The AAPS Journal (2021) 23: 61
DOI: 10.1208/s12248-021-00587-9

Review Article

Theme: Celebrating Women in the Pharmaceutical Sciences
Guest Editors: Diane Burgess, Marilyn Morris and Meena Subramanyam

Choroid Plexus and Drug Removal Mechanisms

Austin Sun[1] and Joanne Wang[1,2]

Received 5 January 2021; accepted 24 March 2021; published online 3 May2021

Abstract. Timely and efficient removal of xenobiotics and metabolites from the brain is crucial in maintaining the homeostasis and normal function of the brain. The choroid plexus (CP) forms the blood-cerebrospinal fluid barrier and vitally removes drugs and wastes from the brain through several co-existing clearance mechanisms. The CP epithelial (CPE) cells synthesize and secrete the cerebrospinal fluid (CSF). As the CSF passes through the ventricular and subarachnoid spaces and eventually drains into the general circulation, it collects and removes drugs, toxins, and metabolic wastes from the brain. This bulk flow of the CSF serves as a default and non-selective pathway for the removal of solutes and macromolecules from the brain interstitium. Besides clearance by CSF bulk flow, the CPE cells express several multispecific membrane transporters to actively transport substrates from the CSF side into the blood side. In addition, several phase I and II drug-metabolizing enzymes are expressed in the CPE cells, which enzymatically inactivate a broad spectrum of reactive or toxic substances. This review summarizes our current knowledge of the functional characteristics and key contributors to the various clearance pathways in the CP-CSF system, overviewing recent developments in our understanding of CSF flow dynamics and the functional roles of CP uptake and efflux transporters in influencing CSF drug concentrations.

KEY WORDS: blood-cerebrospinal fluid barrier; cerebrospinal fluid flow; choroid plexus; drug metabolic enzymes; drug transporters.

INTRODUCTION

The mammalian central nervous system (CNS) is separated from the blood circulation by two major permeability barriers: the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). The BBB is formed by the endothelial cells of the brain capillaries, whereas the BCSFB is formed by the choroid plexuses (CP) located in the brain ventricles. While these barriers restrict the free entry of circulating substances into the brain, they do not provide complete protection of the CNS from exposure to environmental substances, drugs, and other potentially harmful compounds. Lipophilic compounds can cross these barriers by passive diffusion whereas hydrophilic substances may enter the brain through transporter-mediated processes. Furthermore, metabolic wastes and neurotoxic species can be generated within the brain by physiological and pathophysiological processes. Thus, timely and efficient removal of xenobiotics and metabolites from the brain is crucial for maintaining the homeostasis and normal function of the brain.

The CP-CSF system utilizes several different mechanisms to maintain healthy brain homeostasis. The CP produces CSF that bathes and cushions the brain. As the CSF flows through the ventricular system and is reabsorbed into the general circulation, drugs, metabolites, and macromolecules are cleared from the brain. The CPE cells can also actively remove solutes from the CSF by transporting them across the BCSFB into the blood circulation. This process is conducted through specific uptake and efflux transporters expressed by the CPE cells. Lastly, the CPE cells also express several

Guest Editors: Diane Burgess, Marilyn Morris and Meena Subramanyam

[1] Department of Pharmaceutics, University of Washington, Health Science Building Room H-272J, Box 357610, Seattle, Washington 98195-7610, USA.
[2] To whom correspondence should be addressed. (e–mail: jowang@uw.edu)

Abbreviations: *ABC*, ATP-binding cassette; *BBB*, blood-brain barrier; *BCSFB*, blood-cerebrospinal fluid barrier; *BCRP*, breast cancer resistance protein; *CNS*, central nervous system; *CP*, choroid plexus; *CYP*, cytochrome P450; *CPE Cells*, choroid plexus epithelial cells; *CSF*, cerebrospinal fluid; *ISF*, interstitial fluid; *GST*, glutathione S-transferase; *fluo-cAMP*, fluorescein-cyclic AMP; Kp_{uu}, unbound partition coefficient; *MAO*, monoamine oxidase; *OAT*, organic anion transporter; *OCT*, organic cation transporter; *PEPT2*, Peptide transporter 2; *PMAT*, plasma membrane monoamine transporter; *MRP*, multidrug resistance-associated protein; *P-gp*, P-glycoprotein; *SLC*, solute carrier; *SULT*, sulfotransferase; *UGT*, UDP-glucuronosyltransferase.

intracellular detoxifying and conjugating enzymes, which enzymatically inactivate reactive or toxic substances. This article provides a current and integrated review of these clearance processes within the CP-CSF system. Following a brief overview of the anatomical features of the CP-CSF system, the functional characteristics and major contributors to the major clearance pathways are summarized. Recent developments in CSF flow dynamics and the impact of CP transporter on CSF drug concentrations are highlighted. The relevance of the CP drug removal mechanisms to the interplay of drug concentrations between the CSF and the brain interstitial fluid is also discussed.

