-acid mimicking drugs, including levodopa, α-methyldopa, baclofen, melphalan, gabapentin and pregabalin. LAT1 is the predominant isoform at the BBB of humans and rodents and in general has greater affinities to system L substrates than LAT2 (Del Amo et al., 2008). It is expressed in both membrane domains of endothelial cells and normally participates in uptake of substrates from blood to brain (Su et al., 2005; Tsuji, 2005; Uchino et al., 2002; Verrey, 2003).

Monocarboxylate transporters: The transfer of lactate, pyruvate and other monocarboxylates across the BBB is facilitated by members of the monocarboxylate transporter (MCT) family (Morris & Felmlee, 2008; Pierre & Pellerin, 2005). MCTs potentially contribute to enhanced brain uptake of HMG-CoA reductase inhibitors that contain a carboxylic acid moiety, such as simvastatin (Tsuji et al., 1993), and of the drug of abuse gamma-hydroxybutyrate (Bhattacharya & Boje, 2004). On the other hand, they may restrict brain distribution of probenecid (Deguchi et al., 1997). Valproic acid is taken up into the brain by a transport system for medium-chain fatty acids (Adkison & Shen, 1996; Cornford et al., 1985) and has been shown to be a MCT substrate and inhibitor (Fischer et al., 2008). Other drugs that contain a carboxylic group in their chemical structure are also potential MCTs substrates.

Nucleoside transporters: The nucleoside transporters are encoded by the SLC28 (concentrative nucleoside transporter, CNT) and SLC29 (equilibrative nucleoside transporter, ENT) gene families. CNTs mediate Na^+-dependent uptake of nucleosides into cells whereas ENTs are Na^+-independent transporters (Kong et al., 2004). In humans, nucleoside transporters are present in the brain, but have not been localized to the BBB (Jennings et al., 2001; Nies, 2007). However, a sodium-dependent CNT3-like system was demonstrated in CP from humans and monkeys (Spector & Johanson, 2007). We have recently shown that the brain-to-plasma concentration ratio of ribavirin is 2.1-fold lower in $Ent1^{(-/-)}$ mice, compared to $Ent1^{(+/+)}$ controls, indicating an important role for Ent1 in the uptake of ribavirin into the mouse brain (Endres et al., 2007). The involvement of nucleoside transporters in the distribution of other nucleoside analog drug into the CNS is currently unknown.

3. Drug interactions at blood-brain interfaces

3.1. Methodological considerations

The impact of drug interactions described in the following section has been assessed by the use of pharmacodynamic outcomes (drug effects), pharmacokinetic outcomes (drug concentrations), or both. Further information on the type of interaction can be gained by measuring the unbound drug concentrations in plasma and brain. The general scientific consensus is that under normal conditions (BBB that is not leaky or disrupted), only unbound drug can be transferred across the BBB. Thus, an interaction associated with altered drug binding to either plasma proteins or brain tissue can be identified at distribution equilibrium by changes in the ratio of the brain-to-plasma total concentration of the drug, but not the ratio of the unbound drug. In contrast, changes in influx or efflux transporter function may be reflected by changes in the brain-to-plasma ratio of unbound drug. For many drugs, the unbound concentration in the extracellular fluid is also more relevant to their actions than their total brain concentration (de Lange & Danhof, 2002).

In many pharmacokinetic studies, assessment of brain-to-blood ratio is based on a single time point measurement, and blood and brain concentrations are sampled before the drug achieves distribution equilibrium between these compartments. Such measurements should be interpreted with caution as they can result in an underestimation or overestimation of the effect of the precipitant drug due to the effect being dependent on the time of sampling (Fig. 2). This problem is avoided when both the precipitant and object drugs are administered to steady-state or when comparisons are made of the ratio brain AUC: plasma AUC.

Commonly used methods to assess brain concentrations of drugs in animal models include 1) systemic administration of the drug, followed by brain homogenization and measurement of drug concentration at a single time point in each animal, 2) continuous monitoring of drug concentrations in brain ISF by a microdialysis probe, 3) *in situ* perfusion, in which drugs are administered directly into cerebral arteries (the influence of systemic factors on brain drug concentrations is minimized), and 4) measurement of brain concentrations by the use imaging techniques, such as positron emission tomography (PET) and magnetic resonance spectroscopy (MRS). In the clinical setting, brain concentrations of drugs have been most commonly determined by the use of microdialysis (under very special circumstances such as brain surgery) or imaging techniques. Imaging studies allow continuous sampling of brain concentrations of drugs over multiple time points. However, in PET, labeled metabolites may complicate the interpretation of brain concentrations. Except for microdialysis, irrespective of the technique utilized (sampling or imaging brain concentration), underestimation of brain-to-plasma concentration ratios can occur if this ratio is not corrected for vascular contamination. This correction will be most important when drug penetration into the brain tissue is low and the drug is highly bound to plasma proteins. For example, correction for vascular concentrations increased the effect of genetic P-gp KO on the brain-to-plasma ratio of digoxin and nelfinavir from 28-fold to 82-fold and from 31-fold to 42-fold, respectively (Choo et al., 2000; Mayer et al., 1997).

Drug CSF concentrations are sometimes used as a surrogate marker for drug concentration in the brain. However, the CSF is a compartment distinct from brain ISF and may not behave in parallel with the brain as a result of the sink effect of CSF turnover and efflux and influx transport at blood-brain interfaces (see above, Section 2) (Shen *et al.*, 2004). For example, Ramback et al. have recently demonstrated that cerebral cortex extracellular fluid concentrations of several antiepileptic drugs were lower than their corresponding CSF concentrations in patients with pharmacoresistant epilepsy (Rambeck *et al.*, 2006). Moreover, large differences may occur in solute concentrations between ventricular, subarachnoid and lumbar CSF (Shen *et al.*, 2004). For example, topotecan concentrations in humans following its systemic administration are lower in lumbar CSF than in ventricular CSF (Baker et al., 1996), whereas an opposite difference has been observed in monkeys with lamivudine (Blaney et al., 1995). For these reasons, CSF-to-ISF drug concentration ratio may be different than unity and vary with time, and interpretation of CSF drug concentrations as indicators of those in brain should be done with caution. An exception is CSF sampling for measurements of concentrations of antibiotics and antivirals, because CSF often serves as a reservoir of the infectious agent (Shen *et al.*, 2004).

3.2. Drug-drug interactions in animal models

3.2.1. Drug interactions that involve multiple mechanisms—Enhancement of BBB tightness is important in pathological conditions that involve global or regional leaky barrier. For example, dexamethasone has been widely used for the treatment of cerebral oedema (Kaal & Vecht, 2004). It is now known that dexamethasone affects fluid and solute transfer across the BBB by multiple mechanisms, including altered blood flow, enhanced tightness of brain endothelial TJs and up-regulation of efflux transporter expression at brain capillaries (Abbott et al., 2006; Bauer et al., 2004; Bauer et al., 2008; Blecharz et al., 2008; Förster et al., 2005; Förster et al., 2006; Harke et al, 2008; Wilkinson et al., 2006). In rodents, corticosteroids decreased the permeability of the chemotherapeutic drugs cisplatin, cyclophosphamide and ifosfamide into brain tumors. However, the mechanistic basis for this interaction has not been investigated in these studies (Straathof et al., 1999; Straathof et al., 1998).

3.2.2. Drug interactions that involve blood-brain barrier tight junctions—An opposite pharmacotherapeutic challenge is tight BBB that impedes drug delivery to the brain.

For example, despite enhanced transfer of chemotherapeutic drugs across leaky capillaries in blood-tumor barriers, variability in drug distribution into the tumor tissue impairs effective chemotheraphy (Bellavance et al., 2008; Gerstner & Fine, 2007; Motl et al., 2006). Pharmacological approaches to enhance otherwise poor CNS penetration of chemotherapeutic drugs include BBB disruption (BBBD) and inhibition of efflux transporters (Bellavance et al., 2008; Gerstner & Fine, 2007).

The concept of osmotic BBBD was developed in 1972 by Rapport et al. (Rapoport et al., 1972). This approach utilizes intracarotid injections of hyperosmolar solutions to draw water out of brain endothelial cells and open TJs. In animal models, osmotic BBBD significantly enhanced the penetration of chemotherapeutic drugs into brain parenchyma, although increments in permeability were greater in the intact brain than in the tumor (Rapoport, 2000). Moreover, the increased CNS penetration of several chemotherapeuric drugs resulted in neurotoxicity, but subsequent studies reported encouraging results with the use of less neurotoxic compounds (Siegal & Zylber-Katz, 2002). In rats and dogs, osmotic BBBD increased the brain and CSF concentrations of methotrexate 10- to 100-fold (Barnett et al., 1995; Muldoon et al., 2007; Neuwelt et al., 1982; Neuwelt et al., 1998; Neuwelt et al., 1980). Of note, dexamethasone (96 mg/m^2/day for 3 days) abolished the effect of BBBD on tumor methotrexate concentrations (Neuwelt et al., 1982). Plasma concentrations of methotrexate in the dexamethasone-treated group were not reported. More recently, the bradykinin agonist cereport (RMP-7) has been utilized to selectively open TJs in brain tumor vasculature, although it can also affect BBB in non-tumor tissue. Intravenous administration of cereport to rats increased the concentration of [^{14}C]-carboplatin in tumor tissue and its antitumor efficacy (Kemper et al., 2004a), and enhanced the central analgesic activity of loperamide (Emerich et al., 1998).

3.2.3. Drug interactions that involve cerebral blood flow—A study in non human primates evaluated the effect of amitriptyline, a tricyclic antidepressant that enhances cerebral blood flow, on the brain delivery of methotrexate. With one exception, the combination did not significantly affect the CSF-to-blood concentration ratio of methotreaxate, compared to methotrexate alone (Table 4) (Heideman *et al.*, 1986).

3.2.4. ABC transporter-mediated interactions

P-glycoprotein: Inhibition of P-gp has been extensively studied in animal models of refractory brain diseases, such as cancer, AIDS dementia and epilepsy. One of the most extensively studied P-gp substrates is paclitaxel, a lipophilic anticancer drug that shows high potency against brain tumors *in vitro*, but is ineffective *in vivo* because it does not cross the BBB (Miller et al., 2008). The 8-fold increase in brain uptake of paclitaxel in Mdr1a$^{(-/-)}$ mice, compared to WT mice, indicates that paclitaxel is removed from the brain by P-gp (Kemper et al., 2003). Several studies compared the effects of P-gp inhibitors (see below, section 3.3.2) on paclitaxel uptake into mouse brain (Table 3) (Hubensack et al., 2008; Kemper et al., 2004b; Kemper et al., 2003). Among these inhibitors, the most potent was elacridar. However, at elacridar plasma concentrations within the clinically achievable range (<900 ng/ml), complete P-gp inhibition was not achieved and the brain uptake of paclitaxel was increased only 5-fold. A single dose of valspodar increased the brain uptake of paclitaxel less than a single dose of elacridar. However, valspodar administration (50 mg/kg, given orally twice over a period of 5 weeks) to mice implanted with human glioblastoma and treated with paclitaxel (3 mg/kg and another 2 mg/kg after valspodar administration) reduced the volume of the tumor by 90% (Fellner et al., 2002). In contrast, cyclosporine and itraconazole decreased paclitaxel brain-to-plasma AUC ratio (0.7 and 0.2, respectively, compared to non-treated mice) (Kemper *et al.*, 2003), perhaps through inhibition of an uptake transporter(s). Although the interaction of paclitaxel with BBB uptake transporters (of rodents or humans) have not been demonstrated, paclitaxel is

recognized by OATP1B3 (Smith et al., 2005) and OATP1B1 (Gui *et al.*, 2008), whereas cyclosporine is an OATP inhibitor (Kajosaari et al., 2005; Regazzi et al., 1993).

In common with paclitaxel, imatinib penetrates poorly into the brain, at least in part because it is a substrate of P-gp and BCRP. However, unlike the incomplete inhibition of paclitaxel uptake into the brain by elacridar, co-administration of elacridar with imatinib increased the brain distribution of imatinib to a greater extent (up to 5-fold) in WT mice than that observed in P-gp$^{(-/-)}$ KO mice (but lesser than in triple KO mice, Bcrp$^{(-/-)}$, Mdr1a$^{(-/-)}$Mdr1b$^{(-/-)}$) (Breedveld et al., 2005; Oostendorp et al., 2009). Valspodar or zosuquidar enhanced the brain uptake of imatinib up to 3-fold (Bihorel et al., 2007). These findings suggest that administering imatinib together with P-gp inhibitors may improve its delivery into the CNS. Whether dual inhibition of P-gp and BCRP at the human BBB will be more effective than selective inhibition of either transporter is currently unknown.

In contrast to the striking effect of P-gp inhibition on brain distribution of paclitaxel and imatinib, the interaction with other chemotherapeutic agents is moderate at best. Single oral doses of valspodar and elacridar increases the brain uptake of docetaxel up to 2.6-fold, whereas cyclosporine decreases it (Kemper et al., 2004c). Valspodar and elacridar increase the brain uptake of vinblastine 3-fold (Cisternino et al., 2001). Multiple other inhibitors (verapamil, cyclosporine, quinidine, amiodarone, nifedipine and trifluoroperazine) had no effect on vinblastine uptake into mouse brain (Arboix et al., 1997; Cisternino et al., 2001). Similarly, the brain ISF-to-plasma AUC ratio of unbound topotecan lactone (also a BCRP substrate) is increased only 1.7- and 1.6-fold by the dual P-gp, BCRP inhibitors gefitinib (Zhuang et al., 2006) or elacridar (de Vries et al., 2007), respectively. These data demonstrate the importance of selecting the appropriate combination of P-gp substrate and chemotherapeutic agent to obtain clinically significant P-gp inhibition at the BBB.

Infection of the CNS with HIV can produce neurological symptoms, but may also lead to development of latent virus reservoir in the CNS and subsequent drug resistance (Löscher & Potschka, 2005). However, virtually all the drugs currently used for the treatment of HIV infections penetrate the CNS poorly. Protease inhibitors are substrates of P-gp and reverse transcriptase inhibitors are substrates of other transport systems, mostly OATs and MRPs (Strazielle & Ghersi-Egea, 2005). The importance of adequate antiretroviral drug concentrations in the CNS led to evaluation of P-gp inhibitors as a therapeutic modality to enhance CNS distribution of antiretroviral protease inhibitors. In rodent studies, the greatest effect of P-gp inhibition was on the brain distribution of nelfinavir, and the most effective inhibitor was zosuquidar (Table 3). The effect of zosuquidar was dose-dependent and increases in brain uptake of nelfinavir were up to 18-fold in mice (Choo et al., 2000) and 29-fold in rats (Anderson et al., 2006) (25- and 80-fold, respectively, when corrected for drug in vasculature) (Table 3). When ritonavir was combined with saquinavir, ritonavir partly inhibited P-gp-mediated efflux of saquinavir from the mouse brain (Huisman *et al.*, 2001).

In analogy to drug resistance in cancer, overexpression of P-gp and other efflux transporters in epileptic foci may play a role in pharmacoresistant epilepsy. However, while it is established that efflux transporters are upregulated in drug-resistant epileptogenic brain tissue in humans and rodents, their role in removal of antiepileptic drugs from the brain is controversial (Anderson & Shen, 2007). Thus, P-gp inhibition by verapamil, administered directly into rat cerebral cortex, modestly increased (up to two-fold) the ISF-to-plasma concentration ratios of phenobarbital, phenytoin, lamotrigine, felbamate, carbamazepine or oxcarbazepine (Clinckers et al., 2005; Potschka et al., 2001, 2002; Potschka & Löscher, 2001). Nevertheless, in rats with induced seizures, cyclosporine and tariquidar reversed resistance to several antiepileptic drugs and increased their brain-to-plasma concentration ratio without changing their plasma pharmacokinetics (Brandt et al., 2006; Clinckers et al., 2005; Mazarati et al., 2003; van Vliet

et al., 2006). Similar to antiepileptic drugs, P-gp inhibition in rodents had only modest effect on CNS distribution of a variety of antidepressnts and antipsychotic agents, including nortriptyline, fluphenazine, amisulpride, risperidone (Linnet & Ejsing, 2008), and rizulide (Milane *et al.*, 2007). Some of these studies assessed possible interactions when the plasma concentrations of the psychotropic drugs were within their therapeutic range. Based on the therapeutic indices of these compounds, Linnet and Ejsing suggested that even complete inhibition of P-gp is unlikely to yield severe toxicity of these compounds and that in most cases possible clinical effects are likely to be limited (Linnet & Ejsing, 2008).

Most of the studies described so far assessed the potential of P-gp inhibition to enhance drug efficacy in the CNS. A second outcome of P-gp inhibition at the BBB is enhanced CNS distribution and adverse effects of P-gp substrate drugs that normally do not cross the BBB and do not have central effects. Examples are the opioid loperamide, the dopaminergic antagonist domperidone and non-sedating antihistamines. In an *in situ* perfusion study, quinidine (100 μM) mimicked the effect of genetic KO of P-gp in mice and enhanced the brain uptake of loperamide 9-fold, indicating near-complete P-gp inhibition (Dagenais et al., 2004). This study also demonstrated that quinidine is a potent and efficient inhibitor of P-gp-mediated efflux of loperamide from the brain, at least in mice. The impact of P-gp on brain or CSF distribution and analgesic effects of other opioids, including morphine, meperidine, fentanyl, methadone and dextromethorphan was much less (Dagenais et al., 2004; Letrent et al., 1999; Lötsch et al., 2002; Marier et al., 2005; Rodriguez et al., 2004). In pigs, cyclosporine increased the brain [^{11}C]-loperamide-radioactivity up to 7-fold, but plasma loperamide concentration were not reported (Passchier et al., 2003). Likewise, co-administration of cyclosporine (at blood concentrations 2.0-3.1 μM) to rats treated with domperidone increased the brain distribution of domperidone and *in vivo* striatal dopaminergic receptor occupancy 2-fold, and enhanced catalepsy 3-fold (Tsujikawa *et al.*, 2003). Another study in rats demonstrated that cyclosporine does not affect the brain uptake of first generation, sedating antihistamines, but increases by several-fold the brain uptake of the second generation antihistamines cetrizine, loratadine, terfenadine and fexofenadine (Obradovic et al., 2007).

One of the best characterized P-gp-based interactions at the BBB is that between cyclosporine and verapamil, mainly because the availability of verapamil labeled with ^{11}C for PET imaging enables non-invasive studies in animals and humans. Following bolus intravenous injection of [^{11}C]-verapamil to mice (Hendrikse et al., 1998) and rats (Bart et al., 2003; Hendrikse et al., 1999; Hsiao et al., 2006), cyclosporine increased the brain:plasma concentration ratio of [^{11}C]-verapamil-radioactivity up to 5-fold and 6-24-fold (up to 27-fold, when corrected for vascular drug), respectively. When compared to the effect of genetic ablation of the transporter, the lower values indicate incomplete P-gp inhibition by cyclosporine at the mouse BBB (Table 3). These results raise two important issues. First, the concentration of the inhibitor achieved in plasma. Second, the time course of the inhibitor. Lower plasma concentration of the inhibitor will yield incomplete inhibition of P-gp. To ascertain the magnitude of maximum inhibition and to determine if this is equivalent to that obtained with genetic ablation of P-gp, an inhibitor concentration-effect study needs to be conducted. Optimally, such a study should be conducted at increasing steady-state concentrations of the inhibitor. Such an experimental design allows interpretation of the increase in brain-to-plasma concentration ratio of the P-gp substrate at each inhibitor steady-state plasma concentration without the confounding effect of constantly changing inhibitor concentration. To allow the timing of P-gp inhibition to be followed, Syvänen and collaborators (Syvänen *et al.*, 2006) used an alternative approach. Cyclosporine was administered as a short bolus injection after the start of verapamil intravenous infusion to obtain steady state concentrations of [^{11}C]-verapamil. By modeling P-gp inhibition, the authors found that cyclosporine effect is associated mainly, but not exclusively, with reduced verapamil transport out of the brain. However, their data did not allow determination of whether the input rate into the brain was also affected. The model predicted that P-gp inhibition at the BBB is

associated with cyclosporine concentrations at the effect compartment (probably brain endothelial cells) rather than in the plasma. Furthermore, it was shown that the onset of P-gp inhibition by cyclosporine is fast and that inhibition is rapidly reversible. This means that the time of administration of the inhibitor with regard to the substrate is crucial for the interaction, at least for the combination of cyclosporine and verapamil.

When quinidine was perfused as a P-gp inhibitor to mice, its maximal effect on verapamil brain uptake was comparable to that of cyclosporine. At 100 μM in the perfusate, quinidine increased the brain uptake of verapamil 5-fold (Dagenais et al., 2004; Zong & Pollack, 2003). However, at 4 μM, a concentration similar to the total quinidine concentration achievable in humans treated for tumors (4.5-5.6 μM) (Raderer & Scheithauer, 1993), quinidine did not increase the brain uptake of verapamil. Rifampin, on the other hand, inhibited verapamil efflux almost completely (Table 3). In a recent study, tariquidar increased the distribution of [^{11}C]-verapamil-radioactivity into rat brain in a dose-dependent manner. Following administration of the highest dose, tariquidar (at blood and brain concentrations 1402 ng/mL and 4131 ng/mL, respectively), enhanced verapamil brain uptake up to 12-fold (Bankstahl et al., 2008). The effect of 50 mg/kg celecoxib on the uptake of [^{11}C]-verapamil into rat brain was only modest (2-fold) (de Vries et al., 2008).

The impact of quinidine on verapamil uptake into the brain was not replicated when verapamil was substituted with digoxin, but this is likely due to the differences in the plasma concentrations of quinidine (Table 3). Whereas genetic KO increased the brain distribution of digoxin 15-28-fold (26-82-fold, when corrected to drug in vasculture) (Fromm et al., 1999;Mayer et al., 1997;Schinkel et al., 1995), quinidine did not affect it (Fromm et al., 1999). Furthermore, in Mdr1a$^{(-/-)}$ mice, co-administration of quinidine decreased digoxin brain uptake (Fromm *et al.*, 1999), perhaps through OATP inhibition (van Montfoort *et al.*, 2001).

Studies in rats assessed additional substrate-inhibitor combinations. Cyclosporine (50 mg/kg) increased up to 4-fold the brain-to-plasma concentration ratio of [^{11}C]-carvedilol radioactivity (Bart et al., 2005). Valspodar increased the brain-to-plasma concentration ratio of free colchicine (Desrayaud et al., 1997) and cyclosporine (Lemaire et al., 1996) ~ 4-fold and 5-fold, respectively. Elacridar increased the brain-to-plasma AUC ratios of the (+) and (-) enantimers of the antimalarial compound mefloquine 2.5- and 1.5-times, respectively (Barraud de Lagerie et al., 2004).

Why do certain P-gp inhibitors significantly block efflux of some P-gp substrate drugs across the BBB, while affecting others only partially? First, other factors (e.g. diffusion, influx transporters or other efflux transporters) may contribute to the distribution into the CNS of those drugs that are less affected (Table 3). In addition, the timing of inhibitor administration may not provide optimal P-gp inhibition. Finally, *in vitro* studies suggest that differences between individual drug combinations may also be explained by allosteric kinetics. For example, in isolated membrane vesicles, verapamil only partially displaces morphine from its binding to P-gp, but completely displaces vinblastine (Callaghan & Riordan, 1993). So far, the molecular mechanisms of substrate binding and transport by P-gp have not been elucidated. However, it has been suggested that P-gp has either one complex substrate binding site or at least two binding sites, the H site for Hoechst 33342 and the R site for rhodamine 123. Furthermore, P-gp-mediated efflux of both Hoechst 333342 and rhodamine 123 can be activated by prazosin, suggesting a third binding site (Shapiro et al., 1999). Similarly, ketoconazole and loperamide activate P-gp mediated efflux of digoxin *in vitro* (Taub et al., 2005), whereas tariquidar inhibits P-gp function by binding at a site which is distinct from the site of interaction of vinblastin and paclitaxel (Martin et al., 1999).

Collectively, the above rodent data suggest that certain P-gp substrate-inhibitor combinations will result in significant DDIs at the BBB. However, studies in non-human primates have shown that the impact of pharmacological P-gp inhibition on the brain distribution of drugs may be lesser than that in rodents (Table 4). For example, we have recently assessed the impact of P-gp inhibition at the BBB of *Macaca nemestrina* using PET. We studied the effect of cyclosporine (12 or 24 mg/kg/h, iv) on [^{11}C]-verapamil plasma and brain kinetics in 4 pregnant macaques. At 6.5 μM and 19.4 μM cyclosporine blood concentration, the brain-to-plasma AUC ratio of [^{11}C]-radioactivity at 9 minutes changed up to 3.2- and 3.7-fold, respectively (Eyal et al., 2009). Our result are consistent with the 2.3-fold increase in cerebrum-to-blood AUC of [^{11}C]-verapamil-radioactivity in male rhesus monkey (*M. mulatta*) following the administration of 20 mg/kg valspodar (Lee et al., 2006). However, these data are in contrast to the results obtained in mice (Hendrikse *et al.*, 1998) or in rats (Hendrikse et al., 1999;Hsiao et al., 2006) administered cyclosporine. In the latter study, at blood cyclosporine concentrations observed in the non-human primates (>15 μM), the increase in brain verapamil radioactivity was in the order of 12-fold (but see also Section 3.3.3).

In a study that assessed [^{11}C]-loperamide as a PET tracer, Zoghbi et al. (Zoghbi *et al.*, 2008) injected the compound to a male rhesus monkey in the presence or the absence of tariquidar (8 mg/kg, iv). Tariquidar increased the peak brain radioactivity 3.7-fold. A similar magnitude of change was observed with the use of the same dose of another P-gp inhibitor, DCPQ (with only 16% increase in plasma radioactivity). Evaluation of [^{11}C]-loperamide disposition in mice (Zoghbi *et al.*, 2008) revealed that the effect of P-gp inhibition on brain uptake of [^{11}C]-loperamide was blunted by non-P-gp substrate radioactive metabolites of [^{11}C]-loperamide. Because [^{11}C]-radiolabeled metabolites of loperamide could also be formed in the non-human primate, the magnitude of P-gp inhibition in the monkey may be underestimated by the non-P-gp substrate metabolites. In addition, the regional distribution of [^{11}C]-loperamide metabolite, [^{11}C]-desmethyl-loperamide, is flow-limited and DCPQ-induced changes in its distribution should be corrected for regional blood flow to reflect P-gp inhibition (Liow et al., 2009). On the other hand, disulfiram (500 mg given orally on the evening before the scan and 500 mg on the morning of the scan) did not affect [^{11}C]-loperamide distribution into the brain (Ryu *et al.*, 2007), although disulfiram metabolites can covalently inactivate P-gp *in vitro* (Loo et al., 2004)

To assess whether changes in brain nelfinavir concentrations, following P-gp inhibition, parallel those in the CSF, nelfinavir (6 mg/kg, intravenous bolus) was administered to 3 cynomolgus monkeys (*Macaca fascicularis*) in the absence and the presence of zosuquidar (3 mg/kg, intravenously). Zosuquidar significantly increased the distribution of nelfinavir into the brain, without a change in its CSF-to-blood concentration ratio. These data suggest that CSF concentration as a surrogate marker for brain drug concentration should be used with caution, especially to assess drug interactions at the BBB (Kaddoumi *et al.*, 2007). Likewise, doxorubicin CSF concentrations in four adult rhesus monkeys were below the limit of detection whether the drug was administered alone or in combination with intravenous cyclosporine (at blood steady state concentrations of 0.5-1.1 μM) (Warren et al., 2000).

Kurdziel et al. (Kurdziel et al., 2003) utilized PET to asses the tissue distribution of [^{18}F]-paclitaxel in the absence and the presence of tariquidar (2 mg/kg, iv bolus) in 3 rhesus monkeys. Despite changes in the distribution of radioactivity into liver, lung, and kidney with tariquidar administration, paclitaxel uptake into the brain was very low and appeared unchanged after the administration of the inhibitor. The reason for this tissue-specificity of the interaction is unknown, although Choo et al have previously demonstrated in mice that P-gp at the BBB is more resistant to inhibition by tariquidar than in other tissues, when loperamide was used as the substrate (Choo et al., 2006). This finding is also in contrast to the 4.3-fold increase in

paclitaxel brain uptake when it was co-administered with tariquidar to mice (Hubensack et al., 2008).

In contrast to the wealth of data on P-gp inhibition, much less is known about the impact of P-gp induction at the BBB. In one of the earlier studies, rats were treated with morphine (20 mg/kg/d, subcutaneously) or dexamethasone (100 mg/kg/d, intraperitoneally) for 5 days. Both compounds decreased the antinociceptive effect of morphine (4-fold and 2-fold decreases, respectively) and enhanced P-glycoprotein expression in the brain, compared to those observed in animals treated with the vehicle. The investigators postulated that enhanced brain P-gp activity following chronic exposure to morphine or dexamethasone may have caused the lower brain concentrations of the drug. Chronic exposure of rat brain endothelial cells to other drugs, including phenobarbital, phenytoin and carbamazepine can also lead to induction of P-gp expression and function in *vitro* and *in vivo* (Lombardo et al., 2008; Wen et al., 2008). Likewise, HIV protease inhibitors have been shown to up-regulate P-gp expression *in vitro* in a human brain endothelial cell line (Zastre *et al.*, 2009).

Reports about expression and activity of transcription factors that regulate the BBB expression of P-gp and other transporters are conflicting. Bauer and colleagues provided evidence that the nuclear receptor pregnane X receptor (PXR) is present in rat brain capillaries, where it can potentially mediate DDIs (Bauer et al., 2004; Bauer et al., 2008). Upon activation by dexamethasone, PXR regulates the expression of P-gp in rat brain capillaries *in vitro* and *in vivo* (Bauer et al., 2004). Dexamethasone not only activates PXR, but also up-regulates PXR expression through activation of glucocorticoid receptors (Narang *et al.*, 2008). In addition to P-gp, dexamethasone upregulated the expression of Mrp2 and GSTπ *in vitro* and *in vivo* and that of Bcrp *in vitro* (Bauer et al., 2008; Narang et al., 2008). On the other hand, Akanuma et al. did not detect PXR in rat brain endothelial cells (Akanuma et al., 2008).

To overcome the species differences in substrate recognition by PXR, the effect of rifampin on brain distribution and antinociceptive activity of methadone were studied in transgenic mice expressing hPXR but lacking mPXR. The mice were treated with rifampin (50 mg/kg, oral, for 3 days). Rifampin was predicted to produce average steady state unbound plasma concentration of 0.34 μg/mL, comparable to that observed in patients undergoing a course of rifampin treatment. Rifampin induced P-gp expression in the liver, intestine and brain endothelial cells. The antinociceptive effect of methadone decreased by 70% when compared with mice treated with the vehicle without any change in plasma methadone concentrations (Bauer et al., 2006).

PXR has been recently detected in a human brain endothelial cell line (Zastre *et al.*, 2009). However, its presence in human brain endothelial cells *in vivo* has not been demonstrated. Dauchy et. al. reported that the transcription factor AhR is expressed in isolated human brain microvessels. In those microvessels, PXR or CAR transcripts (major transcription factors involved in ABC transporters and CYP gene regulation) were almost undetectable (Dauchy et al., 2008).

MRPs: The effect of MRPs inhibition on substrate distribution into the brain is modest at best. For example, perfusion of mdr1a$^{(-/-)}$ mice with probenecid or the specific MRP inhibitor MK571 did not affect the brain transport of etoposide (Cisternino et al., 2003). Similarly, Tunblad and coworkers reported a 1.3-fold increase of morphine steady-state brain-to-blood ratio in rats when probenecid was co-administered (Tunblad et al., 2003). Probenecid enhanced up to two-fold the brain penetration and anticonvulsant activity of phenytoin (a Mrp2 substrate), but not phenobarbital (Potschka *et al.*, 2003). In rabbits (Spector, 1976), dogs (Ramu et al., 1978) and monkeys (Salzer et al., 2001), probenecid retarded the efflux of methotrexate from CSF to blood.

BCRP (ABCG2): In common with MRPs, pharmacological inhibition studies suggest that the role of Bcrp in drug distribution into the brain is limited. For example, inhibition of Bcrp in Mdr1a/b$^{(-/-)}$ mice by elaricidar increases the oral bioavailability and placental transfer of topotecan by more than 6-fold and 2-fold, respectively (Jonker *et al.*, 2000). However, in the same mouse model, elacridar increased only 1.6-fold the brain distribution of topotecan (de Vries et al., 2007). Similarly, by the use of P-gp$^{(-/-)}$ and Bcrp $^{(-/-)}$ mice, it was demonstrated that elacridar completely inhibits P-gp-mediated efflux of topotecan from the brain, but only partially inhibits Bcrp-mediated topotecan efflux. The authors attributed these differences to greater potency of elacridar to inhibit P-gp, compared to BCRP, and to the fact that topotecan is a better substrate for Bcrp than for P-gp. Similarly, Zhuang et al. (Zhuang et al., 2006) found that the brain ISF-to-plasma AUC ratio of topotecan lactone in mice increased 1.6-fold, whereas that of ventricular CSF-to-plasma decreased by 17% in the presence of gefitinib. Despite the modest effect of the inhibitor, these changes are consistent with the “opposite” localization of Bcrp at the BBB and BCSFB (Fig. 1). Likewise, elacridar increased the brain transport coefficients of other BCRP substrates, mitoxantrone and prazosin, 2-fold and 1.5-fold, respectively, in WT mice (Cisternino et al., 2004).

3.2.5. SLC Transporter-mediated interactions

Organic anion transporters: Functional studies in rodents, using non-specific organic anion transporter inhibitors such as probenecid and *p*-aminohippuric acid (PAH) indicate that organic anion transporters and transporting polypeptides may limit brain exposure to drugs. For example, probenecid increases up to 4.4-fold the brain-to-plasma concentration ratio of 6-mercaptopurine in rats (Deguchi et al., 2000), 2.5-fold the CSF-to-plasma ratio of benzylpenicilliin in rabbits (Spector & Lorenzo, 1974) and 2-fold the brain concentrations of hydroxyurea in guinea pigs (Dogruel et al., 2003). In dogs, probenecid reduced the transfer of methotrexate from CSF to blood (Ramu et al., 1978). More specifically, Mori et al. demonstrated that rOAT3 mediates the brain-to-blood efflux of thiopurines, including mercaptopurine. Although methotrexate inhibited rOAT3-mediated transport of 6-mercaptopurine, their co-administration is not likely to affect 6-mercaptopurine brain distribution because methotrexate Ki was 17.5-fold greater than its estimated brain concentration in patients receiving chemotherapy (Mori et al., 2004).

The restricted CNS distribution of antiretroviral drugs, such as zidovudine, didanosine and zalcitabine has been attributed to active efflux systems (Gibbs et al., 2003a; Gibbs & Thomas, 2002; Strazielle & Ghersi-Egea, 2005). Using thymide and inosine as nucleoside transporter inhibitors and probenecid as an OATs inhibitor, it has been shown in rats that this efflux is most likely mediated by OATs and not nucleoside transporters (NTs) (Masereeuw et al., 1994). Studies in rabbits (Wang et al., 1997) and in the rhesus monkey (Cretton et al., 1991) demonstrated that probenecid increases the CSF-to-plasma concentration ratio of zidovudine 7-fold and up to 2.5-fold, respectively. In the rabbit, the effect of probenecid on zidovudine concentrations was slightly greater at brain ISF than at ventricular CSF (Wong et al., 1993). Nevertheless, this interaction cannot be therapeutically used to enhance zidovudine penetration into the CSF in humans, because probenecid is no longer combined with zidovudine due to adverse cutaneous reactions (Petty et al., 1990).

Several studies assessed possible drug interactions, at the blood-brain interfaces, of drugs used in combination in the treatment of HIV infection. In most cases, drug concentrations were measured in plasma and CSF. Although some of these compounds share common transport mechanisms, in particular OATs and OATPs, studies in rodents could not detect interactions between zidovudine and stavudine (d4T) (Yang et al., 1997), zidovudine and zalcitabine (Gibbs & Thomas, 2002), zidovudine, stavudine, lamivudine (3TC), abacavir or hydroxyurea and didanosine (Gibbs et al., 2003a), zidovudine, abacavir, or stavudine and lamivudine (Gibbs et

al., 2003b), abacavir or nevirapine and ritonavir (Anthonypillai et al., 2004) and nevirapine and efavirenz (Gibbs et al., 2006). Similarly, the CSF-to-plasma concentration ratios of zidovudine and didanosine in the monkey did not change when the two drugs were simultaneously administered (Tuntland et al., 1994).

Several studies suggested that beta-lactam antibiotics undergo facilitated diffusion across the BBB through a probenecid-sensitive mechanism and can therefore compete for this uptake mechanism. For example, concurrent administration of ceftazidime to rabbits reduced 2.5-fold the CSF-to-plasma concentration ratio of ampicillin (Okura et al., 1988). In another study in rats, intravenous administration of benzylpenicillin decreased the brain ISF-to-plasma unbound concentration ratios of cefodizime up to 25-fold. CSF concentrations were only minimally changed, consistent with the very slow equilibrium of cefodizime in the CSF (Matsushita *et al.*, 1991). However, a later *in vitro* study could not confirm carrier-mediated uptake of benzylpenicillin across brain endothelial cells (Török et al., 1998).

LAT1: The interaction of levodopa with large neutral amino acids was first noted in humans more than two decades ago (Nutt et al., 1984) (see below, Section 3.3). Administration of large neutral amino acids (intravenously) or high-protein meals (oral) to parkinsonian and non-parkinsonian monkeys prior to levodopa reduces by half the striatal extracellular fluid-to-plasma concentration ratio of levodopa (Alexander et al., 1994). Interestingly, this and other studies demonstrated that beta-adrenergic agonists increase the transport of levodopa into the brain in rats and monkeys without altering regional cerebral blood flow (Alexander et al., 1994; Eriksson & Carlsson, 1982; Takao et al., 1992; Uc et al., 2002), perhaps through beta receptor-mediated enhanced activity of a transporter for L-amino acids in brain endothelial cells (Eriksson & Carlsson, 1982; Kumakura et al., 2004).

MCT: MCT substrates, such as salicylic acid, probenecid, valproic acid and gamma-hydroxybutyrate can potentially compete for brain uptake (Bhattacharya & Boje, 2004; Kang et al., 1990). For example, Kang et al. demonstrated that valproic acid can inhibit the uptake of salicylic acid into rat brain (Kang *et al.*, 1990). Recently, Bhattacharya and Boje concurrently administered gamma-hydroxybutyrate and salicylic acid to rats to test the hypothesis that salycilic acid can be used to treat gamma-hydroxybutyrate intoxication. The doses were predicted by a previous simulation to yield gamma-hydroxybutyrate toxic plasma and brain concentrations and salicylic acid concentrations within the observed therapeutic window. However, as predicted by the simulation, the reduction of gamma-hydroxybutyrate brain exposure was only modest and the time window for salicylate administration was limited. The authors concluded that salicylic acid is more likely to produce an adverse drug interaction with gamma-hydroxybutyrate, when used therapeutically for the treatment of narcolepsy or catalepsy, than to be an antidote for the treatment of gamma-hydroxybutyrate intoxication (Bhattacharya & Boje, 2006).

Nucleoside transporters: Nucleoside transporter-mediated interactions at the BBB have only recently begun to be investigated. A recent abstract reported 2.5-fold decrease in brain AUC of the adenosine receptor agonist tecadenoson when it was co-administered to mice with the ENT1 inhibitor nitrobenzyl-mercaptopurine ribonucleoside (Lepist *et al.*, 2008). When data on expression and activity of nucleoside transporters at BBB becomes available, studies to determine if nucleoside transporters participate in DDIs will be possible.

3.3. Drug interaction at the human blood-brain barrier: what is the evidence?

It has been widely presumed that the impact of DDIs at the human BBB would be as high as those observed in rodents. However, despite the clinical relevance of DDIs at blood-brain

interfaces, due to technical and ethical limitations, to date only a few studies have addressed this issue in humans.

3.3.1. Drug interactions that involve multiple mechanisms—To compare the CNS distribution of cyclophosphamide and ifosfamide, Yule et al (Yule et al., 1997) evaluated the plasma and CSF concentrations of these drugs in 25 pediatric oncology patients. Subjects received cyclophosphamide (125-1000 mg/m^2, intravenously over 1 hr) or constant infusion of ifosfamide (9 g/m^2) over 72 hours. 7 Patients who were treated with cyclophosphamide for non-Hodgkin's lymphoma had significantly higher cyclophosphamide CSF concentrations, compared with 13 patients that were treated for acute lynphoblastic leukemia. The CSF-to-plasma concentration ratio of cyclophosphamide was 3-fold greater in lymphoma than in leukemia patients. The authors suggested that the differences could result from tightening of the BBB by co-administration of dexamethasone for the treatment of acute lymphoblastic leukemia. Similarly, one patient that received dexamethasone had the lowest CSF-to-plasma concentration ratio of ifosfamide. Because dexamethasone decreases BBB permeability by multiple mechanisms (see Section 3.2.1), it may lead to DDIs with respect to drug distribution into the CNS. The clinical significance of this mechanism of DDI is not clear.

3.3.2. Drug interactions that involve blood-brain barrier tight junctions—CSF concentrations have also been utilized to assess the impact of osmotic BBBD on CNS penetration of methotrexate. For example, intra-arterial administration of methotrexate with osmotic BBBD resulted in up to 6-fold enhancement of methotrexate CSF penetration, compared to intravenous or intra-arterially administration (Zylber-Katz *et al.*, 2000) (Table 5). In general, osmotic BBBD improved clinical outcomes of cancer chemotherapy in phase I and phase II studies (Siegal & Zylber-Katz, 2002), but has not been evaluated in larger clinical trials. Currently, concerns still exist regarding efficacy and toxicity of osmotic BBBD. First, whereas osmotic BBBD probably increases the distribution of hydrophilic compounds into the ISF, it may not improve their distribution into the tumor itself, given the abnormalities of tumor microvessels. Second, non-specific BBB disruption can augment neurotoxicity of the chemotherapeutic compounds as well as that of many other substances that normally would not gain access into brain parenchyma (e.g., albumin) (Kemper et al., 2004a; Zylber-Katz et al., 2000). More selective opening of tumor-blood barrier using bradykinin analogues has been studied in pediatric patients with brain tumors, but did not enhance the efficacy of carboplatin in these patients (Prados et al., 2003; Warren et al., 2006). At present, clinical studies on BBBD to improve CNS drug delivery are ongoing, but the utilization of this method is limited to a few centers and this type of DDI is not expected to occur with the use of conventional therapeutic regimens (Bellavance et al., 2008; Kroll & Neuwelt, 1998).

3.3.3. Transporter-mediated drug interactions—Since the discovery that the calcium channel blocker verapamil can restore drug sensitivity in tumor cell lines (Tsuruo, 1981), many agents have been investigated for their ability to inhibit P-gp and therefore reverse the multidrug resistance of tumors (for review, see Breedveld et al., 2006; Shukla et al., 2008; Szakács et al., 2006)). In addition to verapamil, other P-gp inhibitors already in use for other indications, such as cyclosporine and quinidine, were tested in pre-clinical and clinical trials (Raderer & Scheithauer, 1993). However, these compounds had low potencies to inhibit P-gp and the high doses that were used resulted in significant toxicity of the inhibitor. In addition, these agents increased anticancer drug toxicities due to non-selective inhibition of P-gp and hepatic drug metabolizing enzymes (cytochrome P450, in particular CYP3A) in tissues involved in drug absorption, distribution and elimination. Second generation P-gp inhibitors, e.g., valspodar (PSC-833) and biricodar (VX-710), were more potent and had better tolerability but also inhibited the elimination of co-administered cytotoxic agents. For example, valspodar, the most studied second-generation P-gp inhibitor in the clinic, decreased the clearance of concomitantly

administered etoposide and the study was terminated due to excessive mortality (Advani et al., 1999). In a subsequent trial, valspodar demonstrated an overall survival advantage in a subset of subjects (Kolitz *et al.*, 2004). However, the development of valspodar, as well as that of biricodar, has been discontinued due to their pharmacokinetic interactions (Szakács *et al.*, 2006). Third-generation P-gp inhibitors, such as tariquidar (XR-9576), elacridar (GF120918, also a BCRP inhibitor) and zosuquidar (LY335979) inhibit P-gp potently and have been developed to avoid inhibition of hepatic enzymes (Breedveld et al., 2006). Initial studies with tariquidar were stopped early due to toxicity of the chemotherapeutic drug (Szakács *et al.*, 2006). However, further studies are currently evaluating the safety and efficacy of tariquidar in combination with a variety of chemotherapeutic compounds in patients with solid tumors, including brain malignancies (http://health.nih.gov/topic/ClinicalTrials). In general, little toxicity to the central nervous system has been reported in patients treated with P-gp inhibitors, even in those treated with neurotoxic chemotherapeutic compounds (Gottesman et al., 2002).

Despite the generally disappointing results from studies aimed to reverse efflux transporter-mediated drug resistance to anticancer drugs (Breedveld et al., 2006; Raderer & Scheithauer, 1993), whether inhibition of efflux transporters increases delivery and efficacy of chemotherapeutic drugs in brain tumors remains an open question. In a recent study, paclitaxel (175 mg/m^2 intravenously) was administered alone or with high dose tamoxifen (160 mg/m^2 orally twice daily on days 1-5 preceding paclitaxel) to patients with primary or metastatic brain tumors. The median peak CSF-to-plasma paclitaxel concentration ratio was 3.7-fold lower in the group treated with the combination, as compared with administration of paclitaxel alone, possibly due to inhibition of P-gp in the CP (Chen et al., 2006) (Table 5). In another cohort of patients that received the same treatment, between 2 and 3 hours after completing paclitaxel infusion, samples of tumor tissue, brain adjacent to tumor, normal brain and serum were collected during surgical resection of the tumor. After correcting for tumor type, there was no increase in paclitaxel tissue concentration in patients who received tamoxifen. The authors suggested that serum tamoxifen concentrations were too low to inhibit P-gp *in vivo* (Fine et al., 2006).

Several studies investigated the role of P-gp in CNS distribution of antitetroviral drugs in humans by assuming that CSF is a biomarker of drug concentrations in the brain ISF (Table 5). As pointed out in Section 3.1, this assumption is fraught with problems. Khaliq et al (Khaliq et al., 2000) assessed the effect of ketoconazole (200 or 400 mg/d for 10 days, oral) on CSF concentrations of ritonavir or saquinavir (400 mg twice daily, oral, each) in patients infected with HIV. Ketoconazole (at both plasma concentrations of 4.8 and 8.2 μg/mL, 9 and 15.4 μM) increased ritonavir CSF-to-plasma unbound concentration ratio by 2.9-fold (from 0.09 to 0.26). The increase in saquinvir CSF-to-plasma unbound ratio (5.8-fold) was insignificant, probably because of small subject numbers and high interindividual variability in treatment effect. The authors suggested that inhibition of efflux transporters may be used to improve treatment of HIV in the CNS. Similarly, van Praag et al. (van Praag *et al.*, 2000) added ritonavir (100 mg twice daily) to patients treated with zidovudine or stavudine, lamivudine, abacavir, nevirapine or indinavir. Median serum trough concentrations of indinavir increased 5.2-fold, but serum peak concentrations remained unchanged in the presence of ritonavir, indicating decreased elimination half-life of indinavir as a result of inhibition of its systemic clearance by ritonavir. The median indinavir CSF concentration increased from 39 ng/ml to 104 ng/ml. Thus, when normalized by peak plasma concentration, but not by trough concentrations, ritonavir increased 2.6-fold the CSF-to-plasma ratio of indinavir. These results illustrate the importance of study design when interpreting DDIs at the level of CNS concentrations (Table 1). Under steady-state conditions or when complete AUC profiles are characterized, changes in systemic drug concentrations (due to a systemic DDI) should not affect the CSF-to-plasma or brain-to-plasma concentration of the drug and therefore should not confound interpretation of such data. To overcome complications associated with drawing single CSF samples, Haas et al. (Haas *et*

al., 2003) obtained serial CSF and plasma samples from HIV-infected patients for evaluation of CSF-to-plasma AUC ratio. This study demonstrated that the primary mechanism for ritonavir-indinavir interaction was increased plasma concentrations of indinavir resulting from hepatic CYP3A inhibition by ritonavir.

The transporter theory in refractory epilepsy (Löscher & Potschka, 2005) led to the evaluation of P-gp inhibitors as add-on therapies to antiepileptic drugs for the treatment of intractable epilepsy. Two case reports describe reversal of drug resistance in patients with refractory epilepsy treated with multiple anticonvulsants by verapamil (Iannetti et al., 2005; Summers et al., 2004). Subsequent trials in patients with drug-resistant epilepsy substantiated the effect of combined treatment with antiepileptic drugs and verapamil (Löscher & Potschka, 2005). However, the effect of verapamil in these patients could be mediated by mechanisms other than P-gp inhibition. An ongoing clinical trial will assess the effect of adjuvant treatment with carvedilol (used as a P-gp inhibitor) in patients with refractory epilepsy (ClinicalTrials.gov).

In another case report, the addition of colchicine (2 mg, oral, in two days) to verapamil (120 mg/d, oral, slow release) in a patient that was treated with multiple drugs resulted in tetraparesis (Tröger et al., 2005). Excessive colchicine concentrations were measured in both plasma and CSF. These concentrations decreased gradually when colchicine was stopped. Because colchicine CSF-to-serum concentration ratio was 5-fold higher than normal, it was assumed that verapamil induced colchicine accumulation in the CNS by inhibition of P-gp at the BBB.