ANATOMY AND FUNCTIONS OF CHOROID PLEXUS-CSF SYSTEM

The brain ventricular system is highly conserved across mammalian species and consists of a set of interconnected cavities (lateral, third, and fourth ventricles) filled with the CSF (1, 2). The fourth ventricle is continuous with the central canal of the spinal cord, allowing the CSF to flow down the canal and circulate to the subarachnoid space (Fig. 1). The CP is a secretory tissue located in each brain ventricle that continuously secretes CSF into the ventricles. The CP tissues from the lateral, third, and fourth ventricles share similar ultrastructure, consisting of an outer layer of cuboidal epithelial cells surrounding a core of capillaries and connective tissue (Fig. 1). Unlike the endothelial cells forming the BBB, the CP capillaries are fenestrated with leaky inter-endothelial junctions. This facilitates the rapid exchange of water and solutes between the general circulation and interstitial fluids in the subepithelial space. The CPE cells are joined by tight junctions that restrict the free exchange of solutes and macromolecules between the blood and CSF (*i.e.*, the blood-CSF barrier). The CPE cells are polarized with their apical membrane domain facing the CSF and the basolateral membrane facing the blood circulation (Fig. 1). The apical domains of the CPE cells have a brush border lining (microvilli) that effectively enlarges the surface area by 10–30-fold (4).

A primary function of the CP is to manufacture and secrete CSF. This process is accomplished by inorganic ion and water transport across the CP epithelium. A network of ion pumps, ion transporters, and channels expressed at the basolateral and apical membranes of the CPE cells mediate the net flux of Na^+, Cl^-, and HCO_3^- across the CP epithelium from the blood side to the CSF side. This creates an osmotic pressure gradient which then drives the flow of plasma water across the CP membranes into the ventricles (5). On the other hand, there is a net flux of K^+ from the CSF to the blood. Overall, the CSF is 5 mOsM hyperosmolar compared to the plasma, with a 5-mV transepithelial electrical potential difference across the CPE epithelium (5–7).

The CP-CSF system carries out several physiological functions crucial for the hemostasis and normal functions of the brain. The CSF provides buoyancy to the brain, which reduces the effective brain weight by about 70% in humans and protects the brain from the adversity of gravity and mechanical injury (2). The CP is also an entry site for micronutrients and hormones (*e.g.*, vitamin C, folate), which are further carried by CSF to nourish internal brain cells. The CP-CSF system is essential for clearing waste products and xenobiotics from the brain, which is the focus of this review. The CP-CSF system employs three co-existing and intertwined pathways to clear metabolic wastes, protein debris, drugs, and environmental substances from the CNS. These clearance mechanisms, *i.e.*, clearance by bulk CSF flow, transport, and metabolism in CPE cells, will be discussed in detail below. In addition, emerging studies suggest that CP serves as a gateway for immune cell entry into the CNS and also plays an active role in the regulation of neural stem cells (1, 2). CP-associated macrophages are understood to monitor

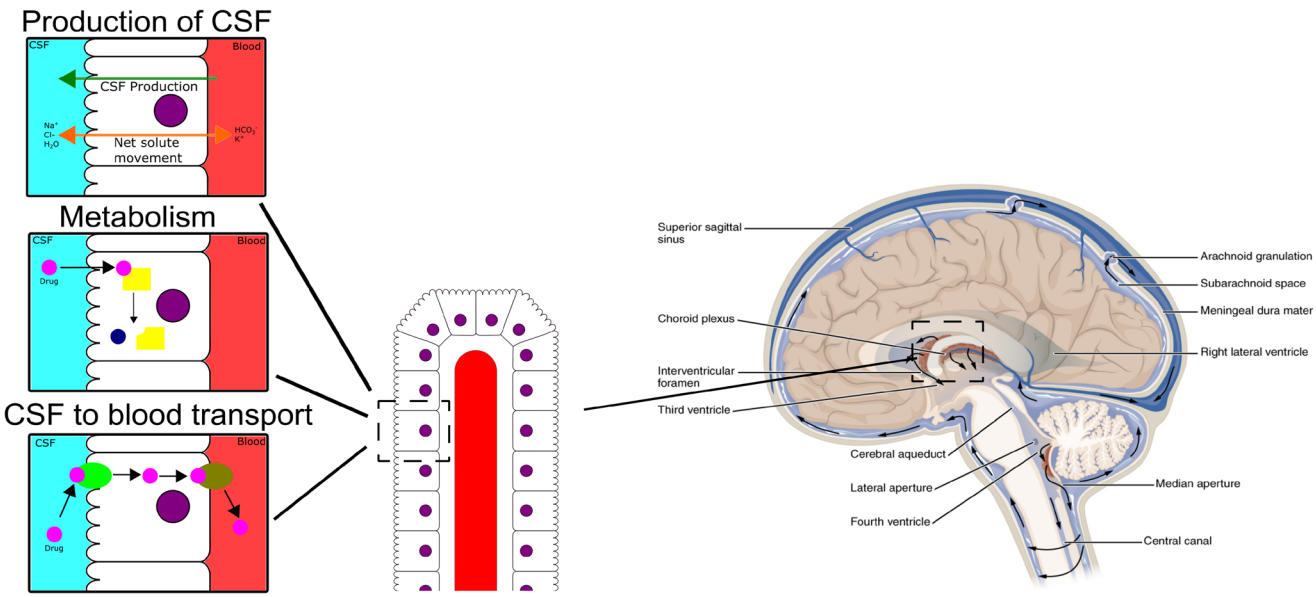

Fig. 1. Schematic representation of the location, structure, and clearance pathways of the CP-CSF system. The blood-CSF barrier is formed by choroid plexus epithelial cells. These cells contribute to drug clearance by production of CSF that drives bulk flow movement out of the CSF, biotransformation by intracellular drug-metabolizing enzymes, and active transport removal by coordinated uptake and efflux out of the CSF. Portions of the figure are adapted from OpenStax Anatomy and Physiology under Creative Commons CC-BY-4.0 License (3)

ventricular CSF in healthy conditions, while memory T cells monitor the subarachnoid and leptomeningeal CSF (8–10). During neuroinflammation, CPE cells respond by recruiting and trafficking leukocytes into the CNS (11).