Based on the remarkable CNS effects of loperamide in P-gp$^{(-/-)}$ KO mice, Sadeque and co-investigators (Sadeque et al., 2000) administered loperamide (16 mg) to eight healthy subjects with or without quinidine sulfate (600 mg), a potent P-gp inhibitor. Opioid-induced respiratory depression served as the marker of central effects of loperamide. In this study, loperamide did not produce respiratory depression when given alone. However, when quinidine was co-administered, respiratory depression occurred. Although the CNS penetration of loperamide was measured indirectly, this study suggested that P-gp at the BBB contributes to the safety of loperamide and that its inhibition may have potential toxic effects. Two subsequent pharmacodynamic studies assessed the effect of quinidine on other opioid drugs. In one, quinidine enhanced the effects of methadone when methadone was administered orally, but not when it was injected intravenously. The investigators concluded that quinidine inhibited intestinal P-gp, as well as methadone metabolism by CYP2D6, but did not inhibit BBB P-gp (Kharasch et al., 2003). In the other study, quinidine (800 mg/kg, oral) did not increase the effect of morphine (7.5 mg, intravenous infusion) on pupil size (Skarke *et al.*, 2004). In contrast, probenecid (500 mg, oral) increased the area under the miotic effect-versus time curve by a factor of 1.2, but also decreased the clearance of morphine's active metabolite, morphine-6-glucoronide. Most recently, Kurnik and co-investigators (Kurnik et al., 2008) assessed the effect of tariquidar (150 mg, iv) on central opioid effects (pupil diameter and sedation) of loperamide (32 mg, orally) and on P-gp activity in T-lymphocytes in nine healthy volunteers. Although tariquidar completely inhibited lymphocyte P-gp activity, it did not significantly affect loperamide's plasma concentrations and CNS effects. Two possible explanations for this tissue selectivity are 1) loperamide's plasma concentrations were not high enough to achieve effective brain concentrations, even if P-glycoprotein is efficiently inhibited by tariquidar; 2) P-gp localized at the BBB is more resistant to inhibition than at the lymphocyte, as has been previously suggested for mice (Choo et al., 2006).

Loperamide is frequently used to treat ritonavir-associated diarrhea in patients with human immunodeficiency virus. Mukawaya et al. (Mukwaya *et al.*, 2005) evaluated respiratory depression and pupil response by loperamide alone (16 mg, oral), with tipranavir (750 mg twice daily, 5.5 days), ritonavir (200 mg twice daily, 5.5 days), or their combination (for 10 days). Tipranavir-containing regimens decreased the plasma AUC of loperamide and its CNS-active

metabolite, *N*-desmethyl-loperamide, whereas ritonavir increased plasma AUC of loperamide and its metabolite 2.2-fold and 1.4-fold, respectively. However, despite the increased plasma exposure to loperamide and its metabolite, there was no clinically relevant change in the respiratory response to carbon dioxide or in pupil diameter between the treatment groups, indicating that ritonavir did not enhance the transfer of loperamide into the CNS. Similarly, Tayrouz et al. (Tayrouz *et al.*, 2001) administered loperamide (16 mg, oral) to 12 healthy volunteers with either 600 mg ritonavir or placebo. Although ritonavir increased 2.7-fold the plasma AUC of loperamide, no central pharmacodynamic effects were observed following coadministration of loperamide with either ritonavir or placebo. Thus, it seems that co-administration of loperamide with ritonavir does not pose particular risks to the patient (Mukwaya et al., 2005; Tayrouz et al., 2001).

To quantitatively measure the impact of P-gp inhibition at the human BBB, we studied the effect of cyclosporine (2.5 mg/kg/h, intravenously) on [^{11}C]-verapamil plasma and brain concentrations in 12 healthy volunteers. At pseudo steady-state 2.8 μM cyclosporine concentration in blood, the brain-to-plasma AUC ratio of [^{11}C]-radioactivity (0-20 min) increased by 88% without a significant change in plasma [^{11}C]-verapamil metabolism or plasma protein binding (Fig. 3) (Sasongko *et al.*, 2005). This increase was modest when compared to the maximal increases reported in rodents (Hendrikse et al., 1999;Hendrikse et al., 1998;Hendrikse & Vaalburg, 2002;Hsiao et al., 2006) and in non-human primates (Eyal et al., 2009) (Section 3.2). When the white matter and gray matter of the human brain were compared, the increase in [^{11}C]-radioactivity distribution was similar (84 %). The difference in magnitude of this DDI at the human versus non-human primates or rodents BBB is partly due to differences in the blood concentrations of the inhibitor, cyclosporine. Indeed, at lower blood concentration of cyclosporine, the magnitude of the [^{11}C]-verapamil-cyclosporine DDI at the rat BBB is smaller (Hsiao et al., 2006). The lower maximal increase in the brain distribution of [^{11}C]-radioactivity in non-human primates, compared to rodents, is likely explained by species differences in the contribution of BBB P-gp activity to the distribution of verapamil into the brain. Thus, although there is an excellent agreement between the interaction observed at the rat and the human BBB at the lower cyclosporine blood concentrations (~3 μM), if the non-human primates is representative of humans, there may be a divergence between the rat and human as the inhibitor concentration is increased and as P-gp inhibition approaches a maximum.

A recent abstract reported the use of PET for evaluation of the pharmacokinetic interactions between quinidine or cyclosporine and loperamide (Passchier *et al.*, 2008). Six healthy volunteers were scanned under baseline conditions or post administration of quinidine (oral, 600 mg, n=3) or cyclosporine (intravenously, 5 mg/kg/hr over 2 hr, n=3). Cyclosporine increased 2-fold the brain uptake of loperamide, but quinidine did not significantly affect it. The authors suggested that in addition to P-gp, other mechanisms are involved in preventing loperamide central action.

An interaction between methotrexate and vincristine has been described in a patient with lymphoma. Vincristine (1.4 mg/m^2) was added at the 23rd hour of a 24-hour intravenous infusion of methotrexate (1.0 g/m^2) on three occasions. Methotrexate plasma concentrations dropped rapidly when the infusion was terminated. However, CSF methotraxate concentrations increased for a few hours following vincristine administration and were 2.5-fold greater compared with methotrexate monotherapy. Thus, vincristine may inhibit methotrexate efflux from the CP (Tejada & Zubrod, 1979). Another vinca alkaloid, vindesine, did not affect the CSF concentration of methotrexate (Tubiana *et al.*, 1985).

The effect of probenecid pretreatment on the CSF kinetics of methotrexate was clinically evaluated in two small studies. In one, a daily dose of probenecid (1250 mg/m^2), did not change

the efflux kinetics of intraventricularly injected methotrexate. However, at 2500 mg/m^2, probenecid (at CSF concentrations 2.4-3.4 μg/ml) extended the terminal half life of methotrexate up to 53% (Bode et al., 1980). Plasma concentrations were not reported. In another study in 4 patients, probenecid decreased methotrexate renal clearance and increased its CSF concentrations 2.8 to 4.2-fold, but did not extend methotrexate CSF half-life. The authors suggested that probenecid concentrations that were high enough to inhibit the renal clearance of methotrexate in humans failed to alter its clearance from the CSF (Howell et al., 1979).

More than two decades ago, Nutt and collaborators investigated whether oscillations in response of patients with Parkinson's disease to levodopa (the “on-off” phenomenon) reflect fluctuations in drug transport across the gut wall and the BBB (Nutt *et al.*, 1984). When phenylalanine, leucine or isoleucine were administered orally to patients during levodopa infusions, the clinical response to levodopa deteriorated, despite a slight increase in plasma levodopa concentration. Glycine and lysine, that use other transport systems to enter the brain, had no effect on the clinical response to levodopa. However, using Michelis-Menten kinetics, del Amo et al. (Del Amo et al., 2008) have recently suggested that LAT-mediated DDIs at the BBB, e.g., interactions between levodopa and melphalan, are improbable (see Section 4.2). This is because, the total plasma concentration of relevant amino acids is in the millimolar range, and their average affinity (Ki) for the transporter is about 70–100μM. These amino acids may saturate the L-system and competitively prevent substrate drug entry into the CNS. On the other hand, the therapeutic plasma concentrations of most drugs that are LAT1 substrates, including levodopa, are in the micromolar range, and are not predicted to saturate LAT1 (Del Amo *et al.*, 2008).

Commonly used drugs and herbal products, such as carbamazepine, rifampin and St. John's Wort, can induce intestinal and hepatic P-gp activity in humans (Giessmann et al., 2004; Kullak-Ublick & Becker, 2003). However, whether these compounds induce P-gp activity at the human BBB remains to be investigated. From a broader point of view, drugs that activate transcriptional mechanisms may enhance expression or function of other transporters at blood-brain interfaces, but currently there are no data in humans to support this assumption.

Based on the above studies, what can we say about the clinical significance of DDIs at the human BBB? Certainly, significant interactions at human blood-brain interfaces are possible under special conditions such as osmotic BBBD or inhibition of P-gp mediated efflux. With respect to the latter, inadvertent drug interactions at the human BBB are likely to be modest when compared with the effect of ablating P-gp activity in rodents. Based on data obtained so far (with [^{11}C]-verapamil or [^{11}C]-loperamide), the effect observed has been ~100% increase in distribution of radioactivity associated with these drugs. Clearly, the rodent models (chemical or genetic knock-out) are not representative of the magnitude of effect observed in the clinic (Table 5 and see below for an explanation for this discrepancy). Nevertheless, doubling the CNS distribution of a P-gp substrate by an inhibitor (e.g cyclosporine) could result in clinically significant DDI, especially when the P-gp substrate has a narrow CNS therapeutic window. It is also important to recognize that verapamil and loperamide may not represent the maximal DDI likely to be observed at the human BBB. This is because other mechanisms (e.g. diffusion) significantly contribute to their CNS distribution. If another drug had been used as a substrate, one where P-gp plays a greater role in preventing its CNS distribution (e.g nelfinavir), the magnitude of the DDI observed at the human BBB might have been greater. For example, when P-gp is ablated in mice, the brain-to-plasma ratio of nelfinavir increases up to 31-fold (Kim et al., 1998) (Table 3). Indeed, preliminary data from our laboratory has shown that at cyclosporine concentrations observed in our human study, the rat brain-to-plasma concentration ratio corrected for vascular volume of nelfinavir increases by 4-fold. Such an increase in humans would most likely be clinically significant. Clearly, additional studies with

other substrates and inhibitors are needed before drawing conclusions about the maximal magnitude of DDIs likely to occur at the human BBB. This call for additional studies is reinforced by data that P-gp demonstrates multiple binding sites (Shapiro et al., 1999;Taub et al., 2005). Thus, the magnitude of drug interactions that involve [^{11}C]-verapamil or [^{11}C]-loperamide might have been more profound if another inhibitor had been used. This brings up another important issue. Because it is impossible to study drug interactions at the human BBB between all drug combinations, it is important that we develop preclinical tools (*in vitro* or *in vivo*) to predict the magnitude of such interactions. The section below is devoted to discussing such strategies.

4. Predictions of drug interactions at the human blood-brain barrier

The important role that P-gp plays in pharmacokinetic drug interactions has been recognized in a recent draft guidance document on the study of DDIs that was developed by the US Food and Drug Administration (FDA) (available at http://www.fda.gov/cder/guidance/6695dft.pdf). This draft states that P-gp "may be appropriate to evaluate during drug development". Many pharmaceutical companies will preclude development of P-gp substrate drug candidates, particularly if the drug is intended for the treatment of CNS disorders. One reason for doing so is a concern for inadvertent but significant drug-drug interactions at the human BBB. Therefore, it is important that preclinical tools (*in vitro* and *in vivo*) be developed to predict the magnitude of such interactions.

4.1. Predictions from animal studies

Extrapolation of data from studies in rodents to humans is based on the assumption that the rodent BBB is representative of the human BBB and that the potency and magnitude of P-gp inhibition by P-gp inhibitors such as cyclosporine and quinidine will be comparable to that at the human BBB. In the case of P-gp$^{(-/-)}$ KO mice, the magnitude of increase in CNS distribution of the candidate drug is viewed as the "worst case scenario", when P-gp activity is completely ablated. Nevertheless, as discussed in previous sections, caution should be taken in interpretation of results from these animal studies due to methodological considerations and species differences in substrate and inhibitor recognition by P-gp. Furthermore, KO mice may develop compensatory mechanisms that enhance drug efflux from the brain. For example, P-gp KO is associated with greater expression of Bcrp at the BBB (Cisternino et al., 2004) that may lead to underestimation of the role of P-gp in drug transport. In addition, species differences in drug binding to plasma proteins and to brain tissue can potentially affect brain-to-plasma concentration ratios when only total concentrations of the drugs are measured (for example, total brain radioactivity in PET studies). Generally, the physico-chemical properties of endothelial cell membrane such as membrane composition are unlikely to differ largely among species. Nevertheless, taken together, such differences between species may lead to discrepancies when making prediction of both maximum inhibition capacity (E_{max}) and potency (IC_{50}) of an inhibitor. For a more detailed discussion of the species differences in P-gp activity, the reader is directed to a recent review by Kim et al. (Kim et al., 2008).

Given these limitations, can we still predict the magnitude of DDIs in the human BBB from studies in rodents? We have begun to answer this question by determining the *in vivo* EC_{50} of P-gp inhibition at the rat BBB using [^{3}H]-verapamil as a model P-gp substrate and cyclosporine as the model P-gp inhibitor (Hsiao et al., 2006). Cyclosporine was administered by an intravenous infusion to achieve pseudo steady-state blood concentrations ranging from 0 to 17.3 μM. The percentage of increase in the brain-to-blood [^{3}H]-radioactivity (relative to verapamil alone) was described by the Hill equation with E_{max}=1290% and EC_{50}=7.9 μM. Previously, using [^{11}C]-verapamil, we have shown that the human brain-to-blood [^{11}C]-radioactivity (at 20 min) was increased by 79% at 2.8 μM cyclosporine pseudo steady-state blood concentration. At an equivalent cyclosporine blood concentration, the rat brain-to-blood

[^{3}H]-radioactivity was increased by a remarkably similar extent of 75% (Fig. 4). As described in previous sections, we propose that genetic KO or complete chemical inhibition of P-gp at the rodent BBB by supratherapeutic concentrations of the inhibitor likely overestimates the magnitude of clinically relevant inadvertent P-gp-based interactions at the human BBB. To accurately predict such drug interactions, the concentration of the P-gp inhibitor used in the rodent studies must be comparable to that observed in the clinic (Hsiao et al., 2006).

Only few studies have assessed the impact of DDI based on transporter induction at the BBB. In this context, it should be stressed that differences exist between species in the potency of transcriptional factors activation. Therefore, compounds recognized by the human PXR, such as rifampin, are not always potent P-gp inducers in rodents. This obstacle may be overcome by the use of transgenic animals, such as the human PXR transgenic mice described by Bauer et al. (Bauer et al., 2006). However, quantitative correlation in induction of P-gp at the BBB between this transgenic mouse and humans has not been investigated.

4.2 Predictions from in vitro data

Commonly used *in vitro* systems for assessment of drug uptake across the BBB include monolayers of cultured brain capillary endothelial cells, either as primary cultures or as immortalized cell lines, and polarized cell lines of non-cerebral origin, stably or transiently overexpressing the transporter of interest. Cell lines that are frequently used in the evaluation of P-gp-mediated drug transport and drug interactions are MDR1-transfected Madin-Darby canine kidney (MDCK) cells or the porcine LLCPK1 cell line, and the human colon adenocarcinoma cell line Caco-2. The ratio between basal-to-apical (BA) and apical-to-basal (AB) transfer across these monolayers (flux ratio) indicates the degree of P-gp-mediated efflux (Cecchelli et al., 2007). Furthermore, Adachi et al. demonstrated that the ratio of transcellular flux ratios in P-gp-positive and negative epithelial cells predicts BBB P-gp activity in mice (Adachi et al., 2001).

While all these established *in vitro* models have played a major role in the study of P-gp activity at the BBB, further improvement of each model may be necessary to address issues such as the tightness of the monolayer, membrane composition, the presence or absence of other transporters (e.g. BCRP, MRPs, OATs, and OCTs), and non-human origin. For example, the sequence homology of mouse and rat Mdr1a with that of the human MDR1 is 87.0% and 86.6%, respectively. Accordingly, the P-gp substrate specificity in rodents may differ from that in humans (Kim et al., 2008). In line with these differences, Suzuyama et al. demonstrated that the *in vitro* IC_{50} of P-gp inhibition by quinidine and verapamil could vary up to 6-fold between species (Suzuyama et al., 2007). Furthermore, some human transporters do not have direct orthologues in rodents (see above, Section 2.3). Moreover, the properties of endothelial cells are modulated by astrocytes and pericytes, and cultured endothelial cells may have different patterns of transporter expression than in the brain. Methods to study transport activity and other BBB functions *in vitro* have been recently summarized in an excellent review (Cecchelli et al., 2007) and will not be further discussed here.

Because of the limitations of these *in vitro* systems, adjustments are necessary for better approximation of the human BBB. For example, scaling factors may be required to better reflect the fold increase of CNS penetration *in vivo*, and *in vitro* systems that utilize serum-free buffer or medium require protein-binding adjustment. For influx transporter-mediated interactions, it is assumed that the extracellular concentration of the inhibitor (i.e. the unbound plasma concentration) is likely to be more representative of the concentration of the inhibitor at the site of interaction. However, currently there are too few examples where both the *in vitro* and *in vivo* drug interaction data are available for such transporters to determine if this hypothesis is correct. Interpretation is more complex with efflux transporters. Neither the unbound nor the total plasma concentration of the inhibitor is necessarily representative of the actual

inhibitor concentration at the binding site. This in itself is not a problem as the reference point for prediction of DDIs will always be the total or unbound plasma concentration. However, the problem arises when the inhibitor is also a substrate of the efflux transporter. In this case, the intracellular (or lipid bilayer) concentration of the inhibitor will depend on the level of expression of the transporter at the BBB or BCSFB. Thus, the IC_{50} or the "apparent" Km of the inhibitor/substrate will depend on the level of P-gp expression (Shirasaka et al., 2008; Xia et al., 2007). For this reason, it is important to match the level of expression of the transporter in the *in vitro* model with that *in vivo*. While it is difficult to determine the latter, the recent development of LC-MS methods to do so appears promising (Kamiie et al., 2008).

Given the complexity of the BBB and BSCFB, very few *in vitro* studies have reported precise quantitative correlations of DDIs from *in vitro* to *in vivo.* The lack of data from human studies further limits the validation of any of the *in vitro* system as a predictive model. Thus, depending on the resource, cost, time available and the purpose of the study intended by each research facility, one or combination of any of the above *in vitro* systems may be chosen. For example, in the discovery preclinical phase for a drug candidate, *in vitro* BBB models focus on high throughput with emphasis on identification of whether a candidate drug is a substrate for a clinically relevant transporter such as P-gp, OATPs etc. Whereas cell lines transfected with a particular transporter gene of interest are useful to determine the role of a particular transporter, cerebral endothelial cells may be more reflective of the actual *in vivo* situation. However, good models of the latter are currently not available.

To conduct an *in vitro* to *in vivo* correlation of DDIs at the human BBB, human data sets on such DDIs must be available. To date, only two data sets are available. Of these, only one has been published, that on ^{11}C- verapamil-cyclosporine interaction (Sasongko et al., 2005). The second, on [^{11}C]-loperamide-cyclosporine interaction has been published only as an abstract (Passchier et al., 2008). To quantitatively predict the first interaction, we developed a high throughput, simple, and cost-effective cell-based assay. This assay was used to determine the potential of putative P-gp inhibitors to inhibit the efflux of verapamil-bodipy, a model P-gp substrate. LLCPK1-MDR1 cells, expressing recombinant human P-gp, or control cells lacking P-gp (LLCPK1) were used in our assay. The *in vivo* potency of the inhibitors was determined by the ratio of the maximal therapeutic plasma concentration (C_{max}) of the drug and *in vitro* EC_{50} for P-gp inhibition. Using this assay, quinine, quinidine, cyclosporine or amprenavir were predicted to be the most potent P-gp inhibitors *in vivo*, at their respective therapeutic maximal unbound plasma concentrations (Table 6). Remarkably, the *in vitro* EC_{50} of cyclosporine (0.6 μM) for inhibition of human P-gp was virtually identical to the unbound EC_{50} of the drug for *in vivo* inhibition of P-gp at the rat BBB (0.5 μM, see below). Moreover, when our *in vivo* data in the rat (see Section 3.2.4.) and *in vitro* data in LLCPK-MDR1 cells are combined, they predict an increase of 129% in [^{11}C]-verapamil distribution into the human brain, a value similar to that observed by us (79 %) using PET (Fig. 4). These data suggest that the rat and our high throughput cell assay appear to predict P-gp drug interactions at the human BBB relatively well. However, additional data (human, rat and *in vitro*) with other inhibitors are needed to generalize beyond the verapamil-cyclosporine interaction. In this regard, we asked if such an *in vitro* system would quantitatively predict the [^{11}C]-loperamide-cyclosporine interaction at the human BBB. Indeed it does. In humans, intravenous infusion of cyclosporine (10 mg/kg/2 hr) increases the brain [^{11}C]-loperamide by ~110% (Passchier et al., 2008). Based on our data (Sasongko et al., 2005), such a cyclosporine infusion rate would result in pseudo steady-state blood concentration of approximately 5.6 μM. The *in vitro* EC_{50} value of cyclosporine for inhibition of human P-gp in MDCK-MDR1 cells using loperamide as a substrate has been reported to be 0.78 ± 0.04 μM (Corkill et al., 2008). Using this value and the range of vascular volume corrected values of fold-change in brain distribution of loperamide reported in knock-out mice (3.8– 22-fold change), we quantitatively predicted the increase in [^{11}C]-loperamide brain distribution at 5.6 μM cyclosporine blood concentration

(Passchier et al., 2008). The increase in [^{11}C]-loperamide CNS distribution in humans predicted at this cyclosporine blood concentration ranged from 56-412%. The actual observed value (110%) falls within this range. Clearly, the large variability in the *in vivo* brain distribution of loperamide suggests that additional studies are required to better define this value. However, these good-to-excellent quantitative predictions give additional credence to the use of this approach to quantitatively predict DDIs at the human BBB. Nevertheless, to generalize beyond interactions with cyclosporine, it is critical that this method be tested with P-gp inhibitors other than cyclosporine.

5. Conclusions

Although DDIs at the blood-brain interfaces can theoretically occur through several mechanisms, the majority of data on such drug interactions involve the ABC efflux transporters, in particular P-gp. Based on studies in rodents, it has been widely postulated that efflux transporters play a vital role at the human BBB in terms of drug delivery and drug interactions. Through PET imaging studies, it is clear that in humans P-gp is important in preventing delivery of drugs to the CNS. However, the magnitude of its contribution is unknown. This is because none of the polymorphic variants of the MDR1 gene result in null activity and it has not been possible to "chemically" knock-out P-gp activity at the human BBB. Using cyclosporine as an inhibitor, it is apparent that at its therapeutic plasma concentrations, it modestly inhibits P-gp activity at the human BBB. It is still not clear whether cyclosporine is representative of other potential P-gp inhibitors and whether [^{11}C]-verapamil or [^{11}C]-loperamide are representative of other P-gp substrates. In fact, literature data suggest that they may not be. For example, the change in the brain distribution of nelfinavir in the KO mice versus WT mice is much greater (~31-fold) than that for verapamil (~9-fold) or loperamide (~20-fold) (Doran et al., 2005; Kim et al., 1998; Zoghbi et al., 2008). Thus drug interactions with P-gp substrates like nelfinavir are likely to be much greater than substrates like verapamil or loperamide. Therefore additional data are needed with other substrates and inhibitors (with higher affinity with P-gp) to map out the maximum boundary for such interactions. However, the data obtained so far strongly suggests that such interactions can be quantitatively predicted by *in vitro* studies and *in vivo* studies in rodents.

Besides the above, there are several other questions that need to be addressed. First, the magnitude of interactions (if any) that involve transporter induction by drugs and nutritional components has not been evaluated in humans. Second, physiological factors, such as age, and certain pathological conditions, such as inflammation and epilepsy, can modify the function of the neurovascular unit and alter BBB permeability. Thus, the impact of drug interactions at the diseased BBB and in vulnerable populations such as pediatric patients, the elderly and pregnant women is currently unknown. Third, interactions may be mediated by yet unidentified transporters and other components of the neurovascular unit. Finally, the therapeutic benefits of targeted modulation of human BBB function have not been established yet. It is hoped that well-designed clinical trials with BBB modulators will improve future treatment of CNS diseases such as malignant tumors, AIDS dementia and epilepsy. For example, identification of the extent and timing of P-gp modulation by selective inhibitors, using non-invasive imaging techniques, will allow administering a substrate drug that normally has poor brain permeability during an appropriate window of time while avoiding unnecessary exposure to the drug.

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Abbreviations

ABC adenosine triphosphate binding cassette

AUC area under the concentration-time curve

BBB blood-brain barrier

BCRP breast cancer resistance protein

BCSFB blood cerebrospinal fluid barrier

CNT concentrative nucleoside transporter

CP choroid plexus

CSF cerebrospinal fluid

DDI drug-drug interaction

ENT equilibrative nucleoside transporter

GST glutathione S-transferase

ISF interstitial fluid

KO knockout

MCT monocarboxylate transporter

MDR multidrug resistance

MRP multidrug resistance-associated protein

OAT organic anion transporter

OATP organic anion transporting polypeptide

OCT OCTN, organic cation transporter

PET
positron emission tomography

P-gp
P-glycoprotein

SLC
solute carrier

TJs
tight junctions

WT
wild type

References

Abbott N, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 2006;7:41–53. [PubMed: 16371949]

Adachi Y, Suzuki H, Sugiyama Y. Comparative studies on in vitro methods for evaluating in vivo function of MDR1 P-glycoprotein. Pharm Res 2001;18:1660–1668. [PubMed: 11785684]

Adkison K, Shen D. Uptake of valproic acid into rat brain is mediated by a medium-chain fatty acid transporter. J Pharmacol Exp Ther 1996;276:1189–1200. [PubMed: 8786552]

Advani R, Saba H, Tallman M, Rowe J, Wiernik P, Ramek J, et al. Treatment of refractory and relapsed acute myelogenous leukemia with combination chemotherapy plus the multidrug resistance modulator PSC 833 (valspodar). Blood 1999;93:787–795. [PubMed: 9920827]

Akanuma S, Hori S, Ohtsuki S, Fujiyoshi M, Terasaki T. Expression of nuclear receptor mRNA and liver X receptor-mediated regulation of ABC transporter A1 at rat blood-brain barrier. Neurochem Int 2008;52:669–674. [PubMed: 17919779]

Alebouyeh M, Takeda M, Onozato M, Tojo A, Noshiro R, Hasannejad H, et al. Expression of human organic anion transporters in the choroid plexus and their interactions with neurotransmitter metabolites. J Pharmacol Sci 2003;93:430–436. [PubMed: 14737013]

Alexander G, Schwartzman R, Grothusen J, Gordon S. Effect of plasma levels of large neutral amino acids and degree of parkinsonism on the blood-to-brain transport of levodopa in naive and MPTP parkinsonian monkeys. Neurology 1994;44:1491–1499. [PubMed: 8058155]

Anderson B, May M, Jordan S, Song L, Roberts M, Leggas M. Dependence of nelfinavir brain uptake on dose and tissue concentrations of the selective P-glycoprotein inhibitor zosuquidar in rats. Drug Metab Dispos 2006;34:653–659. [PubMed: 16434546]

Anderson G, Shen D. Where is the evidence that p-glycoprotein limits brain uptake of antiepileptic drug and contributes to drug resistance in epilepsy? Epilepsia 2007;48:2372–2374. [PubMed: 18088270]

Anthonypillai C, Sanderson R, Gibbs J, Thomas S. The distribution of the HIV protease inhibitor, ritonavir, to the brain, cerebrospinal fluid, and choroid plexuses of the guinea pig. J Pharmacol Exp Ther 2004;308:912–920. [PubMed: 14634041]

Arboix M, Paz O, Colombo T, D'Incalci M. Multidrug resistance-reversing agents increase vinblastine distribution in normal tissues expressing the P-glycoprotein but do not enhance drug penetration in brain and testis. J Pharmacol Exp Ther 1997;281:1226–1230. [PubMed: 9190857]

Aronica E, Gorter J, Ramkema M, Redeker S, Ozbas-Gerceker F, van Vliet E, et al. Expression and cellular distribution of multidrug resistance-related proteins in the hippocampus of patients with mesial temporal lobe epilepsy. Epilepsia 2004;45:441–451. [PubMed: 15101825]

Awasthi S, Hallene K, Fazio V, Singhal S, Cucullo L, Awasthi Y, et al. RLIP76, a non-ABC transporter, and drug resistance in epilepsy. BMC Neurosci 2005;27:61. [PubMed: 16188027]

Babakhanian K, Bendayan M, Bendayan R. Localization of P-glycoprotein at the nuclear envelope of rat brain cells. Biochem Biophys Res Commun 2007;361:301–306. [PubMed: 17651695]

Badagnani I, Castro R, Taylor T, Brett C, Huang C, Stryke D, et al. Interaction of methotrexate with organic-anion transporting polypeptide 1A2 and its genetic variants. J Pharmacol Exp Ther 2006;318:521–529. [PubMed: 16702441]

Baehr C, Reichel V, Fricker G. Choroid plexus epithelial monolayers--a cell culture model from porcine brain. Cerebrospinal Fluid Res 2006;3:13. [PubMed: 17184532]

Baker S, Heideman R, Crom W, Kuttesch J, Gajjar A, Stewart C. Cerebrospinal fluid pharmacokinetics and penetration of continuous infusion topotecan in children with central nervous system tumors. Cancer Chemother Pharmacol 1996;37:195–202. [PubMed: 8529278]

Bankstahl J, Kuntner C, Abrahim A, Karch R, Stanek J, Wanek T, et al. Tariquidar-induced P-glycoprotein inhibition at the rat blood-brain barrier studied with (*R*)-^{11}C-verapamil and PET. J Nucl Med 2008;49:1328–1335. [PubMed: 18632828]

Barnett P, Roman-Goldstein S, Ramsey F, McCormick C, Sexton G, Szumowski J, et al. Differential permeability and quantitative MR imaging of a human lung carcinoma brain xenograft in the nude rat. Am J Pathol 1995;146:436–449. [PubMed: 7856753]

Barraud de Lagerie S, Comets E, Gautrand C, Fernandez C, Auchere D, Singlas E, et al. Cerebral uptake of mefloquine enantiomers with and without the P-gp inhibitor elacridar (GF1210918) in mice. Br J Pharmacol 2004;141:1214–1222. [PubMed: 15023856]

Bart J, Dijkers E, Wegman T, de Vries E, van der Graaf W, Groen H, et al. New positron emission tomography tracer [(^{11})C]carvedilol reveals P-glycoprotein modulation kinetics. Br J Pharmacol 2005;145:1045–1051. [PubMed: 15951832]

Bart J, Willemsen A, Groen H, van der Graaf W, Wegman T, Vaalburg W, et al. Quantitative assessment of P-glycoprotein function in the rat blood-brain barrier by distribution volume of [^{11}C]verapamil measured with PET. Neuroimage 2003;20:1775–1782. [PubMed: 14642487]

Basic S, Hajnsek S, Bozina N, Filipcic I, Sporis D, Mislov D, et al. The influence of C3435T polymorphism of ABCB1 gene on penetration of phenobarbital across the blood-brain barrier in patients with generalized epilepsy. Seizure 2008;17:524–530. [PubMed: 18329296]

Bauer B, Hartz A, Fricker G, Miller D. Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood-brain barrier. Mol Pharmacol 2004;66:413–419. [PubMed: 15322232]

Bauer B, Hartz A, Lucking J, Yang X, Pollack G, Miller D. Coordinated nuclear receptor regulation of the efflux transporter, Mrp2, and the phase-II metabolizing enzyme, GSTpi, at the blood-brain barrier. J Cereb Blood Flow Metab 2008;28:1222–1234. [PubMed: 18349876]

Bauer B, Yang X, Hartz A, Olson E, Zhao R, Kalvass J, et al. In vivo activation of human pregnane X receptor tightens the blood-brain barrier to methadone through P-glycoprotein up-regulation. Mol Pharmacol 2006;70:1212–1219. [PubMed: 16837625]

Beaulieu E, Demeule M, Ghitescu L, Béliveau R. P-glycoprotein is strongly expressed in the luminal membranes of the endothelium of blood vessels in the brain. Biochem J 1997;326(Pt 2):539–544. [PubMed: 9291129]

Belinsky M, Guo P, Lee K, Zhou F, Kotova E, Grinberg A, et al. Multidrug resistance protein 4 protects bone marrow, thymus, spleen, and intestine from nucleotide analogue-induced damage. Cancer Res 2007;67:262–268. [PubMed: 17210706]

Bellavance M, Blanchette M, Fortin D. Recent advances in blood-brain barrier disruption as a CNS delivery strategy. AAPS J 2008;10:166–177. [PubMed: 18446517]

Bendayan R, Ronaldson P, Gingras D, Bendayan M. In situ localization of P-glycoprotein (ABCB1) in human and rat brain. J Histochem Cytochem 2006;54:1159–1167. [PubMed: 16801529]

Betten D, Vohra R, Cook M, Matteucci M, Clark R. Antidote use in the critically ill poisoned patient. J Intensive Care Med 2006;21:255–277. [PubMed: 16946442]

Bhattacharya I, Boje K. GHB (gamma-hydroxybutyrate) carrier-mediated transport across the blood-brain barrier. J Pharmacol Exp Ther 2004;311:92–98. [PubMed: 15173314]

Bhattacharya I, Boje K. Potential gamma-hydroxybutyric acid (GHB) drug interactions through blood-brain barrier transport inhibition: a pharmacokinetic simulation-based evaluation. J Pharmacokinet Pharmacodyn 2006;33:657–681. [PubMed: 16941233]

Bihorel S, Camenisch G, Lemaire M, Scherrmann J. Modulation of the brain distribution of imatinib and its metabolites in mice by valspodar, zosuquidar and elacridar. Pharm Res 2007;24:1720–1728. [PubMed: 17380257]

Blaney S, Daniel M, Harker A, Godwin K, Balis F. Pharmacokinetics of lamivudine and BCH-189 in plasma and cerebrospinal fluid of nonhuman primates. Antimicrob Agents Chemother 1995;39:2779–2782. [PubMed: 8593019]

Blecharz K, Drenckhahn D, Förster C. Glucocorticoids increase VE-cadherin expression and cause cytoskeletal rearrangements in murine brain endothelial cEND cells. J Cereb Blood Flow Metab 2008;28:1139–1149. [PubMed: 18231113]

Bode U, Magrath I, Bleyer W, Poplack D, Glaubiger D. Active transport of methotrexate from cerebrospinal fluid in humans. Cancer Res 1980;40:2184–2187. [PubMed: 7388786]

Bollini P, Riva R, Albani F, Ida N, Cacciari L, Bollini C, et al. Decreased phenytoin level during antineoplastic therapy: a case report. Epilepsia 1983;24:75–78. [PubMed: 6822234]

Brandt C, Bethmann K, Gastens A, Löscher W. The multidrug transporter hypothesis of drug resistance in epilepsy: Proof-of-principle in a rat model of temporal lobe epilepsy. Neurobiol Dis 2006;24:202–211. [PubMed: 16928449]

Breedveld P, Beijnen J, Schellens J. Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. Trends Pharmacol Sci 2006;27:17–24. [PubMed: 16337012]

Breedveld P, Pluim D, Cipriani G, Wielinga P, van Tellingen O, Schinkel A, et al. The effect of Bcrp1 (Abcg2) on the in vivo pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): implications for the use of breast cancer resistance protein and P-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. Cancer Res 2005;65:2577–2582. [PubMed: 15805252]

Brightman M, Reese T. Junctions between intimately apposed cell membranes in the vertebrate brain. J Cell Biol 1969;40:648–677. [PubMed: 5765759]

Bronger H, König J, Kopplow K, Steiner H, Ahmadi R, Herold-Mende C, et al. ABCC drug efflux pumps and organic anion uptake transporters in human gliomas and the blood–tumor barrier. Cancer Res 2005;65:11419–11428. [PubMed: 16357150]

Brunner M, Langer O, Sunder-Plassmann R, Dobrozemsky G, Muller U, Wadsak W, et al. Influence of functional haplotypes in the drug transporter gene *ABCB1* on central nervous system drug distribution in humans. Clin Pharmacol Ther 2005;78:182–190. [PubMed: 16084852]

Calatozzolo C, Gelati M, Ciusani E, Sciacca F, Pollo B, Cajola L, et al. Expression of drug resistance proteins Pgp, MRP1, MRP3, MRP5 and GST-pi in human glioma. J Neurooncol 2005;74:113–121. [PubMed: 16193381]

Callaghan R, Riordan J. Synthetic and natural opiates interact with P-glycoprotein in multidrug-resistant cells. J Biol Chem 1993;268:16059–16064. [PubMed: 8101846]

Cecchelli R, Berezowski V, Lundquist S, Culot M, Renftel M, Dehouck M, et al. Modelling of the blood-brain barrier in drug discovery and development. Nat Rev Drug Discov 2007;6:650–661. [PubMed: 17667956]

Ceravolo R, Piccini P, Bailey D, Jorga K, Bryson H, Brooks D. ^{18}F-dopa PET evidence that tolcapone acts as a central COMT inhibitor in Parkinson's disease. Synapse 2002;43:201–207. [PubMed: 11793426]

Chen C, Hanson E, Watson J, Lee J. P-glycoprotein limits the brain penetration of nonsedating but not sedating H1-antagonists. Drug Metab Dispos 2003a;31:312–318. [PubMed: 12584158]

Chen C, Liu X, Smith B. Utility of Mdr1-gene deficient mice in assessing the impact of P-glycoprotein on pharmacokinetics and pharmacodynamics in drug discovery and development. Curr Drug Metab 2003b;4:272–291. [PubMed: 12871045]

Chen J, Balmaceda C, Bruce J, Sisti M, Huang M, Cheung Y, et al. Tamoxifen paradoxically decreases paclitaxel deposition into cerebrospinal fluid of brain tumor patients. J Neurooncol 2006;76:85–92. [PubMed: 16402278]

Chen, Y.; Sahik, N.; Yang, Z.; Chen, C.; Elmquist, W. ABCB1 and ABCG2 mediated transport and brain distribution of dasatimib. Paper presented at the 15th North American Regional ISSX meeting; 2008.

Choo E, Leake B, Wandel C, Imamura H, Wood A, Wilkinson G, et al. Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. Drug Metab Dispos 2000;28:655–660. [PubMed: 10820137]

Choo EF, Kurnik D, Muszkat M, Ohkubo T, Shay SD, Higginbotham JN, et al. Differential in vivo sensitivity to inhibition of P-glycoprotein located in lymphocytes, testes, and the blood-brain barrier. J Pharmacol Exp Ther 2006;317:1012–1018. [PubMed: 16537797]

Cisternino S, Mercier C, Bourasset F, Roux F, Scherrmann J. Expression, up-regulation, and transport activity of the multidrug-resistance protein Abcg2 at the mouse blood-brain barrier. Cancer Res 2004;64:3296–3301. [PubMed: 15126373]

Cisternino S, Rousselle C, Dagenais C, Scherrmann J. Screening of multidrug-resistance sensitive drugs by in situ brain perfusion in P-glycoprotein-deficient mice. Pharm Res 2001;18:183–190. [PubMed: 11405289]

Cisternino S, Rousselle C, Lorico A, Rappa G, Scherrmann J. Apparent lack of Mrp1-mediated efflux at the luminal side of mouse blood–brain barrier endothelial cells. Pharm Res 2003;20:904–909. [PubMed: 12817896]

Clinckers R, Smolders I, Meurs A, Ebinger G, Michotte Y. Quantitative *in vivo* microdialysis study on the influence of multidrug transporters on the blood-brain barrier passage of oxcarbazepine: concomitant use of hippocampal monoamines as pharmacodynamic markers for the anticonvulsant activity. J Pharmacol Exp Ther 2005;314:725–731. [PubMed: 15860570]

Cole C, Gottfried O, Gupta D, Couldwell W. Total intravenous anesthesia: advantages for intracranial surgery. Neurosurgery 2007;61(5 Suppl 2):369–377. [PubMed: 18091252]

Cooray H, Blackmore C, Maskell L, Barrand M. Localisation of breast cancer resistance protein in microvessel endothelium of human brain. Neuroreport 2002;13:2059–2063. [PubMed: 12438926]

Cordon-Cardo C, O'Brien J, Boccia J, Casals D, Bertino J, Melamed M. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 1990;38:1277–1287. [PubMed: 1974900]

Corkill, G.; Turner, D.; Pufong, B.; Gill, H.; Fessey, R.; Dilworth, C. In Vitro Evaluation of P-glycoprotein Inhibition using Loperamide as a Probe Substrate. Paper presented at the 10th European ISSX Meeting; 2008.

Cornford E, Diep C, Pardridge W. Blood-brain barrier transport of valproic acid. J Neurochem 1985;44:1541–1550. [PubMed: 3921665]

Cretton E, Schinazi R, McClure H, Anderson D, Sommadossi J. Pharmacokinetics of 3′-azido-3′-deoxythymidine and its catabolites and interactions with probenecid in rhesus monkeys. Antimicrob Agents Chemother 1991;35:801–807. [PubMed: 1854160]

Cvetkovic M, Leake B, Fromm M, Wilkinson G, Kim R. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. Drug Metab Dispos 1999;27:866–871. [PubMed: 10421612]

Dagenais C, Graff C, Pollack G. Variable modulation of opioid brain uptake by P-glycoprotein in mice. Biochem Pharmacol 2004;67:269–276. [PubMed: 14698039]

Daood M, Tsai C, Ahdab-Barmada M, Watchko J. ABC Transporter (P-gp/ABCB1, MRP1/ABCC1, BCRP/ABCG2) Expression in the Developing Human CNS. Neuropediatrics 2008;39:211–218. [PubMed: 19165709]

Dauchy S, Dutheil F, Weaver R, Chassoux F, Daumas-Duport C, Couraud P, et al. ABC transporters, cytochromes P450 and their main transcription factors: expression at the human blood-brain barrier. J Neurochem 2008;107:1518–1528. [PubMed: 19094056]

Dauchy S, Miller F, Couraud P, Weaver R, Weksler B, Romero I, et al. Expression and transcriptional regulation of ABC transporters and cytochromes P450 in hCMEC/D3 human cerebral microvascular endothelial cells. Biochem Pharmacol 2009;77:897–909. [PubMed: 19041851]

de Lange E. Potential role of ABC transporters as a detoxification system at the blood-CSF barrier. Adv Drug Deliv Rev 2004;56:1793–1809. [PubMed: 15381334]

de Lange E, Danhof M. Considerations in the use of cerebrospinal fluid pharmacokinetics to predict brain target concentrations in the clinical setting: implications of the barriers between blood and brain. Clin Pharmacokinet 2002;41:691–703. [PubMed: 12162757]

de Vries E, Doorduin J, Vellinga N, van Waarde A, Dierckx R, Klein H. Can celecoxib affect P-glycoprotein-mediated drug efflux? A microPET study. Nucl Med Biol 2008;35:459–466. [PubMed: 18482683]

de Vries N, Zhao J, Kroon E, Buckle T, Beijnen J, van Tellingen O. P-glycoprotein and breast cancer resistance protein: two dominant transporters working together in limiting the brain penetration of topotecan. Clin Cancer Res 2007;13:6440–6449. [PubMed: 17975156]

Dean M, Annilo T. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Annu Rev Genomics Hum Genet 2005;6:123–142. [PubMed: 16124856]

Deeley R, Westlake C, Cole S. Transmembrane transport of endo- and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins. Physiol Rev 2006;86:849–899. [PubMed: 16816140]

Deguchi Y, Nozawa K, Yamada S, Yokoyama Y, Kimura R. Quantitative evaluation of brain distribution and blood-brain barrier efflux transport of probenecid in rats by microdialysis: possible involvement of the monocarboxylic acid transport system. J Pharmacol Exp Ther 1997;280:551–560. [PubMed: 9023263]

Deguchi Y, Yokoyama Y, Sakamoto T, Hayashi H, Naito T, Yamada S, et al. Brain distribution of 6-mercaptopurine is regulated by the efflux transport system in the blood-brain barrier. Life Sci 2000;66:649–662. [PubMed: 10794520]

Del Amo E, Urtti A, Yliperttula M. Pharmacokinetic role of L-type amino acid transporters LAT1 and LAT2. Eur J Pharm Sci 2008;35:161–174. [PubMed: 18656534]

Deleu D, Northway M, Hanssens Y. Clinical pharmacokinetic and pharmacodynamic properties of drugs used in the treatment of Parkinson's disease. Clin Pharmacokinet 2002;41:261–309. [PubMed: 11978145]

Desrayaud S, Guntz P, Scherrmann J, Lemaire M. Effect of the P-glycoprotein inhibitor, SDZ PSC 833, on the blood and brain pharmacokinetics of colchicine. Life Sci 1997;61:153–163. [PubMed: 9217274]

Dogruel M, Gibbs J, Thomas S. Hydroxyurea transport across the blood-brain and blood-cerebrospinal fluid barriers of the guinea-pig. J Neurochem 2003;87:76–84. [PubMed: 12969254]

Dombrowski S, Desai S, Marroni M, Cucullo L, Goodrich K, Bingaman W, et al. Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. Epilepsia 2001;42:1501–1506. [PubMed: 11879359]

Doran A, Obach R, Smith B, Hosea N, Becker S, Callegari E, et al. The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. Drug Metab Dispos 2005;33:165–174. [PubMed: 15502009]

Ehrlich, P. Das sauerstuffbedürfnis des organismus. Berlin: Hirschwald; 1885.

Ejsing T, Pedersen A, Linnet K. P-glycoprotein interaction with risperidone and 9-OH-risperidone studied in vitro, in knock-out mice and in drug-drug interaction experiments. Hum Psychopharmacol 2005;20:493–500. [PubMed: 16118767]

Ekins S, Kim R, Leake B, Dantzig A, Schuetz E, Lan L, et al. Application of three-dimensional quantitative structure-activity relationships of P-glycoprotein inhibitors and substrates. Mol Pharmacol 2002;61:974–981. [PubMed: 11961114]

Elkind N, Szentpétery Z, Apáti A, Ozvegy-Laczka C, Várady G, Ujhelly O, et al. Multidrug transporter ABCG2 prevents tumor cell death induced by the epidermal growth factor receptor inhibitor Iressa (ZD1839, Gefitinib). Cancer Res 2005;65:1770–1777. [PubMed: 15753373]

Emerich D, Snodgrass P, Pink M, Bloom F, Bartus R. Central analgesic actions of loperamide following transient permeation of the blood brain barrier with Cereport (RMP-7). Brain Res 1998;801:259–266. [PubMed: 9729419]

Endres, C.; Govindarajan, R.; Moss, A.; Unadkat, J. The equilibrative nucleoside transporter 1 (Ent1) significantly contributes to the disposition of ribavirin in the mouse. Paper presented at the AAPS Annual Meeting and Exposition; 2007.

Endres C, Hsiao P, Chung F, Unadkat J. The role of transporters in drug interactions. Eur J Pharm Sci 2006;27:501–517. [PubMed: 16364611]

Enokizono J, Kusuhara H, Ose A, Schinkel A, Sugiyama Y. Quantitative investigation of the role of breast cancer resistance protein (Bcrp/Abcg2) in limiting brain and testis penetration of xenobiotic compounds. Drug Metab Dispos 2008;36:995–1002. [PubMed: 18322075]

Eriksson T, Carlsson A. Isoprenaline increases brain concentrations of administered L-dopa and L-tryptophan in the rat. Psychopharmacology (Berl) 1982;77:98–100. [PubMed: 6812125]

Eyal S, Chung F, Muzi M, Link J, Mankoff D, Kaddoumi A, O'Sullivan F, Hebert M, Unadkat J. Simultaneous PET imaging of P-glycoprotein inhibition in multiple tissues in the pregnant non-human primate. J Nucl Med. 2009in press

Fellner S, Bauer B, Miller D, Schaffrik M, Fankhänel M, Spruss T, et al. Transport of paclitaxel (Taxol) across the blood-brain barrier in vitro and in vivo. J Clin Invest 2002;110:1309–1318. [PubMed: 12417570]

Fine R, Chen J, Balmaceda C, Bruce J, Huang M, Desai M, et al. Randomized study of paclitaxel and tamoxifen deposition into human brain tumors: implications for the treatment of metastatic brain tumors. Clin Cancer Res 2006;12:5770–5776. [PubMed: 17020983]

Fischer W, Praetor K, Metzner L, Neubert R, Brandsch M. Transport of valproate at intestinal epithelial (Caco-2) and brain endothelial (RBE4) cells: Mechanism and substrate specificity. Eur J Pharm Biopharm 2008;2008:486–492. [PubMed: 18577448]

Förster C, Silwedel C, Golenhofen N, Kietz S, Mankertz J, Drenckhahn D. Occludin as direct target for glucocorticoid-induced improvement of blood brain barrier properties in a murine in vitro system. J Physiol 2005;565(Part 2):475–486. [PubMed: 15790664]

Förster C, Waschke J, Burek M, Leers J, Drenckhahn D. Glucocorticoid effects on microvascular endothelial barrier permeability are brain specific. J Physiol 2006;573(Part 2):413–425. [PubMed: 16543270]

Fromm M, Kim R, Stein C, Wilkinson G, Roden D. Inhibition of P-glycoprotein-mediated drug transport: A unifying mechanism to explain the interaction between digoxin and quinidine. Circulation 1999;99:552–557. [PubMed: 9927403]

Gao B, Stieger B, Noe B, Fritschy J, Meier P. Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. J Histochem Cytochem 1999;47:1255–1264. [PubMed: 10490454]

Gazzin S, Strazielle N, Schmitt C, Fevre-Montange M, Ostrow J, Tiribelli C, et al. Differential expression of the multidrug resistance-related proteins ABCb1 and ABCc1 between blood-brain interfaces. J Comp Neurol 2008;510:497–507. [PubMed: 18680196]

Gerstner E, Fine R. Increased permeability of the blood-brain barrier to chemotherapy in metastatic brain tumors: establishing a treatment paradigm. J Clin Oncol 2007;25:2306–2312. [PubMed: 17538177]

Ghersi-Egea J, Leninger-Muller B, Suleman G, Siest G, Minn A. Localisation of drug-metabolizing enzyme activities to blood-brain interfaces and circumventricular organs. J Neurochem 1994;62:1089–1096. [PubMed: 8113796]

Ghersi-Egea J, Strazielle N, Murat A, Jouvet A, Buénerd A, Belin M. Brain protection at the blood-cerebrospinal fluid interface involves a glutathione-dependent metabolic barrier mechanism. J Cereb Blood Flow Metab 2006;26:1165–1175. [PubMed: 16395287]

Gibbs J, Gaffen Z, Thomas S. Nevirapine Uptake into the Central Nervous System of the Guinea Pig: An in Situ Brain Perfusion Study. J Pharmacol Exp Ther 2006;317:746–751. [PubMed: 16424147]

Gibbs J, Jayabalan P, Thomas S. Mechanisms by which 2′,3′-dideoxyinosine (ddI) crosses the guinea-pig CNS barriers; relevance to HIV therapy. J Neurochem 2003a;84:725–734. [PubMed: 12562517]

Gibbs J, Rashid T, Thomas S. Effect of transport inhibitors and additional anti-HIV drugs on the movement of lamivudine (3TC) across the guinea pig brain barriers. J Pharmacol Exp Ther 2003b; 306:1035–1041. [PubMed: 12766261]

Gibbs J, Thomas S. The distribution of the anti-HIV drug, 2′3′-dideoxycytidine (ddC), across the blood-brain and blood-cerebrospinal fluid barriers and the influence of organic anion transport inhibitors. J Neurochem 2002;80:392–404. [PubMed: 11905988]

Giessmann T, May K, Modess C, Wegner D, Hecker U, Zschiesche M, et al. Carbamazepine regulates intestinal P-glycoprotein and multidrug resistance protein MRP2 and influences disposition of talinolol in humans. Clin Pharmacol Ther 2004;76:192–200. [PubMed: 15371980]

Giri N, Shaik N, Pan G, Terasaki T, Mukai C, Kitagaki S, et al. Investigation of the role of breast cancer resistance protein (Bcrp/Abcg2) on pharmacokinetics and central nervous system penetration of abacavir and zidovudine in the mouse. Drug Metab Dispos 2008;36:1476–1484. [PubMed: 18443033]

Goldmann E. Vitalfarbung am zentralnervensystem. Abh Konigl Preuss Akad Wiss 1913;1:1–60.