CLEARANCE VIA CSF FLOW

CSF Composition and Flow Dynamic

In humans, about 500–600 mL of CSF is produced each day. The majority of the CSF is produced by the CPs, with the remainder of CSF derived from drainage of brain interstitial fluid and passage across the blood arachnoid barrier (12–14). The CSF is not an ultrafiltrate of the plasma (15, 16) and is slightly hypertonic compared to the plasma (5). Compared to the plasma, the CSF has lower protein and cell density, with slightly altered ion and solute concentrations. The Na^+, $HCO3^-$, Cl^- and Mg^{2+} ion concentrations are slightly higher in the CSF while K^+ and Ca^{2+} concentrations are lower (12). The protein concentration in the CSF is two to three orders of magnitude lower than that in blood, ranging from 15 to 45 mg/dL in young adults (17, 18). Around 20% of the total CSF protein originates from the brain, with the remainder derived from the plasma (19). The CSF also contains a small circulating cell population, estimated to be 5 cells per mL (20). This population includes leukocytes associated with immune surveillance.

Historically, the net circulation of CSF is thought to be unidirectional. Following production, the CSF flows from the lateral ventricles through the interventricular foramina into the third ventricle. The CSF flows outward through the cerebral aqueduct to the fourth ventricle, where it then travels through the median aperture into the cisterna magna and the greater cranial subarachnoid space. From there, the CSF can also flow caudally into the spinal subarachnoid space (20). The CSF is cleared through reabsorption at the arachnoid villi or by drainage along the cranial nerve sheaths into the lymphatics (14, 20). The flow of CSF is pulsatile, corresponding with the cardiac cycle (21). While the net movement of CSF is directional from the ventricles to subarachnoid spaces, the second-to-second pulsatile movement enables mixing of contents between the different CSF compartments (14, 22, 23).

The average flow rate of CSF is estimated to be 0.37 mL/min in humans, based on ventriculo-cisternal perfusion measures (24–26). The CSF flow rate is also age-dependent. Older populations have been shown to have impaired CSF production and flow (27–30). Additionally, CSF production is further reduced in patients with neurodegenerative disorders (31–33). Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis are characterized by misfolded proteins or harmful protein aggregation. Given the significance of CSF bulk flow in clearing such wastes from the CNS, there is substantial interest in studying the role of CSF production and flow in pathogenesis of these diseases.

Recent evidence has also pointed to a relationship between sleep cycles and CSF production and flow patterns. A study utilizing magnetic resonance imaging to visualize CSF flow found that CSF production followed the circadian cycle, with increased production during nighttime (21). In addition, a study by Alperin *et al*. suggested that a supine position increased the oscillatory CSF volume relative to the upright position (34). This contributed to the hypothesis that sleep contributes to healthy brain function by increasing CSF-mediated clearance of waste products out of the CNS. A study published in 2019 explored CSF flow characteristics in humans during sleep (35). The authors were able to observe unique patterns of CSF pulsatile flow during non-rapid eye movement (REM) sleep. While CSF typically flows directionally out of the ventricles in an awake state, an opposing pulsating flow of CSF into the ventricles was observed in subjects experiencing non-REM, slow-wave sleep (35). This reversal of CSF flow during slow-wave sleep is thought to facilitate the mixing of subarachnoid and ventricular fluids, which may facilitate communication between fluid compartments and the overall clearance of waste products (36).

CSF and Brain ISF Exchange and the Glymphatic System Hypothesis

The cells of the brain parenchyma are surrounded by the interstitial fluid (ISF), which is essential for cell-cell communication and maintaining healthy brain homeostasis. Unlike other organs, the brain lacks a true lymphatic system to assist in the removal of interstitial metabolic waste products (37, 38). Instead, the CSF maintains an analogous role for the CNS, responsible for the clearance of metabolic wastes and cellular debris from the brain (14). The brain ISF, containing debris and waste products from brain cells, exchanges with the CSF. The CSF renews the ISF and removes waste products by continuously flowing out of the brain. While this concept is widely appreciated, how fluid and solute exchange between the CSF and the ISF occurs has not been fully understood.

It was previously assumed that CSF and brain ISF freely exchanged with one another and were in rapid equilibrium. This led to the notion that the BCSFB could act as a drug delivery pathway to the CNS and brain parenchyma (39). However, failures in drug development based on this rationale led to this notion falling out of favor. Subsequent research began to unveil that the exchange between CSF and brain ISF was much more nuanced than previously assumed. In some studies, diffusion appeared to dominate, whereby molecular size of the solute and distance from the CSF system dictated the clearance rate from the brain ISF (40–42). However, other studies found that compounds, regardless of molecular size, disappeared from the brain ISF at similar rates, suggesting a bulk flow (*i.e*., advection) of ISF into the CSF (37, 43–45).