Gottesman M, Fojo T, Bates S. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2002;2:48–58. [PubMed: 11902585]

Gottesman M, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 1993;62:385–427. [PubMed: 8102521]

Gui C, Miao Y, Thompson L, Wahlgren B, Mock M, Stieger B, et al. Effect of pregnane X receptor ligands on transport mediated by human OATP1B1 and OATP1B3. Eur J Pharmacol 2008;584:57–65. [PubMed: 18321482]

Haas D, Johnson B, Nicotera J, Bailey V, Harris V, Bowles F, et al. Effects of ritonavir on indinavir pharmacokinetics in cerebrospinal fluid and plasma. Antimicrob Agents Chemother 2003;47:2131–2137. [PubMed: 12821458]

Haga S, Hinoshita E, Ikezaki K, Fukui M, Scheffer G, Uchiumi T, et al. Involvement of the multidrug resistance protein 3 in drug sensitivity and its expression in human glioma. Jpn J Cancer Res 2001;92:211–219. [PubMed: 11223551]

Hagenbuch B, Meier P. Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. Pflugers Arch 2004;447:653–665. [PubMed: 14579113]

Hanyu H, Hirao K, Shimizu S, Iwamoto T, Koizumi K, Abe K. Favourable effects of nilvadipine on cognitive function and regional cerebral blood flow on SPECT in hypertensive patients with mild cognitive impairment. Nucl Med Commun 2007;28:281–287. [PubMed: 17325591]

Harke N, Leers J, Kietz S, Drenckhahn D, Förster C. Glucocorticoids regulate the human occludin gene through a single imperfect palindromic glucocorticoid response element. Mol Cell Endocrinol 2008;295:39–47. [PubMed: 18782596]

Hawkins B, Davis T. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev 2005;57:173–185. [PubMed: 15914466]

Hediger M, Romero M, Peng J, Rolfs A, Takanaga H, Bruford E. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Introduction. Pflugers Arch 2004;447:465–468. [PubMed: 14624363]

Heideman R, Balis F, Zimm S, Lester C, Poplack D. The effect of amitriptyline on the central nervous system penetration of methotrexate. Cancer Drug Deliv 1986;3:219–222. [PubMed: 3567842]

Hendrikse N, de Vries E, Eriks-Fluks L, van der Graaf W, Hospers G, Willemsen A, et al. A new *in vivo* method to study P-glycoprotein transport in tumors and the blood-brain barrier. Cancer Res 1999;59:2411–2416. [PubMed: 10344751]

Hendrikse N, Schinkel A, de Vries E, Fluks E, Van der Graaf W, Willemsen A, et al. Complete in vivo reversal of P-glycoprotein pump function in the blood-brain barrier visualized with positron emission tomography. Br J Pharmacol 1998;124:1413–1418. [PubMed: 9723952]

Hendrikse N, Vaalburg W. Dynamics of multidrug resistance: P-glycoprotein analyses with positron emission tomography. Methods 2002;27:228–233. [PubMed: 12183111]

Howell S, Olshen R, Rice J. Effect of probenecid on cerebrospinal fluid methotrexate kinetics. Clin Pharmacol Ther 1979;26:641–646. [PubMed: 498706]

Hsiao P, Bui T, Ho R, Unadkat J. In Vitro to In Vivo Prediction of P-glycoprotein Based Drug Interactions at the Human and Rodent Blood-Brain Barrier. Drug Metab Dispos 2008;36:481–484. [PubMed: 18057117]

Hsiao P, Sasongko L, Link J, Mankoff D, Muzi M, Collier A, et al. Verapamil P-glycoprotein transport across the rat blood-brain barrier: cyclosporine, a concentration inhibition analysis, and comparison with human data. J Pharmacol Exp Ther 2006;317:704–710. [PubMed: 16415090]

Hubensack M, Müller C, Höcherl P, Fellner S, Spruss T, Bernhardt G, et al. Effect of the ABCB1 modulators elacridar and tariquidar on the distribution of paclitaxel in nude mice. J Cancer Res Clin Oncol 2008;134:597–607. [PubMed: 17932689]

Huber R, Gao B, Sidler Pfaendler M, Zhang-Fu W, Leuthold S, Hagenbuch B, et al. Characterization of two splice variants of human organic anion transporting polypeptide 3A1 (OATP3A1) isolated from human brain. Am J Physiol Cell Physiol 2007;292:C795–806. [PubMed: 16971491]

Hugnet C, Cadore J, Buronfosse F, Pineau X, Mathet T, Berny P. Loperamide poisoning in the dog. Vet Hum Toxicol 1996;38:31–33. [PubMed: 8825747]

Huisman M, Smit J, Wiltshire H, Hoetelmans R, Beijnen J, Schinkel A. P-glycoprotein limits oral availability, brain, and fetal penetration of saquinavir even with high doses of ritonavir. Mol Pharmacol 2001;59:806–813. [PubMed: 11259625]

Iannetti P, Spalice A, Parisi P. Calcium-channel blocker verapamil administration in prolonged and refractory status epilepticus. Epilepsia 2005;46:967–969. [PubMed: 15946342]

Inano A, Sai Y, Nikaido H, Hasimoto N, Asano M, Tsuji A, et al. Acetyl-L-carnitine permeability across the blood-brain barrier and involvement of carnitine transporter OCTN2. Biopharm Drug Dispos 2003;24:357–365. [PubMed: 14595704]

Jennings L, Hao C, Cabrita M, Vickers M, Baldwin S, Young J, et al. Distinct regional distribution of human equilibrative nucleoside transporter proteins 1 and 2 (hENT1 and hENT2) in the central nervous system. Neuropharmacology 2001;40:722–731. [PubMed: 11311901]

Jonker J, Schinkel A. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). J Pharmacol Exp Ther 2004;308:2–9. [PubMed: 14576340]

Jonker J, Smit J, Brinkhuis R, Maliepaard M, Beijnen J, Schellens J, et al. Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. J Natl Cancer Inst 2000;92:1651–1656. [PubMed: 11036110]

Joo E, Hong S, Tae W, Han S, Seo D, Lee K, et al. Effect of lamotrigine on cerebral blood flow in patients with idiopathic generalised epilepsy. Eur J Nucl Med Mol Imaging 2006;33:724–729. [PubMed: 16528524]

Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976;455:152–162. [PubMed: 990323]

Kaal E, Vecht C. The management of brain edema in brain tumors. Curr Opin Oncol 2004;16:593–600. [PubMed: 15627023]

Kaddoumi A, Choi S, Kinman L, Whittington D, Tsai C, Ho R, et al. Inhibition of P-glycoprotein activity at the primate blood-brain barrier increases the distribution of nelfinavir into the brain but not into the cerebrospinal fluid. Drug Metab Dispos 2007;35:1459–1462. [PubMed: 17591677]

Kajosaari L, Niemi M, Neuvonen M, Laitila J, Neuvonen P, Backman J. Cyclosporine markedly raises the plasma concentrations of repaglinide. Clin Pharmacol Ther 2005;78:388–399. [PubMed: 16198658]

Kalaria R, Mitchell M, Harik S. Monoamine oxidases of the human brain and liver. Brain 1988;111:1441–51. [PubMed: 3208065]

Kamiie J, Ohtsuki S, Iwase R, Ohmine K, Katsukura Y, Yanai K, et al. Quantitative atlas of membrane transporter proteins: development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel in-silico peptide selection criteria. Pharm Res 2008;25:1469–1483. [PubMed: 18219561]

Kang Y, Terasaki T, Tsuji A. Acidic drug transport in vivo through the blood-brain barrier. A role of the transport carrier for monocarboxylic acids. J Pharmacobiodyn 1990;13:158–163. [PubMed: 2117062]

Kemper E, Boogerd W, Thuis I, Beijnen J, van Tellingen O. Modulation of the blood-brain barrier in oncology: therapeutic opportunities for the treatment of brain tumours? Cancer Treat Rev 2004a; 30:415–423. [PubMed: 15245774]

Kemper E, Cleypool C, Boogerd W, Beijnen J, van Tellingen O. The influence of the P-glycoprotein inhibitor zosuquidar trihydrochloride (LY335979) on the brain penetration of paclitaxel in mice. Cancer Chemother Pharmacol 2004b;53:173–178. [PubMed: 14605863]

Kemper E, van Zandbergen A, Cleypool C, Mos H, Boogerd W, Beijnen J, et al. Increased penetration of paclitaxel into the brain by inhibition of P-Glycoprotein. Clin Cancer Res 2003;97:2849–2855. [PubMed: 12855665]

Kemper E, Verheij M, Boogerd W, Beijnen J, van Tellingen O. Improved penetration of docetaxel into the brain by co-administration of inhibitors of P-glycoprotein. Eur J Cancer 2004c;40:1269–1274. [PubMed: 15110893]

Khaliq Y, Gallicano K, Venance S, Kravcik S, Cameron D. Effect of ketoconazole on ritonavir and saquinavir concentrations in plasma and cerebrospinal fluid from patients infected with human immunodeficiency virus. Clin Pharmacol Ther 2000;68:637–646. [PubMed: 11180024]

Kharasch E, Hoffer C, Whittington D, Sheffels P. Role of P-glycoprotein in the intestinal absorption and clinical effects of morphine. Clin Pharmacol Ther 2003;74:543–554. [PubMed: 14663457]

Kido Y, Tamai I, Ohnari A, Sai Y, Kagami T, Nezu J, et al. Role of P-glycoprotein in the intestinal absorption and clinical effects of morphine. Clin Pharmacol Ther 2003;74:543–554. [PubMed: 14663457]

Kim I, Booth-Genthe C, Ambudkar S. Relationship between drugs and functional activity of various mammalian P-glycoproteins (ABCB1). Mini Rev Med Chem 2008;8:193–200. [PubMed: 18336339]

Kim R, Fromm M, Wandel C, Leake B, Wood A, Roden D, et al. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J Clin Invest 1998;101:289–294. [PubMed: 9435299]

Klaassen C, Lu H. Xenobiotic transporters: ascribing function from gene knockout and mutation studies. Toxicol Sci 2008;101:186–196. [PubMed: 17698509]

Koepsell H, Endou H. The SLC22 drug transporter family. Pflugers Arch 2004;447:666–676. [PubMed: 12883891]

Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. Pharm Res 2007;24:1227–1251. [PubMed: 17473959]

Kolitz J, George S, Dodge R, Hurd D, Powell B, Allen S, et al. Dose escalation studies of cytarabin, daunorubicin, and etoposide with and without multidrug resistant modulation with PSC833 in untreated adults with acute myeloid leukemia younger than 60 years; final induction results of Cancer and Leukemia Group B Study 9621. J Clin Oncol 2004;22:4290–4301. [PubMed: 15514371]

Kong W, Engel K, Wang J. Mammalian nucleoside transporters. Curr Drug Metab 2004;5:63–84. [PubMed: 14965251]

Kroll R, Neuwelt E. Outwitting the blood-brain barrier for therapeutic purposes: osmotic opening and other means. Neurosurgery 1998;42:1083–1099. [PubMed: 9588554]

Kullak-Ublick GA, Becker MB. Regulation of drug and bile salt transporters in liver and intestine. Drug Metab Rev 2003;35:305–317. [PubMed: 14705863]

Kumakura Y, Danielsen E, Reilhac A, Gjedde A, Cumming P. Levodopa effect on [^{18}F]fluorodopa influx to brain: normal volunteers and patients with Parkinson's disease. Acta Neurol Scand 2004;110:188–195. [PubMed: 15285777]

Kurdziel KA, Kiesewetter DO, Carson RE, Eckelman WC, Herscovitch P. Biodistribution, radiation dose estimates, and in vivo Pgp modulation studies of 18F-paclitaxel in nonhuman primates. J Nucl Med 2003;44:1330–1339. [PubMed: 12902425]

Kurnik D, Sofowora G, Donahue J, Nair U, Wilkinson G, Wood A, et al. Tariquidar, a Selective P-glycoprotein Inhibitor, Does Not Potentiate Loperamide's Opioid Brain Effects in Humans despite Full Inhibition of Lymphocyte P-glycoprotein. Anesthesiology 2008 2008;109:1092–1099.

Kusuhara H, Sugiyama Y. Active efflux across the blood-brain barrier: role of the solute carrier family. NeuroRx 2005;2:73–85. [PubMed: 15717059]

Langer O, Müller M. Methods to assess tissue-specific distribution and metabolism of drugs. Curr Drug Metab 2004;5:463–481. [PubMed: 15578942]

Långsjö J, Maksimow A, Salmi E, Kaisti K, Aalto S, Oikonen V, et al. S-ketamine anesthesia increases cerebral blood flow in excess of the metabolic needs in humans. Anesthesiology 2005;103:258–268. [PubMed: 16052107]

Lee G, Dallas S, Hong M, Bendayan R. Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. Pharmacol Rev 2001;53:569–596. [PubMed: 11734619]

Lee Y, Kusuhara H, Jonker J, Schinkel A, S Y. Investigation of efflux transport of dehydroepiandrosterone sulfate and mitoxantrone at the mouse blood-brain barrier: a minor role of breast cancer resistance protein. J Pharmacol Exp Ther 2005;312:44–52. [PubMed: 15448171]

Lee YJ, Maeda J, Kusuhara H, Okauchi T, Inaji M, Nagai Y, et al. In vivo evaluation of P-glycoprotein function at the blood-brain barrier in nonhuman primates using [^{11}C]verapamil. J Pharmacol Exp Ther 2006;316:647–653. [PubMed: 16293715]

Leggas M, Adachi M, Scheffer G, Sun D, Wielinga P, Du G, et al. Mrp4 confers resistance to topotecan and protects the brain from chemotherapy. Mol Cell Biol 2004;24:7612–7621. [PubMed: 15314169]

Lemaire M, Bruelisauer A, Guntz P, Sato H. Dose-dependent brain penetration of SDZ PSC 833, a novel multidrug resistance-reversing cyclosporin, in rats. Cancer Chemother Pharmacol 1996;38:481–486. [PubMed: 8765444]

Lepist, E.; Zhang, E.; Tackaberry, T.; Damaraju, V.; Gati, W.; Yao, S., et al. Transmembrane transport of CVT-510 is mediated by recombinant hENT1 in vitro and by mENT1 in vivo. Paper presented at the 15th North American Regional ISSX meeting; 2008.

Letrent S, Pollack G, Brouwer K, Brouwer K. Effects of a potent and specific P-glycoprotein inhibitor on the blood-brain barrier distribution and antinociceptive effect of morphine in the rat. Drug Metab Dispos 1999;27:827–834. [PubMed: 10383928]

Levy, R.; Thummel, K.; Trager, W.; Hansten, P.; Eichelbaum, M. Metabolic Drug Interactions. Philadelphia: Lippincott Williams & Wilkins; 2000.

Lin J, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. Clin Pharmacokinet 2003;42:59–98. [PubMed: 12489979]

Lindvall M, Hardebo J, Owman C. Barrier mechanisms for neutrotransmitter monoamines in the choroid plexus. Acta Physiol Scand 1980;108:215–221. [PubMed: 6103637]

Linnet K, Ejsing T. A review on the impact of P-glycoprotein on the penetration of drugs into the brain. Focus on psychotropic drugs. Eur Neuropsychopharmacol 2008;18:157–169. [PubMed: 17683917]

Liow J, Kreisl W, Zoghbi S, Lazarova N, Seneca N, Gladding R, et al. P-Glycoprotein Function at the Blood-Brain Barrier Imaged Using ^{11}C-N-Desmethyl-Loperamide in Monkeys. J Nucl Med 2009;50:108–115. [PubMed: 19091890]

Liu X, Chen C, Smith B. Progress in brain penetration evaluation in drug discovery and development. J Pharmacol Exp Ther 2008;325:349–356. [PubMed: 18203948]

Lombardo L, Pellitteri R, Balazy M, Cardile V. Induction of nuclear receptors and drug resistance in the brain microvascular endothelial cells treated with antiepileptic drugs. Curr Neurovasc Res 2008;5:82–92. [PubMed: 18473823]

Loo T, Bartlett M, Clarke D. Disulfiram metabolites permanently inactivate the human multidrug resistance P-glycoprotein. Mol Pharm 2004;1:426–433. [PubMed: 16028354]

Löscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci 2005;6:591–602. [PubMed: 16025095]

Lötsch J, Schmidt R, Vetter G, Schmidt H, Niederberger E, Geisslinger G, et al. Increased CNS uptake and enhanced antinociception of morphine-6-glucuronide in rats after inhibition of P-glycoprotein. J Neurochem 2002;83:241–248. [PubMed: 12423235]

Mao Q, Unadkat J. Role of the breast cancer resistance protein (ABCG2) in drug transport. AAPS J 2005;7:E118–133. [PubMed: 16146333]

Marier J, Deschênes J, Hage A, Seliniotakis E, Gritsas A, Flarakos T, et al. Enhancing the uptake of dextromethorphan in the CNS of rats by concomitant administration of the P-gp inhibitor verapamil. Life Sci 2005;77:2911–2926. [PubMed: 15964599]

Martin C, Berridge G, Mistry P, Higgins C, Charlton P, Callaghan R. The molecular interaction of the high affinity reversal agent XR9576 with P-glycoprotein. Br J Pharmacol 1999;128:403–411. [PubMed: 10510451]

Masereeuw R, Jaehde U, Langemeijer M, de Boer A, Breimer D. In vitro and in vivo transport of zidovudine (AZT) across the blood-brain barrier and the effect of transport inhibitors. Pharm Res 1994;11:324–330. [PubMed: 8165196]

Matsushita H, Suzuki H, Sugiyama Y, Sawada Y, Iga T, Kawaguchi Y, et al. Facilitated transport of cefodizime into the rat central nervous system. J Pharmacol Exp Ther 1991;259:620–625. [PubMed: 1941610]

Mayer U, Wagenaar E, Dorobek B, Beijnen J, Borst P, Schinkel A. Full blockade of intestinal P-glycoprotein and extensive inhibition of blood-brain barrier P-glycoprotein by oral treatment of mice with PSC833. J Clin Invest 1997;100:2430–2436. [PubMed: 9366556]

Mazarati A, Wasterlain C, Shinmei S. Enhancement of antiepileptic drug treatment of experimental status epilepticus through inhibition of P-glycoprotein. Epilepsia 2003;44:109.

Mealey K, Bentjen S, Gay J, Cantor G. Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. Pharmacogenetics 2001;11:727–733. [PubMed: 11692082]

Mealey K, Greene S, Bagley R, Gay J, Tucker R, Gavin P, et al. P-glycoprotein contributes to the blood-brain, but not blood-cerebrospinal fluid, barrier in a spontaneous canine p-glycoprotein knockout model. Drug Metab Dispos 2008;36:1073–1079. [PubMed: 18332085]

Mealey K, Northrup N, Bentjen S. Increased toxicity of P-glycoprotein-substrate chemotherapeutic agents in a dog with the MDR1 deletion mutation associated with ivermectin sensitivity. J Am Vet Med Assoc 2003;223:1453–1455. 1434. [PubMed: 14627096]

Miecz D, Januszewicz E, Czeredys M, Hinton B, Berezowski V, Cecchelli R, et al. Localization of organic cation/carnitine transporter (OCTN2) in cells forming the blood-brain barrier. J Neurochem 2008;104:113–123. [PubMed: 17995936]

Milane A, Fernandez C, Vautier S, Bensimon G, Meininger V, Farinotti R. Minocycline and riluzole brain disposition: interactions with p-glycoprotein at the blood-brain barrier. J Neurochem 2007;103:164–173. [PubMed: 17635670]

Miller D, Bauer B, Hartz A. Modulation of P-glycoprotein at the blood-brain barrier: opportunities to improve central nervous system pharmacotherapy. Pharmacol Rev 2008;60:196–209. [PubMed: 18560012]

Mori S, Ohtsuki S, Takanaga H, Kikkawa T, Kang Y, Terasaki T. Organic anion transporter 3 is involved in the brain-to-blood efflux transport of thiopurine nucleobase analogs. J Neurochem 2004;90:931–941. [PubMed: 15287899]

Morris M, Felmlee M. Overview of the Proton-coupled MCT (SLC16A) Family of Transporters: Characterization, Function and Role in the Transport of the Drug of Abuse gamma-Hydroxybutyric Acid. AAPS J 2008;10:311–321. [PubMed: 18523892]

Mosyagin I, Runge U, Schroeder H, Dazert E, Vogelgesang S, Siegmund W, et al. Association of ABCB1 genetic variants 3435C>T and 2677G>T to ABCB1 mRNA and protein expression in brain tissue from refractory epilepsy patients. Epilepsia 2008;49:1555–1561. [PubMed: 18494787]

Motl S, Zhuang Y, Waters C, Stewart C. Pharmacokinetic considerations in the treatment of CNS tumours. Clin Pharmacokinet 2006;45:871–903. [PubMed: 16928151]

Mukwaya G, MacGregor T, Hoelscher D, Heming T, Legg D, Kavanaugh K, et al. Interaction of ritonavir-boosted tipranavir with loperamide does not result in loperamide-associated neurologic side effects in healthy volunteers. Antimicrob Agents Chemother 2005;49:4903–4910. [PubMed: 16304151]

Muldoon L, Soussain C, Jahnke K, Johanson C, Siegal T, Smith Q, et al. Chemotherapy delivery issues in central nervous system malignancy: a reality check. J Clin Oncol 2007;25:2295–2305. [PubMed: 17538176]

Nagata Y, Kusuhara H, Imaoka T, Endou H, Sugiyama Y. Involvement of rat organic anion transporter 3 in the uptake of an organic herbicide, 2,4-dichlorophenoxyacetate, by the isolated rat choroid plexus. J Pharm Sci 2004;93:2724–2732. [PubMed: 15389674]

Narang V, Fraga C, Kumar N, Shen J, Throm S, Stewart C, et al. Dexamethasone increases expression and activity of multidrug resistance transporters at the rat blood-brain barrier. Am J Physiol Cell Physiol 2008;295:C440–450. [PubMed: 18524938]

Neuwelt E, Barnett P, Bigner D, Frenkel E. Effects of adrenal cortical steroids and osmotic blood-brain barrier opening on methotrexate delivery to gliomas in the rodent: the factor of the blood-brain barrier. Proc Natl Acad Sci U S A 1982;79:4420–4423. [PubMed: 6289301]

Neuwelt E, Barnett P, McCormick C, Remsen L, Kroll R, Sexton G. Differential permeability of a human brain tumor xenograft in the nude rat: impact of tumor size and method of administration on optimizing delivery of biologically diverse agents. Clin Cancer Res 1998;4:1549–1555. [PubMed: 9626476]

Neuwelt E, Frenkel E, Rapoport S, Barnett P. Effect of osmotic blood-brain barrier disruption on methotrexate pharmacokinetics in the dog. Neurosurgery 1980;7:36–43. [PubMed: 6774280]

Nicolazzo J, Charman S, Charman W. Methods to assess drug permeability across the blood-brain barrier. J Pharm Pharmacol 2006;58:281–293. [PubMed: 16536894]

Nies A. The role of membrane transporters in drug delivery to brain tumors. Cancer Lett 2007;254:11–29. [PubMed: 17275180]

Nies A, Jedlitschky G, König J, Herold-Mende C, Steiner H, Schmitt H, et al. Expression and immunolocalization of the multidrug resistance proteins, MRP1-MRP6 (ABCC1-ABCC6), in human brain. Neuroscience 2004;129:349–360. [PubMed: 15501592]

Noé B, Hagenbuch B, Stieger B, Meier P. Isolation of a multispecific organic anion and cardiac glycoside transporter from rat brain. Proc Natl Acad Sci U S A 1997;94:10346–10350. [PubMed: 9294213]

Nutt J, Woodward W, Hammerstad J, Carter J, Anderson J. The “on-off” phenomenon in Parkinson's disease. Relation to levodopa absorption and transport. N Engl J Med 1984;310:483–488. [PubMed: 6694694]

Obradovic T, Dobson G, Shingaki T, Kungu T, Hidalgo I. Assessment of the first and second generation antihistamines brain penetration and role of P-glycoprotein. Pharm Res 2007;24:318–327. [PubMed: 17180728]

Ohtsuki S, Takizawa T, Takanaga H, Hori S, Hosoya K, Terasaki T. Localization of organic anion transporting polypeptide 3 (oatp3) in mouse brain parenchymal and capillary endothelial cells. J Neurochem 2004;90:743–749. [PubMed: 15255953]

Okura K, Haruta T, Kobayashi Y. Drug transfer into cerebrospinal fluid after simultaneous administration of ampicillin with ceftriaxone or ceftazidime in rabbits with Staphylococcus aureus meningitis. J Antimicrob Chemother 1988;22:207–211. [PubMed: 3053552]

Oostendorp R, Buckle T, Beijnen J, van Tellingen O, Schellens J. The effect of P-gp (Mdr1a/1b), BCRP (Bcrp1) and P-gp/BCRP inhibitors on the in vivo absorption, distribution, metabolism and excretion of imatinib. Invest New Drugs 2009;27:31–40. [PubMed: 18449471]

Passchier J, Comley R, Salinas C, Rabiner E, Gunn R, Cunningham V, et al. Blood brain barrier permeability of [^{11}C]loperamide in humans under normal and impaired P-glycoprotein function. J Nucl Med 2008;49(Supp 1):221P.