In 2013, Iliff *et al*. published a seminal paper on the "glymphatic system," a term coined to describe brain CSF-ISF exchange routes along the perivascular spaces (46). The authors demonstrated that cisternal CSF injection of fluorescent tracers into mice led to tracer accumulation alongside cerebral surface arteries. At later time points, tracers accumulated along parenchymal venules and cerebral veins. Furthermore, knockout of Aquaporin-4 significantly reduced the movement of the tracers used. Based on these observations, a brain-wide glymphatic system was proposed for fluid movement in the brain, which includes the para-arterial influx of subarachnoid CSF into the brain interstitium, followed by

the clearance of ISF along large draining veins. Aquaporin-4, a water channel protein expressed at the astrocytic end feet, is thought to provide the driving force for the continuous movement of fluid through the glymphatic system. This system is thought to play a critical role in the clearance of interstitial solutes and macromolecules including $A\beta_{1-40}$ from the brain.

Since the work of Iliff *et al*., the idea and significance of the glymphatic pathway has been a subject of great interest and debate. Xie *et al*. demonstrated that mice showed increased glymphatic exchange between CSF and brain ISF when asleep through fluorescent tracer infusions (47). They additionally demonstrated that Aβ, the main component of amyloid plaques characteristic of Alzheimer's disease, was cleared from the brain faster in sleeping mice (47). A study by Smith *et al*. demonstrated that loss of Aquaporin-4 did not change transport of fluorescent tracers from the subarachnoid space to the brain (48). However, a later study by Mestre *et al*. demonstrated that loss of Aquaporin-4 did indeed decrease tracer transport across four independently generated knockout lines (49). Some groups contend that diffusion between the CSF and brain ISF is still the predominant mechanism of solute exchange (14, 42, 48, 50, 51). There is still no consensus on the relative contribution of glymphatic flow to total CSF/brain ISF exchange (14, 42). Regardless, debates surrounding the glymphatic hypothesis have renewed discussions and interests in the relationship between brain ISF and CSF, which is also relevant to drug distribution kinetics in the brain and the prediction of CNS pharmacokinetics.

TRANSPORTER-MEDIATED CLEARANCE

Membrane transporters can have a large influence on the disposition of drugs. These drug transporters have been extensively studied in the small intestine, liver, and kidney, where they contribute to the systemic absorption and clearance of their drug substrates (52). In peripheral tissues, transporters can determine local tissue concentrations, impacting drug efficacy or tissue-specific toxicity (53). The BCSFB expresses xenobiotic transporters responsible for the elimination of harmful compounds from the CSF. The apical (CSF-facing) membranes of the CP epithelial cells have microvillous structures, increasing the absorptive surface area and thus enabling efficient extraction of xenobiotics from the ventricular CSF. Clearance across the BCSFB typically occurs in a stepwise manner. First, uptake transporters on the apical membrane transport a substrate from the CSF into the CPE cells. Efflux transporters on the basolateral (blood-facing) membrane then remove the substrate out of the cell and into the blood, where they can be further delivered to the liver or the kidney for systemic elimination.

There are two superfamilies of transporters, the ATP-binding cassette transporters (ABC) and the solute carrier transporters (SLC) (54). The ABC transporters utilize ATP hydrolysis to actively efflux substrate out of cells. There are about 48 ABC transporter members in humans. The SLC transporters are a more diverse superfamily of transporters, consisting of over 400 members. Some SLC transporters utilize electrochemical or ion gradients to actively transport substrates across the membrane while others operate as facilitative transporters (55). Previous gene profiling studies have indicated the presence of several multispecific xenobiotic ABC and SLC transporters at the choroid plexus (56–58). We previously performed Slc transporter gene profiling in the mouse brain and our results showed that Slc transporter expression profiles at the BCSFB are substantially different from those at the BBB and other brain areas (56, 57). Recently, absolute protein expression levels of xenobiotic transporters have been determined using quantitative targeted proteomic methods in several different species (59–61).

Current methods to study BCSFB transport function *in vitro* or *ex vivo* include transport assays using conditionally immortalized CPE cells, primary culture of CPE cells, and freshly isolated CP tissue (62). While cell-based systems are straightforward for drug transport analyses using standard uptake or transwell assays, immortalized CP cell lines and primary CP cells have been demonstrated to have lower expression of xenobiotic transporters and weakened tight junction formation (63). Freshly isolated CP tissues from preclinical species maintain the tissue physiology, barrier integrity, and transporter expression reflected *in vivo*, and transport studies in isolated CP tissues have contributed to much of our understanding of the functional transport systems at the BCSFB. However, human CP tissues are difficult to obtain and cannot typically be studied using this approach. Furthermore, the anatomy of the isolated tissue only allows for the study of CSF-to-blood drug transport *in vitro*. Recently, a human pluripotent stem-cell-derived organoid model has been developed for CP (64). The organoids developed tight junctions and produced CSF-like fluid in a self-contained compartment. They also appear to express xenobiotic transporters previously observed at the CP (64). This newly established CP organoid system represents a novel and promising *in vitro* approach to characterize human CP function and aid in the study of drug transport at the BCSFB.

In this section, we highlight the ABC and SLC drug transporters whose functional roles at the BCSFB have been unequivocally established by studies in transporter knockout mice. These include the Slc uptake transporters organic anion transporter 3 (OAT3), peptide transporter 2 (PEPT2), and plasma membrane monoamine transporter (PMAT), and the ABC efflux transporters multidrug resistance-associated proteins (MRPs) 1 and 4. These transporters compose the pathways of clearance for organic anions, small peptides, and organic cations (Fig. 2). It is important to note that other ABC and SLC xenobiotic transporters have been indicated to be expressed at the BCSFB, although their functional roles in drug transport at the BCSFB are less characterized. These include Bcrp, P-gp, and Oatp1a4/5 (58, 59, 65). Bcrp, Oatp1a5, and P-gp are expressed on the apical membrane of the CPE cells, while Oatp1a4 is expressed on the basolateral membrane of the CPE cells (66). However, there is also conflicting data in the literature regarding P-gp and Bcrp, where limited or no expression of P-gp and Bcrp at the BCSFB were observed (58, 60, 63, 67). Monocarboxylate transporters (MCT/Mct) 1, 3, and 8 are also expressed at the BCSFB (59, 68). This family of transporters is important for hormone, nutrient, and amino acid transport (69). (For a general review of transporter expression and localization at the BCSFB, see the review by Morris *et al*. (68)).