Passchier J, Lawrie K, Bender D, Fellows I, Gee A. [^{11}C]Loperamide as highly sensitive PET probe for measuring changes in P-glycoprotein functionallity. J Labelled Comp Radiopharm 2003;46(Supp 1):S84–S94.

Pauli-Magnus C, Feiner J, Brett C, Lin E, Kroetz D. No effect of MDR1 C3435T variant on loperamide disposition and central nervous system effects. Clin Pharmacol Ther 2003;74:487–498. [PubMed: 14586389]

Petty B, Kornhauser D, Lietman P. Zidovudine with probenecid: a warning. Lancet 1990;335:1044–1045. [PubMed: 1970102]

Pierre K, Pellerin L. Monocarboxylate transporters in the central nervous system: distribution, regulation and function. J Neurochem 2005;94:1–14. [PubMed: 15953344]

Potschka H, Fedrowitz M, Loscher W. Multidrug resistance protein MRP2 contributes to the blood-brain barrier function and restricts antiepileptic drug activity. J Pharmacol Exp Ther 2003;306:124–131. [PubMed: 12663688]

Potschka H, Fedrowitz M, Löscher W. P-glycoprotein and multidrug resistance-associated protein are involved in the regulation of extracellular levels of the major antiepileptic drug carbamazepine in the brain. Neuroreport 2001;12:3557–3560. [PubMed: 11733711]

Potschka H, Fedrowitz M, Löscher W. P-Glycoprotein-mediated efflux of phenobarbital, lamotrigine, and felbamate at the blood-brain barrier: evidence from microdialysis experiments in rats. Neurosci Lett 2002;327:173–176. [PubMed: 12113905]

Potschka H, Löscher W. In vivo evidence for P-glycoprotein-mediated transport of phenytoin at the blood-brain barrier of rats. Epilepsia 2001;42:1231–1240. [PubMed: 11737157]

Prados M, Schold SJ, Fine H, Jaeckle K, Hochberg F, Mechtler L, et al. A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. Neuro Oncol 2003;5:96–103. [PubMed: 12672281]

Raderer M, Scheithauer W. Clinical trials of agents that reverse multidrug resistance. A literature review. Cancer 1993;72:3553–3563. [PubMed: 8252468]

Raichle M, MacLeod A, Snyder A, Powers W, Gusnard D, Shulman G. A default mode of brain function. Proc Natl Acad Sci U S A 2001;98:676–682. [PubMed: 11209064]

Rambeck B, Jürgens U, May T, Pannek H, Behne F, Ebner A, et al. Comparison of brain extracellular fluid, brain tissue, cerebrospinal fluid, and serum concentrations of antiepileptic drugs measured intraoperatively in patients with intractable epilepsy. Epilepsia 2006;47:681–694. [PubMed: 16650134]

Ramu A, Fusner J, Blaschke T, Glaubiger D. Probenecid inhibition of methotrexate-cerebrospinal fluid pharmacokinetics in dogs. Cancer Treat Rep 1978;62:1465–1470. [PubMed: 581360]

Rao V, Dahlheimer J, Bardgett M, Snyder A, Finch R, Sartorelli A, et al. Choroid plexus epithelial expression of MDR1 P-glycoprotein and multidrug resistance-associated protein contribute to the blood–cerebrospinal-fluid drug-permeability barrier. Proc Natl Acad Sci USA 1999;96:3900–3905. [PubMed: 10097135]

Rapoport S. Osmotic opening of the blood-brain barrier: principles, mechanism, and therapeutic applications. Cell Mol Neurobiol 2000;20:217–230. [PubMed: 10696511]

Rapoport S, Hori M, Klatzo I. Testing of a hypothesis for osmotic opening of the blood-brain barrier. Am J Physiol 1972;223:323–331. [PubMed: 5046750]

Rautio J, Humphreys J, Webster L, Balakrishnan A, Keogh J, Kunta J, et al. In vitro p-glycoprotein inhibition assays for assessment of clinical drug interaction potential of new drug candidates: a recommendation for probe substrates. Drug Metab Dispos 2006;34:786–792. [PubMed: 16455806]

Redzic Z, Segal M. The structure of the choroid plexus and the physiology of the choroid plexus epithelium. Adv Drug Deliv Rev 2004;56:1695–1716. [PubMed: 15381330]

Reese T, Karnovsky M. Fine structural localization of a blood-brain barrier to exogenous per-oxidase. J Cell Biol 1967;34:207–217. [PubMed: 6033532]

Regazzi M, Iacona I, Campana C, Raddato V, Lesi C, Perani G, et al. Altered disposition of pravastatin following concomitant drug therapy with cyclosporin A in transplant recipients. Transplant Proc 1993;25:2732–2734. [PubMed: 8356729]

Richard K, Hume R, Kaptein E, Stanley E, Visser T, Coughtrie M. Sulfation of thyroid hormone and dopamine during human development: ontogeny of phenol sulfotransferases and arylsulfatase in liver, lung, and brain. J Clin Endocrinol Metab 2001;86:2734–2742. [PubMed: 11397879]

Rizwan A, Burckhardt G. Organic anion transporters of the SLC22 family: biopharmaceutica, physiological, and pathological roles. Pharm Res 2007;24:450–470. [PubMed: 17245646]

Rizzi M, Caccia S, Guiso G, Richichi C, Gorter J, Aronica E, et al. Limbic seizures induce P-glycoprotein in rodent brain: functional implications for pharmacoresistance. J Neurosci 2002;22:5833–5839. [PubMed: 12122045]

Roberts L, Black D, Raman C, Woodford K, Zhou M, Haggerty J, et al. Subcellular localization of transporters along the rat blood-brain barrier and blood-cerebral-spinal fluid barrier by in vivo biotinylation. Neuroscience 2008a;155:423–438. [PubMed: 18619525]

Roberts L, Woodford K, Zhou M, Black D, Haggerty J, Tate E, et al. Expression of the thyroid hormone transporters MCT8 (SLC16A2) and OATP14 (SLCO1C1) at the blood-brain barrier. Endocrinology 2008b;149:6251–6261. [PubMed: 18687783]

Rodriguez M, Ortega I, Soengas I, Suarez E, Luka sJ, Calvo R. Effect of P-glycoprotein inhibition on methadone analgesia and brain distribution in the rat. J Pharm Pharmacol 2004;56:367–374. [PubMed: 15025862]

Ryu Y, Liow J, Zoghbi S, Fujita M, Collins J, Tipre D, et al. Disulfiram inhibits defluorination of (^{18})F-FCWAY, reduces bone radioactivity, and enhances visualization of radioligand binding to serotonin 5-HT_{1A} receptors in human brain. J Nucl Med 2007;48:1154–1161. [PubMed: 17574977]

Sadeque AJ, Wandel C, He H, Shah S, Wood AJ. Increased drug delivery to the brain by P-glycoprotein inhibition. Clin Pharmacol Ther 2000;68:231–237. [PubMed: 11014404]

Saito T, Zhang Z, Tokuriki M, Ohtsubo T, Shibamor iY, Yamamoto T, et al. Cyclosporin A inhibits the extrusion pump function of p-glycoprotein in the inner ear of mice treated with vinblastine and doxorubicin. Brain Res 2001;901:265–270. [PubMed: 11368976]

Salama N, Kelly E, Bu iT, Ho R. The impact of pharmacologic and genetic knockout of P-glycoprotein on nelfinavir levels in the brain and other tissues in mice. J Pharm Sci 2005;94:1216–1225. [PubMed: 15858856]

Salzer W, Widemann B, McCully C, Adamson PC, Balis FM. Effect of probenecid on ventricular cerebrospinal fluid methotrexate pharmacokinetics after intralumbar administration in nonhuman primates. Cancer Chemother Pharmacol 2001;48:235–40. [PubMed: 11592346]

Sare G, Gray L, Bath P. Effect of antihypertensive agents on cerebral blood flow and flow velocity in acute ischaemic stroke: systematic review of controlled studies. J Hypertens 2008;26:1058–1064. [PubMed: 18475140]

Sartor L, Bentjen S, Trepanier L, Mealey K. Loperamide toxicity in a collie with the MDR1 mutation associated with ivermectin sensitivity. J Vet Intern Med 2004;18:117–118. [PubMed: 14765742]

Sasongko L, Link J, Muzi M, Mankoff D, Yang X, Collier A, et al. Imaging P-glycoprotein transport activity at the human blood-brain barrier with positron emission tomography. Clin Pharmacol Ther 2005;77:503–514. [PubMed: 15961982]

Schinkel A. P-Glycoprotein, a gatekeeper in the blood-brain barrier. Adv Drug Deliv Rev 1999;36:179–194. [PubMed: 10837715]

Schinkel A, Mayer U, Wagenaar E, Mol C, van Deemter L, Smit J, et al. Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci U S A 1997;94:4028–4033. [PubMed: 9108099]

Schinkel A, Smit J, van Tellingen O, Beijnen J, Wagenaar E, van Deemter L, et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 1994;77:491–502. [PubMed: 7910522]

Schinkel A, Wagenaar E, Mol C, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 1996;97:2517–2524. [PubMed: 8647944]

Schinkel A, Wagenaar E, van Deemter L, Mol C, Borst P. Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Invest 1995;96:1698–1705. [PubMed: 7560060]

Schlageter K, Molnar P, Lapin G, Groothuis D. Microvessel organization and structure in experimental brain tumors: microvessel populations with distinctive structural and functional properties. Microvasc Res 1999;58:312–328. [PubMed: 10527772]

Shaik N, Giri N, Pan G, Elmquist W. P-glycoprotein-mediated active efflux of the anti-HIV1 nucleoside abacavir limits cellular accumulation and brain distribution. Drug Metab Dispos 2007;35:2076–2085. [PubMed: 17709369]

Shapiro A, Fox K, Lam P, Ling V. Stimulation of P-glycoprotein-mediated drug transport by prazosin and progesterone. Evidence for a third drug-binding site. Eur J Biochem 1999;259:841–850. [PubMed: 10092872]

Shen D, Artru A, Adkison K. Principles and applicability of CSF sampling for the assessment of CNS drug delivery and pharmacodynamics. Adv Drug Deliv Rev 2004;56:1825–1857. [PubMed: 15381336]

Shinotoh H, Iyo M, Yamada T, Inoue O, Suzuki K, Itoh T, et al. Detection of benzodiazepine receptor occupancy in the human brain by positron emission tomography. Psychopharmacology (Berl) 1989;99:202–207. [PubMed: 2552484]

Shirasaka Y, Masaoka Y, Kataoka M, Sakuma S, Yamashita S. Scaling of in vitro membrane permeability to predict P-glycoprotein-mediated drug absorption in vivo. Drug Metab Dispos 2008;36:916–922. [PubMed: 18276834]

Shukla S, Wu C, Ambudkar S. Development of inhibitors of ATP-binding cassette drug transporters: present status and challenges. Expert Opin Drug Metab Toxicol 2008;4:205–223. [PubMed: 18248313]

Siegal T, Zylber-Katz E. Strategies for increasing drug delivery to the brain: focus on brain lymphoma. Clin Pharmacokinet 2002;41:171–186. [PubMed: 11929318]

Sills G, Kwan P, Butler E, de Lange E, van den Berg D, Brodie M. P-glycoprotein-mediated efflux of antiepileptic drugs: preliminary studies in mdr1a knockout mice. Epilepsy Behav 2002;3:427–432. [PubMed: 12609264]

Skarke C, Jarrar M, Schmidt H, Kauert G, Langer M, Geisslinger G, et al. Effects of ABCB1 (multidrug resistance transporter) gene mutations on disposition and central nervous effects of loperamide in healthy volunteers. Pharmacogenetics 2003;13:651–660. [PubMed: 14583678]

Skarke C, Langer M, Jarrar M, Schmidt H, Geisslinger G, Lötsch J. Probenecid interacts with the pharmacokinetics of morphine-6-glucuronide in humans. Anesthesiology 2004;101:1394–1399. [PubMed: 15564947]

Smith N, Acharya M, Desai N, Figg W, Sparreboom A. Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. Cancer Biol Ther 2005;4:815–818. [PubMed: 16210916]

Soranzo N, Kelly L, Martinian L, Burley M, Thom M, Sali A, et al. Lack of support for a role for RLIP76 (RALBP1) in response to treatment or predisposition to epilepsy. Epilepsia 2007;48:674–683. [PubMed: 17437410]

Spanaki M, Siegel H, Kopylev L, Fazilat S, Dean A, Liow K, et al. The effect of vigabatrin (gamma-vinyl GABA) on cerebral blood flow and metabolism. Neurology 1999;53:1518–1522. [PubMed: 10534261]

Spector R. Inhibition of methotrexate transport from cerebrospinal fluid by probenecid. Cancer Treat Rep 1976;60:913–916. [PubMed: 1009521]

Spector R, Johanson C. The origin of deoxynucleosides in brain: implications for the study of neurogenesis and stem cell therapy. Pharm Res 2007;24:859–867. [PubMed: 17372683]

Spector R, Lorenzo A. The effects of salicylate and probenecid on the cerebrospinal fluid transport of penicillin, aminosalicyclic acid and iodide. J Pharmacol Exp Ther 1974;188:55–65. [PubMed: 4809276]

Stewart P, Béliveau R, Rogers K. Cellular localization of P-glycoprotein in brain versus gonadal capillaries. J Histochem Cytochem 1996;44:679–685. [PubMed: 8675989]

Straathof C, van den Bent M, Loos W, Vecht C, Schellens J. The accumulation of topotecan in 9L glioma and in brain parenchyma with and without dexamethasone administration. J Neurooncol 1999;42:117–122. [PubMed: 10421068]

Straathof C, van den Bent M, Ma J, Schmitz P, Kros J, Stoter G, et al. The effect of dexamethasone on the uptake of cisplatin in 9L glioma and the area of brain around tumor. J Neurooncol 1998;37:1–8. [PubMed: 9525832]

Strazielle N, Ghersi-Egea J. Demonstration of a coupled metabolism-efflux process at the choroid plexus as a mechanism of brain protection toward xenobiotics. J Neurosci 1999;19:6275–6289. [PubMed: 10414957]

Strazielle N, Ghersi-Egea J. Factors affecting delivery of antiviral drugs to the brain. Rev Med Virol 2005;15:105–133. [PubMed: 15546130]

Su T, Feng M, Weber M. Mediation of highly concentrative uptake of pregabalin by L-type amino acid transport in Chinese hamster ovary and Caco-2 cells. J Pharmacol Exp Ther 2005;313:1406–1415. [PubMed: 15769862]

Sugawara I, Hamada H, Tsuruo T, Mori S. Specialized localization of P-glycoprotein recognized by MRK 16 monoclonal antibody in endothelial cells of the brain and the spinal cord. Jpn J Cancer Res 1990;81:727–730. [PubMed: 1975805]

Summers M, Moore J, McAuley J. Use of verapamil as a potential P-glycoprotein inhibitor in a patient with refractory epilepsy. Ann Pharmacother 2004;38:1631–1634. [PubMed: 15328394]

Sura P, Sura R, Enayetallah A, Grant D. Distribution and Expression of Soluble Epoxide Hydrolase in Human Brain. J Histochem Cytochem 2008;56:551–559. [PubMed: 18319271]

Suzuyama N, Katoh M, Takeuchi T, Yoshitomi S, Higuchi T, Asashi S, et al. Species differences of inhibitory effects on P-glycoprotein-mediated drug transport. J Pharm Sci 2007;96:1609–1618. [PubMed: 17094122]

Sweet D, Miller D, Pritchard J. Ventricular choline transport: a role for organic cation transporter 2 expressed in choroid plexus. J Biol Chem 2001;276:41611–41619. [PubMed: 11553644]

Sweet D, Miller D, Pritchard J, Fujiwara Y, Beier D, Nigam S. Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 (Slc22a8) knockout mice. J Biol Chem 2002;277:26934–26943. [PubMed: 12011098]

Syvänen S, Blomquist G, Sprycha M, Höglund A, Roman M, Eriksson O, et al. Duration and degree of cyclosporin induced P-glycoprotein inhibition in the rat blood-brain barrier can be studied with PET. Neuroimage 2006;32:1134–1141. [PubMed: 16857389]

Szakács G, Paterson J, Ludwig J, Booth-Genthe C, Gottesman M. Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006;5:219–234. [PubMed: 16518375]

Tachikawa M, Watanabe M, Hori S, Fukaya M, Ohtsuki S, Asashima T, et al. Distinct spatio-temporal expression of ABCA and ABCG transporters in the developing and adult mouse brain. J Neurochem 2005;95:294–304. [PubMed: 16181433]

Takano A, Kusuhara H, Suhara T, Ieiri I, Morimoto T, Lee Y, et al. Evaluation of in vivo P-glycoprotein function at the blood-brain barrier among MDR1 gene polymorphisms by using ^{11}C-verapamil. J Nucl Med 2006;47:1427–1433. [PubMed: 16954549]

Takao Y, Kamisaki Y, Itoh T. Beta-adrenergic regulation of amine precursor amino acid transport across the blood brain barrier. Eur J Pharmacol 1992;215:245–251. [PubMed: 1356793]

Takenaka K, Morgan J, Scheffer G, Adachi M, Stewart C, Sun D, et al. Substrate overlap between Mrp4 and Abcg2/Bcrp affects purine analogue drug cytotoxicity and tissue distribution. Cancer Res 2007;67:6965–6972. [PubMed: 17638908]

Taub M, Podila L, Ely D, Almeida I. Functional assessment of multiple P-glycoprotein (P-gp) probe substrates: influence of cell line and modulator concentration on P-gp activity. Drug Metab Dispos 2005;33:1679–1687. [PubMed: 16093365]

Tayrouz Y, Ganssmann B, Ding R, Klingmann A, Aderjan R, Burhenne J, et al. Ritonavir increases loperamide plasma concentrations without evidence for P-glycoprotein involvement. Clin Pharmacol Ther 2001;70:405–414. [PubMed: 11719726]

Tejada F, Zubrod C. Vincristine effect on methotrexate cerebrospinal fluid concentration. Cancer Treat Rep 1979;63:143–145. [PubMed: 311246]

Thiebaut F, Tsuruo T, Hamada H, Gottesman M, Pastan I, Willingham M. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci U S A 1987;84:7735–7738. [PubMed: 2444983]

Thompson S, Koszdin K, Bernards C. Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. Anesthesiology 2000;92:1392–1399. [PubMed: 10781286]

Toornvliet R, van Berckel BN, Luurtsema G, Lubberink M, Geldof AA, Bosch TM, et al. Effect of age on functional P-glycoprotein in the blood-brain barrier measured by use of (*R*)-[(^{11})C]verapamil and positron emission tomography. Clin Pharmacol Ther 2006;79:540–548. [PubMed: 16765142]

Török M, Huwyler J, Drewe J, Gutmann H, Fricker G. Transport of the beta-lactam antibiotic benzylpenicillin and the dipeptide glycylsarcosine by brain capillary endothelial cells in vitro. Drug Metab Dispos 1998;26:1144–1148. [PubMed: 9806958]

Tröger U, Lins H, Scherrmann J, Wallesch C, Bode-Böger S. Tetraparesis associated with colchicine is probably due to inhibition by verapamil of the P-glycoprotein efflux pump in the blood-brain barrier. BMJ 2005;331:613. [PubMed: 16148013]

Tsuji A. Small molecular drug transfer across the blood-brain barrier via carrier-mediated transport systems. NeuroRx 2005;2:54–62. [PubMed: 15717057]

Tsuji A, Saheki A, Tamai I, Terasaki T. Transport mechanism of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors at the blood-brain barrier. J Pharmacol Exp Ther 1993;267:1085–1090. [PubMed: 8263769]

Tsuji A, Terasaki T, Takabatake Y, Tenda Y, Tamai I, Yamashima T, et al. P-glycoprotein as the drug efflux pump in primary cultured bovine brain capillary endothelial cells. Life Sci 1992;51:1427–37. [PubMed: 1357522]

Tsujikawa K, Dan Y, Nogawa K, Sato H, Yamada Y, Murakami H, et al. Potentiation of domperidone-induced catalepsy by a P-glycoprotein inhibitor, cyclosporin A. Biopharm Drug Dispos 2003;24:105–114. [PubMed: 12673668]

Tsuruo T. Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res 1981;41:1967–1972. [PubMed: 7214365]

Tubiana N, Lena N, Barbet J, Imbert A, Lejeune C, Maraninchi D, et al. Methotrexate-vindesine association in leukemia: pharmacokinetic study. Med Oncol Tumor Pharmacother 1985;2:99–102. [PubMed: 3864001]

Tunblad K, Jonsson E, Hammarlund-Udenaes M. Morphine blood-brain barrier transport is influenced by probenecid co-administration. Pharm Res 2003;20:618–623. [PubMed: 12739770]

Tuntland T, Ravasco R, al-Habet S, Unadkat J. Efflux of zidovudine and 2′,3′-dideoxyinosine out of the cerebrospinal fluid when administered alone and in combination to Macaca nemestrina. Pharm Res 1994;11:312–317. [PubMed: 8165194]

Uc E, Dienel G, Cruz N, Harik S. beta-Adrenergics enhance brain extraction of levodopa. Mov Disord 2002;17:54–59. [PubMed: 11835439]

Uchino H, Kanai Y, Kim D, Wempe M, Chairoungdua A, Morimoto E, et al. Transport of amino acid-related compounds mediated by L-type amino acid transporter 1 (LAT1): insights into the mechanisms of substrate recognition. Mol Pharmacol 2002;61:729–737. [PubMed: 11901210]

Upton R. Cerebral uptake of drugs in humans. Clin Exp Pharmacol Physiol 2007;34:695–701. [PubMed: 17600543]

van Montfoort J, Müller M, Groothuis G, Meijer D, Koepsell H, Meier P. Comparison of “type I” and “type II” organic cation transport by organic cation transporters and organic anion-transporting polypeptides. J Pharmacol Exp Ther 2001;298:110–115. [PubMed: 11408531]

van Praag R, Weverling G, Portegies P, Jurriaans S, Zhou X, Turner-Foisy M, et al. Enhanced penetration of indinavir in cerebrospinal fluid and semen after the addition of low-dose ritonavir. AIDS 2000;14:1187–1194. [PubMed: 10894283]

van Vliet E, Redeker S, Aronica E, Edelbroek P, Gorter J. Expression of multidrug transporters MRP1, MRP2, and BCRP shortly after status epilepticus, during the latent period, and in chronic epileptic rats. Epilepsia 2005;46:1569–1580. [PubMed: 16190927]

van Vliet E, van Schaik R, Edelbroek P, Redeker S, Aronica E, Wadman W, et al. Inhibition of the multidrug transporter P-glycoprotein improves seizure control in phenytoin-treated chronic epileptic rats. Epilepsia 2006;47:672–680. [PubMed: 16650133]

Vecht C, Wagner G, Wilms E. Interactions between antiepileptic and chemotherapeutic drugs. Lancet Neurol 2003;2:404. [PubMed: 12849118]

Venneri A. Imaging treatment effects in Alzheimer's disease. Magn Reson Imaging 2007;25:953–968. [PubMed: 17446028]

Verrey F. System L: heteromeric exchangers of large, neutral amino acids involved in directional tranport. Pflugers Arch 2003;445:529–533. [PubMed: 12634921]

Wang J, DeVane C, Gibson B, Donovan J, Markowitz J, Zhu H. Population pharmacokinetic analysis of drug-drug interactions among risperidone, bupropion, and sertraline in CF1 mice. Psychopharmacology (Berl) 2006;183:490–499. [PubMed: 16283256]

Wang Y, Wei Y, Sawchuk R. Zidovudine transport within the rabbit brain during intracerebroventricular administration and the effect of probenecid. J Pharm Sci 1997;86:1484–1490. [PubMed: 9423165]

Warren K, Jakacki R, Widemann B, Aikin A, Libucha M, Packer R, et al. Phase II trial of intravenous lobradimil and carboplatin in childhood brain tumors: a report from the Children's Oncology Group. Cancer Chemother Pharmacol 2006;58:343–347. [PubMed: 16408203]

Warren K, Patel M, McCully C, Montuenga L, Balis F. Effect of P-glycoprotein modulation with cyclosporin A on cerebrospinal fluid penetration of doxorubicin in non-human primates. Cancer Chemother Pharmacol 2000;45:207–212. [PubMed: 10663638]

Wen T, Liu Y, Yang H, Liu H, Liu X, Wang G, et al. Effect of 21-day exposure of phenobarbital, carbamazepine and phenytoin on P-glycoprotein expression and activity in the rat brain. J Neurol Sci 2008;270:99–106. [PubMed: 18440557]

Westholm D, Rumbley J, Salo D, Rich T, Anderson G. Organic anion-transporting polypeptides at the blood-brain and blood-cerebrospinal fluid barriers. Curr Top Dev Biol 2008;80:135–70. [PubMed: 17950374]

Wijnholds J, deLange E, Scheffer G, van den Berg D, Mol C, van der Valk M, et al. Multidrug resistance protein 1 protects the choroid plexus epithelium and contributes to the blood–cerebrospinal fluid barrier. J Clin Invest 2000;105:279–285. [PubMed: 10675353]

Wilkinson I, Jellineck D, Levy D, Giesel F, Romanowski C, Miller B, et al. Dexamethasone and enhancing solitary cerebral mass lesions: alterations in perfusion and blood-tumor barrier kinetics shown by magnetic resonance imaging. Neurosurgery 2006 2006;58:640–646.