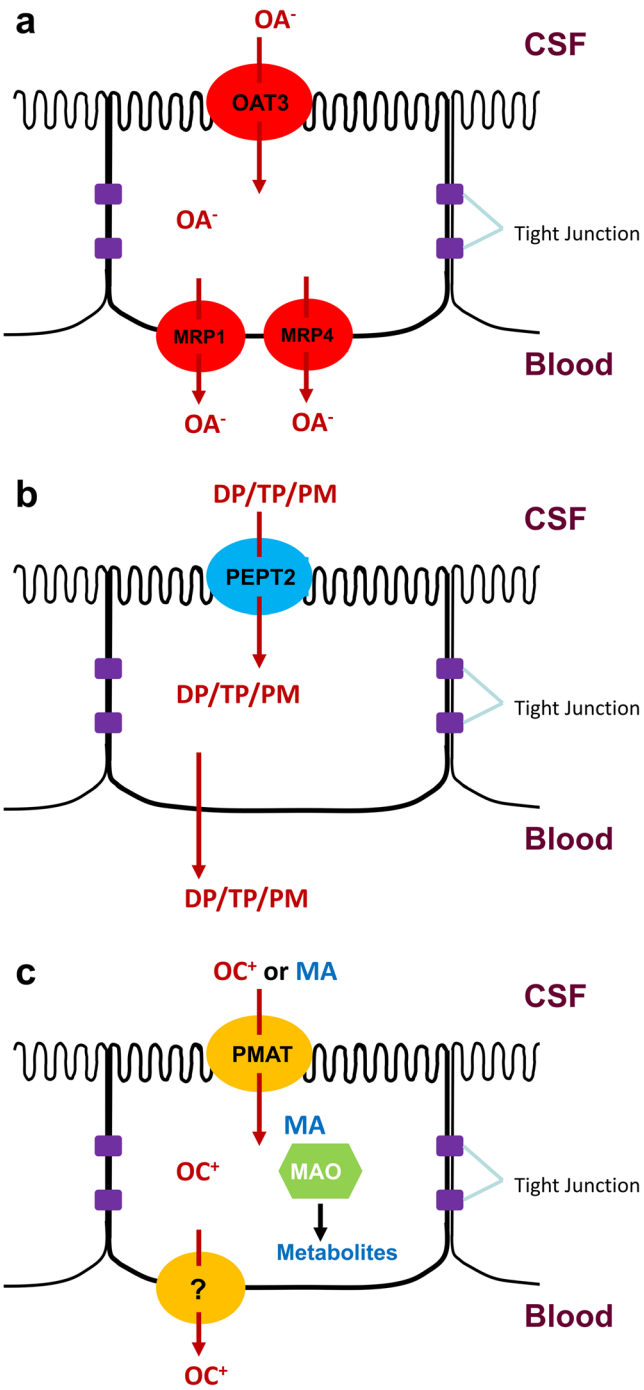

Fig. 2. Cellular models for **a** organic anion, **b** oligopeptides and peptidomimetic, and **c** monoamine/organic cation transport at the blood-CSF barrier. OA^-, organic anion; SP, small peptide; PM, peptidomimetic; OC^+, organic cation; MA, monoamine; MAO, monoamine oxidase

Uptake Transporters

OAT3 (SLC22A8)

OAT3 is a tertiary active uptake transporter that operates through dicarboxylate exchange, typically utilizing alpha-ketoglutarate as a counterion (70). Outside of the BCSFB, OAT3 is expressed in the kidney, brain capillary endothelium, and retina (71). OAT3, alongside OAT1, is basolaterally expressed in the kidney proximal tubules and plays a major role in mediating tubular secretion of small organic anion compounds from the blood to urine. Drug substrates include antineoplastic drugs, antiviral drugs, antibiotics, and diuretics (Table I). Oat3 knockout studies indicate reduced clearance of methotrexate and penicillin G due to diminished renal secretion (98, 99). The International Transport Consortium and the Food and Drug Administration recognize OAT3, alongside OAT1, as determinants of clinical drug interactions (54).

Active removal of organic anions from the CSF has been observed in humans since the 1960s (100–103). Compounds like penicillin G and methotrexate were observed to have much lower CSF levels compared to blood when systemically administered, limiting the utility of these compounds for the treatment of meningitis and brain cancer. Penicillin G and methotrexate clearance from the CSF were both observed to be sensitive to probenecid inhibition (101, 104). Soon after the discovery of the organic anion transporter family, Oat3 was found to be expressed at the apical membrane of the CPE cells and confirmed to be functionally active through knockout studies, responsible for apical uptake of substrates from the CSF (72, 105). Radiolabel uptake studies using para-aminohippurate, a prototype Oat substrate, showed marked reduction in Oat3 knockout CP tissue compared to wild-type controls. Transport was shown to be sodium-dependent and sensitive to probenecid inhibition, consistent with Oat function. Confocal imaging studies indicated significant accumulation of fluorescein in wild-type CP, while accumulation in the Oat3 knockout CP was significantly reduced (105, 106). In wild-type CP, fluorescein primarily accumulated in the blood capillaries adjacent to the CP, suggesting a coupled efflux system exists at the basolateral membrane, leading to a CSF-to-blood directional clearance of small organic anions. As discussed below, members of the MRP families are most likely to mediate the efflux step of organic anions at the basolateral membrane.

PEPT2 (SLC15A2)

PEPT2 is a member of the proton-coupled oligopeptide transporter family (SLC15) first identified in 1995 (107). PEPT2 is a secondary active uptake transporter driven by the proton gradient through proton co-transport (108). PEPT2 primarily transports di- and tri-peptides alongside peptidomimetic drugs. Drug substrates include penicillin and cephalosporin antibiotics (Table I). PepT2 is primarily expressed at the kidney and the CP, but transcripts have also been observed in the lungs, mammary glands, and spleen (108). PepT2 is the predominant peptide transporter at the kidney, responsible for tubular reabsorption of filtered di- and tri-peptide substrates. Knockout of PepT2 results in 2–3-fold reductions in systemic exposure of its substrates due to increased renal clearance. This has been observed for GlySar, cefadroxil, and carnosine in pharmacokinetic studies utilizing PepT2 KO mice (109–112).

PepT2 is localized on the apical membrane of CPE cells, responsible for uptake of substrates from the CSF (83, 113). In CP tissues isolated from PepT2 knockout mice, uptake of GlySar, a probe substrate of Pept2, was reduced by 90%,

Table I. Localization and Selected Drug Substrates of Drug Transporters at the Blood-CSF Barrier with Known Function

Transporter	BCSFB localization	Drug class	Example drug substrates	Km (μM)
OAT3	Apical (72)	Antineoplastic	Methotrexate	10.9 (73)
			Topotecan	56.5 (74)
		Antiviral	Zidovudine	145.1 (75)
		Antibiotic	Benzylpenicillin	52.1 (76)
			Cefaclor	Transported (77)
		Diuretic	Hydrochlorothiazide	134 (78)
			Furosemide	21.5 (79)
PMAT	Apical (80)	Antihyperglycemic	Metformin	1320 (81)
		Beta blocker	Atenolol	907 (82)
PEPT2	Apical (83)	Antibiotic	Cefadroxil	150.8 (84)
			Cefaclor	70.2 (85)
			Amoxicillin	1040 (85)
MRP1	Basolateral (65)	Antineoplastic	Daunorubicin	Transported (86)
			Vincristine	Transported (86)
			Methotrexate	2150 (87)
		Statin	Atorvastatin	Transported (88)
		Antiviral	Ritonavir	Transported (89)
		Antibiotic	Cefadroxil	3900 (90)
MRP4	Basolateral (91)	Antineoplastic	Methotrexate	220 (92)
			Topotecan	1.66 (93)
			6-mercaptopurine	Transported (94)
		Antiviral	Adefovir	1000+ (95)
		Antibiotic	Ceftizoxime	18 (96)
			Cefazolin	80 (96)
		Diuretic	Furosemide	Transported (97)

demonstrating that PepT2 is the primary di- and tri-peptide uptake transporter at the BCSFB (114). While coupled uptake and efflux have been observed for small organic anions at the BCSFB, there does not appear to be a coupled basolateral efflux system for small peptide drugs. A study characterizing transport properties of PepT2 in rat CP epithelial tissue and primary culture cells observed unsaturable cellular efflux of cefadroxil, indicating that basolateral efflux may occur by passive diffusion (115). Functional characterization of PepT2 has also been studied through *in vivo* pharmacokinetic studies. In Pept2 knockout mice, CSF:blood ratio of cefadroxil increased by 6-fold as compared to wild-type animals (111). More recently, using *in vivo* intracerebral microdialysis, it was shown that PEPT2 ablation significantly increased the brain ISF and CSF levels of cefadroxil after IV infusion (116). Together, these studies demonstrated that PEPT2 at the CP contributes to BCSFB-mediated drug clearance from the CSF and limits brain exposure to cefadroxil and possibly other di- and tri-peptide drugs.

PMAT (SLC29A4)

PMAT is a newer multispecific organic cation transporter first cloned and characterized by our laboratory (117). Initially suspected to transport nucleosides, PMAT was found to transport a wide range of organic cation compounds. Its substrate profile includes monoamine neurotransmitters, neurotoxins, and cationic drugs, with substrate overlap with the organic cation transporters (OCT) in the SLC22 family (118) (Table I). PMAT also strongly interacts with HIV protease inhibitors; lopinavir acts as a potent and selective PMAT inhibitor (119). Transport by PMAT is electrogenic, utilizing the membrane potential gradient as its driving force (81, 117, 118, 120). PMAT mRNA is highly expressed in the brain, although lower levels of mRNA transcripts are also found in the other tissues (117, 121, 122). Within the brain, PMAT is broadly distributed with highest expression found the CP, cerebral cortex, hippocampus, and cerebellum (121). PMAT is believed to play a role in regulation of monoamine neurotransmission, while also contributing to tissue-specific disposition of organic cation drugs and toxins (118).