Wong S, Van Belle K, Sawchuk R. Distributional transport kinetics of zidovudine between plasma and brain extracellular fluid/cerebrospinal fluid in the rabbit: investigation of the inhibitory effect of probenecid utilizing microdialysis. J Pharmacol Exp Ther 1993;264:899–909. [PubMed: 8437131]

Xia C, Milton M, Gan L. Evaluation of drug-transporter interactions using in vitro and in vivo models. Curr Drug Metab 2007;8:341–363. [PubMed: 17504223]

Yang Z, Brundage R, Barbhaiya R, Sawchuk R. Microdialysis studies of the distribution of stavudine into the central nervous system in the freely-moving rat. Pharm Res 1997;14:865–872. [PubMed: 9244142]

Yonezawa A, Masuda S, Yokoo S, Katsura T, Inui K. Cisplatin and oxaliplatin, but not carboplatin and nedaplatin, are substrates for human organic cation transporters (SLC22A1-3 and multidrug and toxin extrusion family). J Pharmacol Exp Ther 2006;319:879–886. [PubMed: 16914559]

Yule S, Price L, Pearson A, Boddy A. Cyclophosphamide and ifosfamide metabolites in the cerebrospinal fluid of children. Clin Cancer Res 1997;3:1985–1992. [PubMed: 9815588]

Zastre J, Chan G, Ronaldson P, Ramaswamy M, Couraud P, Romero I, et al. Up-regulation of P-glycoprotein by HIV protease inhibitors in a human brain microvessel endothelial cell line. J Neurosci Res 2009;87:1023–1036. [PubMed: 18855943]

Zhang W, Mojsilovic-Petrovic J, Andrade M, Zhang H, Ball M, Stanimirovic D. The expression and functional characterization of ABCG2 in brain endothelial cells and vessels. FASEB J 2003;17:2085–2087. [PubMed: 12958161]

Zhang Y, Schuetz J, Elmquist WF, Miller D. Plasma membrane localization of multidrug resistanceassociated protein homologs in brain capillary endothelial cells. J Pharmacol ExpTher 2004;311:449–455.

Zhuang Y, Fraga C, Hubbard K, Hagedorn N, Panetta J, Waters C, et al. Topotecan central nervous system penetration is altered by a tyrosine kinase inhibitor. Cancer Res 2006;66:11305–11313. [PubMed: 17145877]

Zoghbi S, Liow J, Yasuno F, Hong J, Tuan E, Lazarova N, et al. ^{11}C-loperamide and its N-desmethyl radiometabolite are avid substrates for brain permeability-glycoprotein efflux. J Nucl Med 2008;49:649–656. [PubMed: 18344435]

Zong J, Pollack G. Modulation of P-glycoprotein transport activity in the mouse blood-brain barrier by rifampin. Pharmacol Exp Ther 2003;306:556–562.

Zylber-Katz E, Gomori J, Schwartz A, Lossos A, Bokstein F, Siegal T. Pharmacokinetics of methotrexate in cerebrospinal fluid and serum after osmotic blood-brain barrier disruption in patients with brain lymphoma. Clin Pharmacol Ther 2000;67:631–641. [PubMed: 10872645]

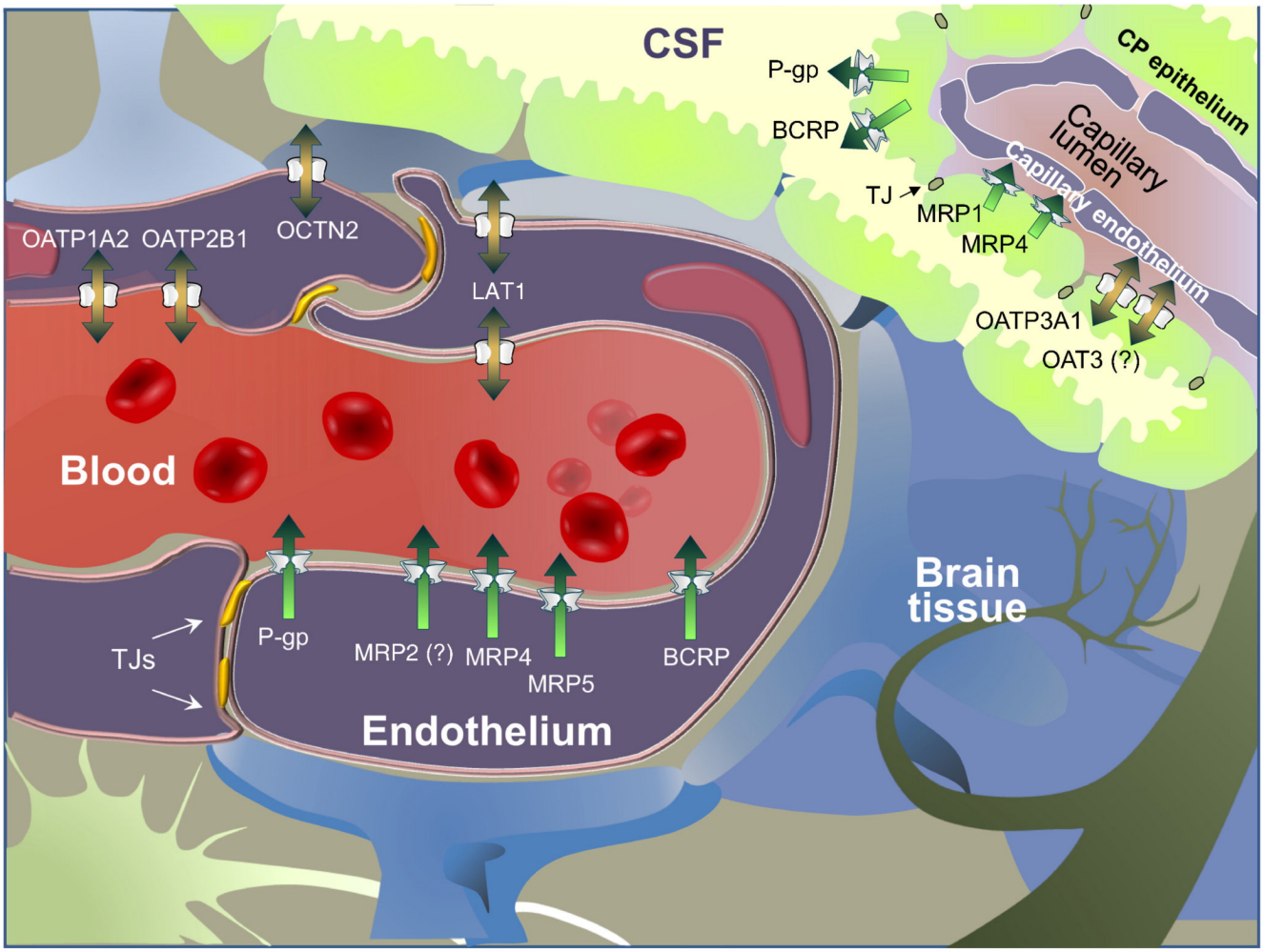

Fig. 1. The localization of transporters at blood-brain barriers

The blood-brain barrier (BBB) is formed by capillary endothelial cells, sealed together by tight junctions. The blood-cerebrospinal fluid barrier (BCSFB) is formed by epithelial cells of the choroid plexus (CP), and tight junctions limit drug transfer between blood and CSF. Under normal conditions, these two anatomical barriers make the brain almost inaccessible to polar drugs, unless they are transferred into the CNS by influx transport systems. Some of these transporters can transfer drugs bidirectionally, down their concentration gradients. Efflux transporters at the luminal membranes of the BBB and the BCSFB remove drugs from brain interstitial fluid back to blood or into the CSF, respectively, thereby preventing them from producing CNS effects. The role of efflux transporters located on the abluminal membranes is unclear. In addition, several drug metabolizing enzymes can potentially form an enzymatic barrier to drug distribution into the brain. BCRP, breast cancer resistance protein; LAT, L-amino acid transporter; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCTN, organic cation/carnitine transporter; P-gp, P-glycoprotein.

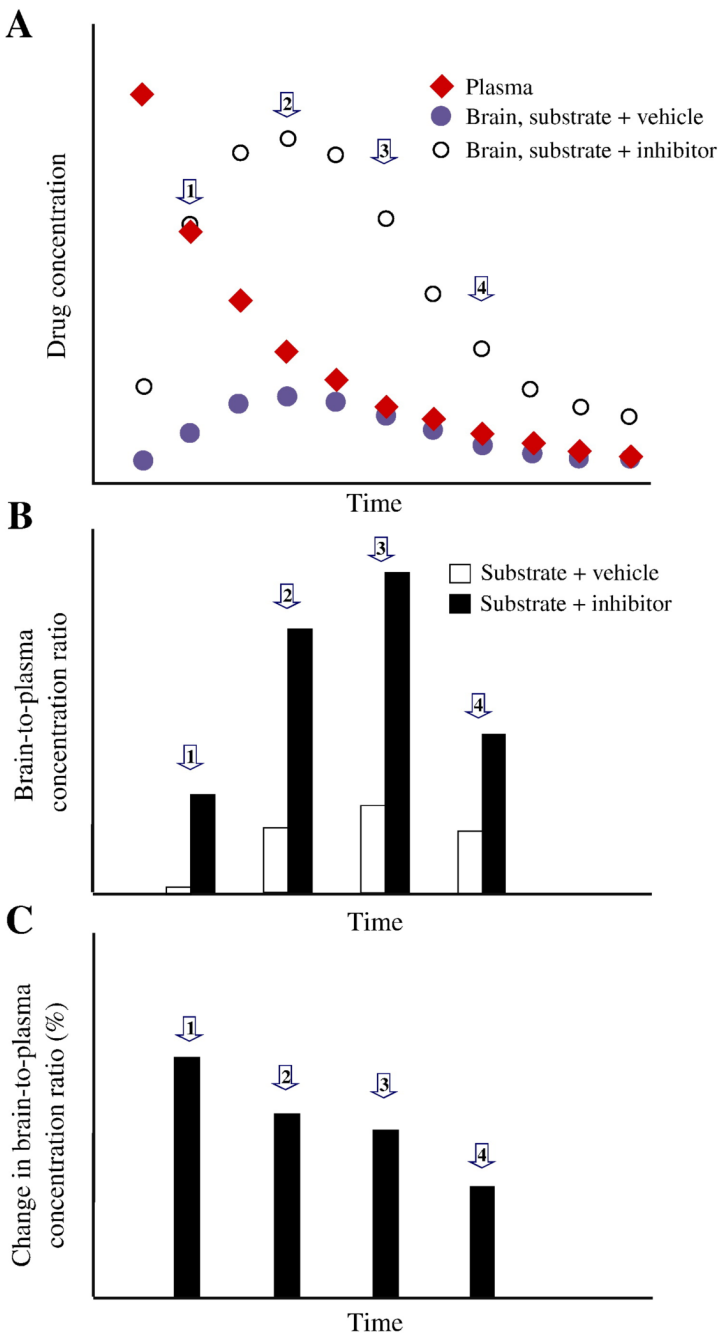

Fig. 2. The effect of sampling time on blood-to-brain concentration ratio

Shown is a simulation of plasma and brain concentrations of a P-gp substrate drug in the presence and the absence of a P-gp inhibitor. For simplification, plasma concentrations of the substrate drug remain unchanged with co-administration of the inhibitor. A. Substrate drug concentrations in brain and plasma. B. Brain-to-plasma ratios of substrate drug concentrations. C. Fold-change in brain-to-plasma concentrations of the substrate with co-administration of the inhibitor. Arrows represent different scenarios of sampling time: 1, 2, 3 - sampling both plasma and brain before distribution equilibrium has been achieved. 4 -plasma is sampled as in (3), but brain sampling is delayed.

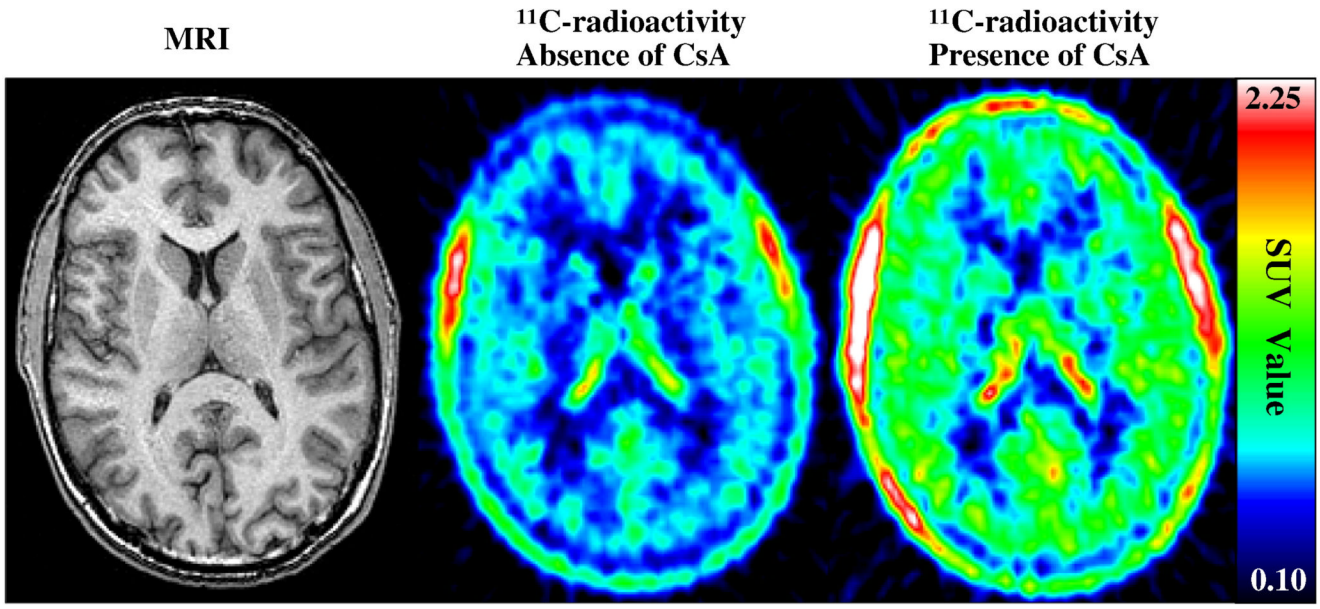

Fig. 3. Positron-emission tomography visualization of a drug-drug interaction at the human BBB

A magnetic resonance image (MRI) showing a normal human brain (A) and a corresponding positron emission tomography (PET) image after [^{11}C]-verapamil administration in the absence (B) or presence (C) of the P-gp inhibitor cyclosporine are shown. Images indicate increased regional uptake (green to red areas) of ^{11}C-radioactivity into the brain in the presence of cyclosporine (from Sasongko *et al.*, 2005).

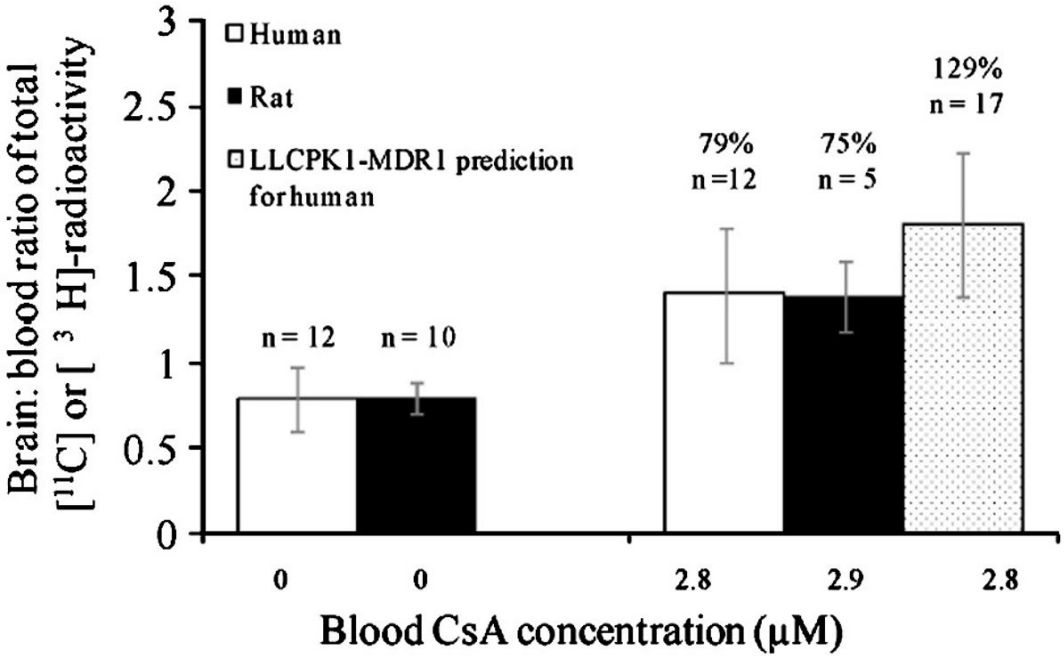

Fig. 4. Prediction of P-gp inhibition at the human BBB from *in vitro* data and from *in vivo* studies in rodents

At identical pseudo steady-state cyclosporine blood concentrations, the brain-to-blood concentration ratio of total verapamil-radioactivity in the rat (gray bars) is virtually identical to that previously obtained in our PET imaging study in humans (open bars). Our *in vitro* studies predict an increase in brain-to-blood radioactivity that is similar to that observed in humans at the same unbound cyclosporine blood concentration. The numerical values above the each bar represent the percent increase in brain-to-blood total radioactivity (relative to the absence of cyclosporine) produced by pseudo steady-state cyclosporine blood concentrations of ~3 μM. N = the number of subjects/experiments per cyclosporine blood concentration (from Hsiao et al., 2008)

Table 1

Examples of different types of drug interactions that affect CNS drug concentrations and activity in humans

Type of interaction	Classification	Example	Putative mechanism of interaction	Outcome	Reference
Altered transfer of object drug across the BBB	Pharmacokinetic	Verapamil-cyclosporine	Inhibition of P-glycoprotein-mediated efflux of verapamil from brain to plasma	Increased brain concentrations of verapamil	(Sasongko *et al.*, 2005)
Altered plasma concentrations of object drug	Pharmacokinetic	Phenytoin-carmustine, vinblastin, methotrexate	Enhanced systemic metabolism of phenytoin by vinca alkaloids	Decreased plasma concentration of phenytoin, recurrent seizures	(Bollini et al., 1983; Vecht et al., 2003)
Altered pharmacokinetics of object drug within the CNS	Pharmacokinetic	Levodopa-tolcapone	Inhibition of levodopa metabolism in brain parencyma*	Improved efficacy of levodopa	(Ceravolo et al., 2002; Deleu et al., 2002)
Altered binding of object drug to target site(s) in the CNS	Pharmacodynamic	Benzodiazepines-flumazenil	Inhibition of benzodiazepine binding to GABAergic receptors	Reversal of benzodiazepine overdose	(Betten et al., 2006; Shinotoh et al., 1989)

* Tolcapone inhibits peripheral metabolism of levodopa as well

Table 2

Selected clinically relevant P-gp substrates and inhibitors

Analgesics	**H_2-receptor antagonists**	**Cardioactive Drugs**
Asimadoline	Cimetidine	Verapamil
Methadone	Ranitidine	Diltiazem
		Digoxin
	Antigout agents	Quinidine
Anticancer drugs	Colchicine	Amiodarone
Vinblastine		
Paclitaxel	**Antidiarrheal agents**	
Doxorubicin	Loperamide	**Antihypertensives**
Daunorubicin		Losartan
Epirubicin	**Antiemetics**	Atorvastatin
Bisantrene	Domperidone	
Mitoxantrone	Ondansetron	**Immunosuppressant**
Etoposide		Cyclosporine A
Teniposide	**Antifungals**	FK506
Actinomycin D	Ketoconazole	Tacrolimus
	Itraconazole	
HIV protease inhibitors		**Corticosteroids**
Saquinavir	**Antihistamines**	Dexamethasone
Ritonavir	Fexofenadine	Hydrocortisone
Nelfinavir	Cetirizine	Corticosterone
Indinavir	Desloratadine	Triamcinolone
Lopinavir		
Amprenavir	**Antipsychotic drugs**	**Antibiotics**
	Risperidone	Erythromycin
β blockers		Gramicidin D
Talinolol		Valinomycin

Inhibitors		
1st Generation	*2nd Generation*	*3rd Generation*
Verapamil	Dexverapamil	LY-335979 (zosuquidar)
Nicardipine	Emopamil	GF-120918 (elacridar)
Quinacrine	Gallopamil	XR-9576 (tariquidar)
Cyclosporine A	PSC-833 (valspodar)	R-101933 (Laniquidar)
	VX-710 (biricodar)	OC144-093

Table 3

Effect of pharmacological inhibition or genetic modulation on brain uptake of P-gp substrate drugs in mice