Work conducted in our laboratory has established the expression and function of PMAT at the blood-CSF barrier. PMAT is expressed and localized on the apical membrane of CPE cells, responsible for uptake of substrates from the CSF into CPE cells (80). Prior to the discovery of PMAT, organic cation transporter 2 (OCT2) was thought to be a mediator of organic cation uptake at the BCSFB. In particular, Sweet *et al.* showed that mRNA expression of Oct2 by RT-PCR and demonstrated an apical localization when GFP-tagged rat Oct2 was transfected into rat CP (123). However, protein expression and activity of endogenous Oct2 were never demonstrated in the rat CP. Our laboratory and others were unable to confirm Oct2 expression at the BCSFB (58, 59, 80). Using quantitative real-time PCR, we compared multispecific organic cation transporter expression at both the human and mouse CP tissues (80). Our results showed that PMAT was expressed at much higher levels than any other transporter tested; other organic cation transporters, including Oct1/2/3 and Mate1/2, were found to be minimally expressed. Protein expression of PMAT was further confirmed by Western blot

and immunostaining (80). To further establish the role of PMAT at the BCSFB, we developed a Pmat knockout mouse model. Using this model, we observed significant decreases in organic cation and monoamine uptake in isolated CP from Pmat knockouts. Inhibitors of the Octs and neurotransmitter transporters had no effect on CP uptake of organic cations. These results clearly demonstrated that PMAT, rather than the OCTs, is responsible for the organic cation uptake in the CP (80). By removing its substrates from the CSF, PMAT could play an important role in protecting the brain from cationic neurotoxins and other potentially toxic organic cations.

Currently, it is unknown how organic cations are effluxed out of the CPE cells at the basolateral membrane. MATE1/2, the proton-organic cation exchangers responsible for the final OC excretion step in renal tubular cells, are minimally expressed in CPE cells and thus may not play a significant role in OC efflux at the BCSFB. Miller *et al.* previously suggested that basolateral efflux of organic cations may occur through a vesicular transport mechanism independent of a carrier-mediated pathway (124).

Efflux Transporters

MRP1 (ABCC1)

The MRP subfamily transporters act as primary active ATP-dependent efflux transporters of endogenous substrates and xenobiotics. The first member of the MRP subfamily, MRP1, was first cloned and identified in 1992 (125). Initially found to efflux chemotherapeutics in cancer cells, MRP1 was later found to be ubiquitously expressed in the body with a wide substrate profile and capable of transporting both hydrophobic and anionic molecules. Endogenous substrates include glutathione or other anionic conjugates, while xenobiotic substrates include antineoplastic, antibiotic, and antiviral drugs (126) (Table I).

MRP1 has been primarily studied in the context of multidrug resistance in cancer. MRP1 overexpression in cancer tissues is associated with reduced overall survival and relapse propensity in cancer patients (127). Although MRP1 is ubiquitously expressed in the body, it does not appear to play a significant role in systemic drug disposition. However, tissue-specific protective effects have been observed with MRP1. MRP1 expressed in the heart has a cardioprotective role during chemotherapy, where polymorphisms in MRP1 expression have been linked to drug-induced cardiac dysfunction and toxicity (127–130).

Mrp1 is expressed and localized to the basolateral membrane of CPE cells, consistent with a role in CPE-to-blood transport of drugs out of the CSF and preventing drug entry into the CSF from general circulation (65, 67). Mrp1 activity was initially demonstrated by Rao *et al.*, who demonstrated that ^{99m}Tc-sestamibi transport across rat CP was sensitive to inhibition by MK-571, an inhibitor of MRPs (65). The role of Mrp1 at the BCSFB was further evaluated in an *in vivo* study in Mrp1 deficient mice by Wijnholds *et al.* (131). In Mrp1 KO mice, a 10-fold increase in CSF accumulation of etoposide, a chemotherapeutic agent, was observed after intravenous administration of the drug (131). In contrast, there is no change in total brain and plasma drug levels. These data suggest that Mrp1 at the BCSFB plays an important role in determining drug levels in the CSF, either by limiting drug entry from the blood and/or by promoting drug efflux from the CSF.

MRP4 (ABCC4)

MRP4, first identified and cloned in 1997, also has a wide tissue distribution, though not as ubiquitous as MRP1 (132). Outside of the CP, MRP4 is notably expressed in the liver, kidney, and blood-brain barrier (133, 134). In the liver, MRP4 is located at the sinusoidal membrane and mediates efflux of compounds from the hepatocytes into the blood, which represents an important protective function under cholestasis (135). In the kidney, MRP4 is apically localized in proximal tubule epithelial cells, where it contributes to the active tubular secretion of a range of small anionic drugs (133). Substrates of MRP4 include nucleoside analogues, diuretics, antivirals, antibiotics, and antineoplastic drugs (133) (Table I).