	Genetic KO of P-gp			Pharmacological inhibition of P-gp			
Substrate drug	**Brain uptake ratio in WT, untreated mice (value corrected for drug in vasculature)**	**Fold change in brain uptake ratio in P-gp KO vs. control mice, assessed parameter (value corrected for drug in vasculature)**	**References**	**Inhibitor (dose, route of administration)**	**Inhibitor concentration (site of measurement)**	**Fold change in brain uptake ratio in inhibitor-treated vs. control mice (value corrected for drug in vasculature)**	**References**
Paclitaxel	0.5 (0.5)	7.9 (8.1), AUC (brain 0-24 h, plasma 0-8 h)	(Kemper *et al.*, 2003)	**Cyclosporine** (50 mg/kg, PO)		0.7 (0.7), AUC	(Kemper *et al.*, 2003)
				Itraconazole (50 mg/kg, PO)		0.2 (0.1), AUC	(Kemper *et al.*, 2003)
				Valspodar (12.5 mg/kg, PO)	< 900 ng/mL (plasma)	2.4 (2.5), AUC	(Kemper *et al.*, 2003)
				Elacridar (100 mg/kg, PO)		4.8 (5.0), AUC	(Kemper *et al.*, 2003)
				Elacridar (100 mg/kg × 1 + 50 mg/kg × 2, PO)		5.2 (5.3), AUC	(Kemper *et al.*, 2003)
				Elacridar (50 mg/kg, PO)	10-51 nmol/g (brain) (~ 3-fold lower in plasma)	>2.5, Conc.	(Hubensack *et al.*, 2008)
				Zosuquidar (80 mg/kg, PO)	~100-600 ng/mL (plasma)	3.2 (3.3), AUC (brain 0-24 h, plasma 0-8 h)	(Kemper et al., 2004b)
	<1	Values not available, Conc.	(Hubensack *et al.*, 2008)	**Tariquidar** (50 mg/kg, PO)	3-9 nmol/g (brain) (~2-fold greater in plasma)	>4.5, Conc.	(Hubensack *et al.*, 2008)
Docetaxel	0.3 (0.3)	6.0 (6.3), AUC (brain 0-24 h, plasma 0-8 h)	(Kemper et al., 2004c)	**Cyclosporine** (50 mg/kg, PO)		0.7 (0.7), AUC (brain 0-24 h, plasma 0-8 h)	(Kemper et al., 2004c)
				Valspodar (25 mg/kg, PO)		1.9 (2.0), AUC (brain 0-24 h, plasma 0-8 h)	(Kemper et al., 2004c)
				Elacridar (25 mg/kg, PO)		2.6 (2.7), AUC (brain 0-24 h, plasma 0-8 h)	(Kemper et al., 2004c)
Vinblastine	1.7 (1.7)	11.2 (11.3), Conc.	(Schinkel et al., 1994)	**Verapamil** (1 mg/kg, IV)		(~1.0) [a], K_{net}	(Cisternino *et al.*, 2001)
		(2.7) [a,c], K_{net}	(Cisternino *et al.*, 2001)	**Valspodar** (10 mg/kg, IV)		(~3.0) [a], K_{net}	(Cisternino *et al.*, 2001)
	0.6	3.3 (3.4)	(Saito *et al.*, 2001)	**Elacridar** (10 mg/kg, IV)		(~3.0) [a], K_{net}	(Cisternino *et al.*, 2001)

	Genetic KO of P-gp			Pharmacological inhibition of P-gp			
Substrate drug	**Brain uptake ratio in WT, untreated mice (value corrected for drug in vasculature)**	**Fold change in brain uptake ratio in P-gp KO vs. control mice, assessed parameter (value corrected for drug in vasculature)**	**References**	**Inhibitor (dose, route of administration)**	**Inhibitor concentration (site of measurement)**	**Fold change in brain uptake ratio in inhibitor-treated vs. control mice (value corrected for drug in vasculature)**	**References**
Vincristine		(1) [a,c], K_{net}	(Cisternino *et al.*, 2001)				
Topotecan	0.3 (0.3)	2.0 (2.1), AUC	(de Vries et al., 2007)	**Elacridar** (50 and 100 mg/kg, PO)		1.6 (1.7), AUC	(de Vries et al., 2007)
				Gefitinib (200 mg/kg, PO) ~1.3-12 μg/mL		1.7 [b], AUC	(Zhuang et al., 2006)
Imatinib		(5.5) [a], K_{net}	(Bihorel et al., 2007)	**Valspodar** (IA)	25 μM (perfusate)	(2.6) [a], K_{net}	(Bihorel *et al.*, 2007)
				Zosuquidar (IA)	5 μM (perfusate)	(2.5) [a], K_{net}	(Bihorel *et al.*, 2007)
				Elacridar (IA)	25 μM (perfusate)	(4.5) [a], K_{net}	(Bihorel *et al.*, 2007)
		(3.6), Conc.-to-AUC ratio	(Breedveld *et al.*, 2005)	**Elacridar** (100 mg/kg, PO)		(4.2), Conc.-to-AUC ratio	(Breedveld *et al.*, 2005)
				Elacridar (100 mg/kg, PO)		5.0, Conc.	(Oostendorp *et al.*, 2009)
	< 0.1	2.3, Conc.	(Oostendorp *et al.*, 2009)	**Pantoprazole** (40 mg/kg, IV)		(1.8), Conc.-to-AUC ratio	(Breedveld *et al.*, 2005)
				Pantoprazole (40 mg/kg, PO)		1.8, conc.	(Oostendorp *et al.*, 2009)
Nelfinavir	0.1 (0.1)	30.9 (41.7), Conc. (4 hr post IV dose, 3 mg/kg, total radioactivity)	(Kim et al., 1998)	**Cyclosporine** (25 mg/kg, IV)		3.4 (4.0), Conc.	(Choo et al., 2000)
				Quinidine (50 mg/kg, IV)		0.6 (0.5), Conc.	(Choo et al., 2000)
	0.1 (0.1)	21.8 (28.3), Conc. (2 hr post IV dose, 5 mg/kg, HPLC)	(Choo et al., 2000)	**Verapamil** (12.5 mg/kg, IV)		1.0 (1.0), Conc.	(Choo et al., 2000)
	0.9 (0.9)	16.1 (16.5), Conc (2 hr post IV dose, 10 mg/kg, HPLC)	(Salama et al. 2005)	**Ketoconazole** (50 mg/kg, IV)		2.1 (2.4), Conc.	(Choo et al., 2000)
				Zosuquidar (50 mg/kg, IV)		18.1 (25.1), Conc.	(Choo et al., 2000)

	Genetic KO of P-gp			Pharmacological inhibition of P-gp			
Substrate drug	**Brain uptake ratio in WT, untreated mice (value corrected for drug in vasculature)**	**Fold change in brain uptake ratio in P-gp KO vs. control mice, assessed parameter (value corrected for drug in vasculature)**	**References**	**Inhibitor (dose, route of administration)**	**Inhibitor concentration (site of measurement)**	**Fold change in brain uptake ratio in inhibitor-treated vs. control mice (value corrected for drug in vasculature)**	**References**
				Valspodar (12.5 mg/kg, IV)		10.3 (13.3), Conc.	(Choo et al., 2000)
				Elacridar (10 mg/kg, IV)		8.7 (8.9), Conc.	(Salama *et al.*, 2005)
Saquinavir	0.1 (0.1)	6.7 (7.4), Conc.	(Kim et al., 1998)	**Zosuquidar** (50 mg/kg, IV)		~ 3.0, Conc.	(Choo et al., 2000)
Indinavir	0.1 (0.1)	9.6 (12.3), Conc.	(Kim et al., 1998)	**Zosuquidar** (50 mg/kg, IV)		~ 2.0, Conc.	(Choo et al., 2000)
Amprenavir	~0.3	~ 4.0, Conc.	(Choo et al., 2000)	**Zosuquidar** (50 mg/kg, IV)		~ 2.0, Conc.	(Choo et al., 2000)
Abacavir	0.2	10.9, AUC	(Shaik et al., 2007)	**Elacridar** (10 mg/kg, IV)		3.4 [c], Conc.	(Giri *et al.*, 2008)
				Zosuquidar (25 mg/kg, IV)		2.9 [c], Conc.	(Giri *et al.*, 2008)
Phenytoin	~ 0.25-0.3	~ 1.3, Conc.	(Sills *et al.*, 2002)				
	0.5-1.5	1.6, Conc.	(Rizzi *et al.*, 2002)				
Phenobarbital	~ 0.27-0.4	~ 1.3, Conc.	(Sills *et al.*, 2002)				
Carbamazepine	~ 0.15- 0.6	~0.9, Conc.	(Sills *et al.*, 2002)				
	2.1	0.9, Conc.	(Rizzi *et al.*, 2002)				
Topiramate	~ 0.3- 0.5	~2.0, Conc.	(Sills *et al.*, 2002)				

	Genetic KO of P-gp			Pharmacological inhibition of P-gp			
Substrate drug	**Brain uptake ratio in WT, untreated mice (value corrected for drug in vasculature)**	**Fold change in brain uptake ratio in P-gp KO vs. control mice, assessed parameter (value corrected for drug in vasculature)**	**References**	**Inhibitor (dose, route of administration)**	**Inhibitor concentration (site of measurement)**	**Fold change in brain uptake ratio in inhibitor-treated vs. control mice (value corrected for drug in vasculature)**	**References**
Loratadine	1.6	2.0, AUC	(Chen et al., 2003a)				
Desloratadine	<1	14.1, AUC	(Chen et al., 2003a)				
Hydroxyzine	1.2	3.83, AUC	(Chen et al., 2003a)				
Diphenhydramine	1	9.02, AUC	(Chen et al., 2003a)				
Verapamil	0.4	7.7 (8.0), Conc.	(Hendrikse *et al.*, 1998)	**Cyclosporine** (50 mg/kg, IV)		5.3 (5.5), Conc.	(Hendrikse *et al.*, 1998)
				Quinidine (IA)	100 μM (perfusate)	(5.1), K_{net}/C_{Perf}	(Dagenais *et al.*, 2004)
	0.6	9.2 (9.6), AUC	(Doran et al., 2005)	**Methadone** (IA)	50 μM (perfusate)	(2), K_{net}/C_{Perf}	(Dagenais *et al.*, 2004)
				Meperidine (IA)	50 μM (perfusate)	(1), K_{net}/C_{Perf}	(Dagenais *et al.*, 2004)
		(~7), [a] K_{net}/C_{Perf}	(Zong & Pollack, 2003),	**Rifampin** (IA)	500 μM (perfusate)	(~ 6), K_{net}/C_{Perf}	(Zong & Pollack, 2003),
Quinidine	0.3	(~8), [a] K_{net}/C_{Perf}	(Zong & Pollack, 2003)	**Rifampin** (IA)	500 μM (perfusate)	(~3), K_{net}/C_{Perf}	(Zong & Pollack, 2003)
	0.4	8.3 (8.9), Conc.	(Fromm *et al.*, 1999)	**Rifampin** (IA)	1000 μM (perfusate)	(~4), K_{net}/C_{Perf}	(Zong & Pollack, 2003)
		36.5 (40.8), AUC	(Doran et al., 2005)				
Digoxin	0.06	27.9 (82.3), Conc.	(Mayer *et al.*, 1997)	**Valspodar** (50 mg/kg, PO)	3-4 μg/mL (plasma), 250 ng/g (brain)	7.8 (21.6), Conc.	(Mayer *et al.*, 1997)
	0.1	18.7 (35.5), Conc.	(Schinkel et al., 1995)				
	0.1	15.5 (26.5), Conc.	(Fromm *et al.*, 1999)	**Quinidine** (100 mg/kg, IP)	0.6 μg/mL (~2 μM) (plasma)	1, Conc.	(Fromm *et al.*, 1999)

	Genetic KO of P-gp			Pharmacological inhibition of P-gp			
Substrate drug	**Brain uptake ratio in WT, untreated mice (value corrected for drug in vasculature)**	**Fold change in brain uptake ratio in P-gp KO vs. control mice, assessed parameter (value corrected for drug in vasculature)**	**References**	**Inhibitor (dose, route of administration)**	**Inhibitor concentration (site of measurement)**	**Fold change in brain uptake ratio in inhibitor-treated vs. control mice (value corrected for drug in vasculature)**	**References**
Loperamide	0.3	6.7 (7.1), Conc.	(Schinkel et al., 1996)	**Quinidine** (IA)	100 μM (perfusate)	(9), K_{net}/C_{Perf}	(Dagenais *et al.*, 2004)
		(10.4), K_{net}/C_{Perf}	(Dagenais *et al.*, 2004)				
	0.4 (total radioactivity)	3.7-4.5 (3.8-4.7)[c], Conc.	(Zoghbi *et al.*, 2008)				
	0.2 (loperamide)	13.9-19.7 (15.5-21.7) [c], Conc.	(Zoghbi *et al.*, 2008)				
Morphine	0.5	1.7 (1.7), AUC	(Doran *et al.*, 2005)	**Verapamil** (1 mg/kg, IV)		(~ 1.0) [a], K_{net}	(Cisternino *et al.*, 2001)
		(1.2), K_{net}/C_{Perf}	(Dagenais *et al.*, 2004)	**Valspodar** (10 mg/kg, IV)		(~ 1.0) [a], K_{net}	(Cisternino *et al.*, 2001)
		(1.4) [a], K_{net}	(Cisternino *et al.*, 2001)	**Elacridar** (10 mg/kg, IV)		(~ 1.0) [a], K_{net}	(Cisternino *et al.*, 2001)
Rizulide	2	(1.4), Conc.	(Milane *et al.*, 2007)	**Minocycline** (90 mg/kg/d for 5 d, IP)		(2.1), Conc.	(Milane *et al.*, 2007)
Risperidone	0.8	10.3 (10.5), AUC	(Doran et al., 2005)	**Bupropion** (100 mg/kg, IP)	52 μg/g (brain), 6.7 μg/mL (plasma), 6.3 μg/g (brain), 0.35 μg/mL (plasma),	1, AUC	(Wang et al., 2006)
	1.6, Conc.		(Wang et al., 2006)				
	0.4	13.6 (14.1), Conc.	(Ejsing et al., 2005)	**Sertraline** (10 mg/kg, IP)		1.4, AUC	(Wang et al., 2006)

Brain uptake is the ratio between drug concentrations or area under the concentration-time curve (AUC) values in brain and plasma.

[a] Brain uptake was determined by *in situ* brain perfusion.

[b] Brain uptake was measured by microdialysis.

[c] Values obtained from different brain regions.

Conc., concentrations; C_{perf}, drug concentration in perfusate; HPLC, high-performance liquid chromatograpy; IA, intra-arterial; IP, intra-peritoneal; IV, intravenous; K_{net}, net transport coefficient; KO, knockout; PO, oral; WT, wild type.

Table 4

Summary of studies on drug interactions at non-human primate blood-brain barriers

Object drug	CNS distribution in untreated subjects (evaluated parameter)	Precipitant drug		Fold change in brain uptake ratio in precipitant compound-treated vs. control subjects (value corrected for drug in vasculature)	Fold change in CSF uptake ratio in precipitant compound-treated vs. control subjects	References
		(Dose, route of administration)	Concentration (site of measurement)			
Methotrexate (1.6 mg/kg, IV)	0.02 (ventricular CSF / plasma AUC ratio)	**Amitryptyline** (3 or 6 mg/kg for 1 or 7 days, IM)	100-370 ng/mL (0.4-1.3 μM) (plasma, range in four animals) 1890 ng/mL (6.8 μM) (plasma, one animal)		1.8 (mean of four animals) (7.6, one animal, treated with highest amitryptyline dose)	(Heideman *et al.*, 1986)
Doxorubicin (2 mg/kg, IV infusion alone or 1 mg/kg/hr with inhibitor)	< 0.05 (ventricular CSF / plasma conc. ratio)	**Cyclosporine** (4 mg/kg iv bolus + 12 mg/kg/day for 48 hr, IV infusion)	551-1315 μg/L (0.5-11 μM) (blood)		~1 (CSF conc.below limit of detection)	(Warren et al., 2000)
[^{18}F]-Paclitaxel (1-1.9 nmol, IV)	0.05 (brain / metabolite-corrected plasma AUC ratio)	**Tariquidar** (2 mg/kg, IV)		1.2		(Kurdziel *et al.*, 2003)
Nelfinavir (6 mg/kg, IV)	0.04 (brain / plasma conc. ratio) 0.006 (lumbar CSF / plasma, conc. ratio)	**Zosuquidar** (3 mg/kg, IV)	929 ng/mL (1.5 μM) (plasma) 8.5 ng/mL (0.01 μM) (CSF) 4816 ng/mL (7.6 μM) (brain)	146 (211)	1.2	(Kaddoumi *et al.*, 2007)
[^{11}C]-Loperamide (0.3 nmol/kg, IV)	(brain / plasma AUC ratio)	**DCPQ** (8 mg/kg, IV)		3.0		(Zoghbi *et al.*, 2008)
	(brain AUC)	**Tariquidar** (8 mg/kg, IV)		3.7		(Zoghbi *et al.*, 2008)
[^{11}C]-Loperamide (3.9 nmol, IV)	(brain SUV)	**Disulfiram** (500 mg on previous evening and 500 mg on the morning of the scan, PO)		1		(Ryu *et al.*, 2007)
[^{11}C]-Verapamil (1.4 nmol/kg, IV)	0.64 (brain / plasma AUC ratio)	**Valspodar** (20 mg/kg/2h, IV infusion)		2.3		(Lee et al., 2006)
[^{11}C]-Verapamil (0.1-0.2 μg/kg, IV)	1.00 (brain / plasma conc. ratio)	**Cyclosporine** (24 mg/kg/hr, IV infusion)	19.4 μM (blood)	3.9 (4.0)		(Eyal, et al., 2009)
Zidovudine (33.3 mg/kg, SC)	0.3 (CSF / plasma conc. ratio)	**Probenecid** (100 mg/kg, SC)			2.5	(Cretton et al., 1991)
Zidovudine (1.2 or 24 mg/kg/hr, IV)	0.2 (lumbar / throracic CSF conc. ratio)	**Didanosine** (1.2 or 24 mg/kg/hr, IV)	0.9-11.3 μg/mL (3.8-47.8 μM) (plasma) 0.1-1.2 μg/mL (0.4-5.1 μM) (CSF)		1.1	(Tuntland *et al.*, 1994)

Object drug	CNS distribution in untreated subjects (evaluated parameter)	Precipitant drug		Fold change in brain uptake ratio in precipitant compound-treated vs. control subjects (value corrected for drug in vasculature)	Fold change in CSF uptake ratio in precipitant compound-treated vs. control subjects	References
		(Dose, route of administration)	Concentration (site of measurement)			
Didanosine (1.2 or 24 mg/kg/hr, IV)	0.1 (lumbar / thoracic CSF conc. ratio)	**Zidovudine** (1.2 or 24 mg/kg/hr, IV)	0.5-11.2 μg/mL (1.9-41.9 μM) (plasma) 0.1-2.2 μg/mL (0.4-8.2 μM) (CSF)		0.8-1.3	(Tuntland *et al.*, 1994)
Levodopa / carbidopa (50 mg/5 mg, IV)	8.7 (brain ECF / plasma conc. ratio)	**Large-neutral amino acids** (IV) **or protein** (PO)	591 μM (large neutral amino acids, plasma)	0.5		(Alexander *et al.*, 1994)
Levodopa / carbidopa (50 mg/5 mg, IV)	5.9 (brain ECF / plasma, conc. ratio)	**Isoproterenol** (2 mg/kg, IP)		1.2		(Alexander *et al.*, 1994)

Brain or CSF uptake is the ratio between drug concentrations or area under the concentration-time curve (AUC) in CNS and that in plasma.

Conc., concentrations; DCPQ, (2*R*)-anti-5-{3-[4-(10,11-dichloromethanodibenzo-suber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride; IA, intra-arterial; IM, intra-muscular; IP, intra-peritoneal; IV, intravenous; PO, oral; SC, subcutaneous; SUV, standardized uptake value (tissue exposure to radioactivity normalized by injected dose and subject's weight).

Table 5

Summary of studies on drug interactions at human blood-brain barriers

Object drug	CNS distribution in untreated subjects (evaluated parameter)	Precipitant compound		Effect of precipitant compound		References
		Coumpound (dose, route of administration)	Concentration (site of measurement)	Fold change in brain uptake ratio in precipitant compound-treated vs. control subjects (value corrected for drug in vasculature)	Fold change in CSF uptake ratio in precipitant compound-treated vs. control subjects	
Methotrexate (3.5 g/m^2 IV or 2.8 g/m^2, IA, for control group; 1.4 g/m^2, IA, for BBBD group)	3.5-5.3 (Ventricular CSF / plasma AUC ratio)	**Mannitol** (25%, 3-11 mL/s over 30 s, IA)			4.1-6.2 (vs. IV administration), 3.0-4.5 (vs. IA administration)	(Zylber-Katz *et al.*, 2000)
Paclitaxel (175 mg/m^2, IV)	0.1 (Lumbar CSF or CSF obtained through Ommaya reservoir / plasma conc. ratio)	**Tamoxifen** (160 mg/m^2 BID for 5 days, PO)			0.3	(Chen et al., 2006)
Ritonavir (400 mg BID, PO)	0.09 (Lumbar CSF / plasma unbound conc. ratio, 4-5 hr post-dose)	**Ketoconazole** (200 or 400 mg/day for 10 days, PO)	4.8 and 10.2 μg/mL (9.0-19.2 μM) at 200 and 400 mg/day, respectively (plasma)		2.9	(Khaliq *et al.*, 2000)
Saquinavir (400 mg BID, PO)	0.06 (Lumbar CSF / plasma, unbound conc. ratio, 4-5 hr post-dose)	**Ketoconazole** (200 or 400 mg/day for 10 days, PO)	4.8 and 10.2 μg/mL (9.0-19.2 μM) at 200 and 400 mg/day, respectively (plasma)		5.8	(Khaliq *et al.*, 2000)
Indinavir (1000 mg TID without ritonavir, 800 mg BID or TID, with ritonavir, PO)	0.6 (Lumbar CSF / plasma trough conc. ratio) -0.05 (lumbar CSF / plasma peak conc. ratio)	**Ritonavir** (100 mg BID, PO)			0.5 (plasma trough)-2.6 (plasma peak)	(van Praag *et al.*, 2000)
Indinavir (800 mg TID without ritonavir, 800 mg BID with ritonavir, PO)	0.07 (Lumbar CSF / plasma AUC ratio)	**Ritonavir** (100 mg BID, PO)			1.5	(Haas *et al.*, 2003)
[^{11}C]-Verapamil (3.9 ± 3.4 nmol, <0.12 μg/kg, IV)	0.42 at 20 min, 0.55 at 45 min (brain / plasma AUC ratio)	**Cyclosporine** (2.5 mg/kg/hr for 1 hr, IV infusion)	2.1-3.2 μM (blood)	1.9 (2.0)	1.6	(Sasongko *et al.*, 2005)
[^{11}C]-Loperamide	Brain (uptake clearance)	**Cyclosporine** (5 mg/kg/hr for 2 hr, IV infusion)		2		(Passchier *et al.*, 2008)
[^{11}C]-Loperamide	Brain (uptake clearance)	**Quinidine** (600 mg, PO)		1		(Passchier *et al.*, 2008)

Brain or CSF uptake is the ratio between drug concentrations or area under the concentration-time curve (AUC) in CNS and that in plasma.

BBBD, blood-brain barrier disruption; BID, twice daily; Conc., concentrations; IA, intra-arterial; IM, intramuscular; IV, intravenous; PO, oral; TID, three times daily.

Table 6

In vitro EC_{50} values of P-gp inhibitors compared with those reported previously (Ekins et al., 2002; Rautio et al., 2006)

	EC_{50} (μM) mean ± SD	Reported EC_{50} (μM)	Therapeutic C_{max} (μM)	Cmaxu (μM)	Therapeutic C_{max}/EC_{50}	C_{maxu}/EC_{50}
Cyclosporine A	0.6 ± 0.3	0. 46 ~ 6.2	1.11	0.078	1.78	0.12
Quinidine	0.9 ± 0.2	2 ~ 55	8.94	1.16	9.57	1.24
Quinine	1.2 ± 0.3	NA	18.8	2.44	15.2	1.97
Verapamil	3.8 ± 1.3	1.5~ 61	0.66	0.07	0.17	0.02
Ketoconanzole	2.8 ± 1.2	0.7 ~ 29	8.47	0.085	2.98	0.03
Itraconazole	0.3 ± 0.2	0.5	0.92	0.033	2.98	0.11
Amprenavir	5.8 ± 0.9	> 100	15.15	1.52	2.61	0.26
Indinavir	ND	> 50	9.8	3.82	ND	ND
Nelfinavir	18.1 ± 8.6	1.4 ~ 44	5.64	0.11	0.31	0.01
Ritonavir	12.1 ± 5.0	3.8	15.26	0.31	1.26	0.03
Saquinavir	15.9 ± 4.1	6.5	5.64	0.11	0.35	0.01
Atazanavir	13.2 ± 4.8	NA	7.66	1.07	0.58	0.08
Lopinavir	3.1 ± 0.8	NA	15.59	0.31	5.03	0.10
Tipranavir	2.9 ± 0.9	NA	94.8	0.09	32.39	0.03
Erythromycin	44.6 ± 18	38	4.77	0.76	0.11	0.02
Clarithromycin	26.4 ± 10	NA	3.21	1.86	0.12	0.07

The ratios, therapeutic C_{max}/EC_{50} and C_{maxu}/EC_{50}, provide a rank order of the potency of the drug to produce drug interactions at the human BBB. Fraction unbound values are based on reported human data and drug package inserts. Based on the FDA guidance of 0.1 or greater therapeutic C_{max}/EC_{50} as the screening criteria (http://www.fda.gov/cder/drug/druginteractions/decisiontree.htm#dt_pgp), all the drugs listed above, except indinavir, would be classified as potential in vivo inhibitors of P-gp (Hsiao et al., 2008).

EC_{50}, concentration of drug that produces half-maximal (E_{max}) inhibition of P-gp; C_{max}, maximal therapeutic plasma (or blood) concentrations; C_{maxu}, unbound C_{max}; NA, not available; ND, not determined.