Similar to Mrp1, Mrp4 is expressed and localized to the basolateral membrane of CPE cells, contributing to CPE-to-blood efflux of drugs out of the CSF (91). Mrp4 has been shown to limit the accumulation of topotecan into the CSF (91). Mrp-deficient mice demonstrated 10-fold higher concentrations of topotecan in the CSF compared to wild-type controls (91). However, since Mrp4 is normally expressed at both the BCSFB and the BBB, the relative contribution of Mrp4 at the two barriers to brain drug levels could not be determined. Mrp4 has also been shown to contribute to active removal of compounds from the CSF in *ex vivo* tissue studies (136). Confocal imaging of fluorescein-cyclic AMP (fluo-cAMP), a fluorescent MRP4 probe, in isolated rat CP showed rapid accumulation of fluo-cAMP in subepithelial blood vessel areas, indicating a rapid transcellular flux of the fluorescent probe from the CSF side to the blood side. Transport of fluo-cAMP was insensitive to Mrp1 inhibition but was reduced by Mrp4 selective inhibitors, indicating the involvement of Mrp4 (136). Mrp4 activity and expression have also been demonstrated using immortalized CP epithelial cell lines (63). Recently, Mrp4 expression at the mouse CP was shown to be sex-dependent with male mice displaying a significant reduction in Mrp4 expression (137). Consistent with the expression data, vascular accumulation of fluo-cAMP was markedly reduced in CP tissues isolated from male mice (137). These studies strongly suggested a significant role of Mrp4 in promoting drug efflux at the basolateral membrane at the BCSFB. As many Mrp4 substrates are organic anions also transported by the apical Oat3, it is likely that these transporters form a transepithelial transport pathway to actively remove anionic drugs and metabolites from the CSF (Fig. 2).

ENZYME-MEDIATED CLEARANCE

Enzyme-mediated metabolism in the CPE cells provides another pathway for clearing xenobiotics and bioactive compounds from the CSF. In the liver, the primary site of drug metabolism, metabolism of xenobiotics can be divided into two phases. Phase I metabolisms involve oxidation, reduction, or hydrolysis of the parent drug. The cytochrome P450s (CYPs), especially members of CYP1-3 families, are the major Phase I enzymes involved in drug metabolism in the liver. Phase II metabolisms involve conjugation by

coupling the drug or its metabolites to polar species such as glutathione, sulfate, glucuronic acid, or glycine. Phase I and II metabolisms generally convert more active and/or lipophilic compounds into less reactive and more hydrophilic products that can be more readily excreted from the body. Similar to drug-metabolizing processes in the liver, enzymes within the CPE cells can inactivate drugs and other potentially harmful substances. Expression and activity analyses suggest that CP expresses a number of phase I and II enzymes. In particular, the CPs have been reported to harbor a high level of activities for phase II enzymes including glutathione S-transferase (GST), UDP-glucuronosyltransferase (UGT), and sulfotransferase (SULT), and thus likely represents a major site of xenobiotic metabolism in the brain (138).

Phase I Enzymes

The expression and activities of CYP enzymes have been studied in CP tissues from rats or mice (139, 140). Nonspecific CYP-dependent monooxygenase activity was detected in isolated rat CP tissues (140). However, a recent transcriptomic analysis of more than 88 rat Cyps genes only detected the expression of a few Cyps genes in rat CP, which include Cyp1b1, Cyp2d4, and Cyp2j4 (141). The activities and functional significance of the individual Cyp isoforms have not been demonstrated. Several immunohistochemical and *in situ* hybridization studies showed Cyp1a1 to be present in rat and mouse CPs; however, they appear to be mainly localized in endothelial, but not epithelial, cells of the CP (142). In addition to the CYPs, the expression of several flavin-containing monooxygenases was reported in mouse CP (143), but their functional roles in CP metabolism and detoxification have not been studied.

On the other hand, other phase I enzymes, including the monoamine oxidases (MAOs), epoxide hydrolases, and class III alcohol dehydrogenase appear to be highly expressed and functional in CPE cells (139). In particular, the CP possesses highly functional monoamine oxidases, especially MAO B (144, 145). Located in the outer mitochondrial membrane, MAO catalyzes the oxidative deamination of biogenic and xenobiotic amines. Although most MAO substrates are endogenous amines, certain dietary amines (*e.g.*, tyramine), exogenous neurotoxins (*e.g.*, MPTP), and carcinogens (*e.g.*, dimethylnitrosamine) are known to be metabolized by the MAOs (146). As discussed earlier, the PMAT transporter on the apical membrane is the major transporter responsible for transporting biogenic amines and organic cations from the CSF into CPE cells (80), providing substrate access to the intracellularly located MAOs. It is likely that PMAT and MAO in CPE cells form a coupled system to clear biogenic amines and xenobiotic amines from the CSF.

Phase II Enzymes

High enzymatic glutathione conjugation activities have been reported in both human and rodent CP tissues (147). The glutathione conjugation activities measured in the CP tissue can reach hepatic levels and are much higher than that of other brain regions (139). Consistent with enzyme activity, protein and mRNA analyses have revealed the expression of several GST enzymes in the CP including GST isoforms of the π, μ, and α subfamilies (139, 141, 148). In particular, GSTμ1, GSTμ2, GSTα3, and possibly GSTπ1 were thought to be responsible for the high conjugation activity observed in CP during postnatal development (148). Recently, using a functional knock-down rat model for choroidal glutathione conjugation, Kratzer *et al*. showed that glutathione-dependent enzymatic metabolism in the CP epithelium can effectively inactivate a broad spectrum of compounds, preventing their penetration and accumulation in the CSF *in vivo* (148).

Very high UDP-glucuronosyltransferase activity approaching those in the liver has also been reported in rat CP (140). Similar to the liver, this activity is inducible by exogenous polycyclic aromatic hydrocarbons (149). UGT1A6 is likely responsible for the observed activities as its presence in rat CP was demonstrated by immunostaining (150).

Sulfotransferases catalyze the transfer of the sulfate group to a hydroxy or amine acceptor in a variety of substrates such as steroids, biogenic amines, and therapeutic drugs (151). Sulfation is an important mechanism for regulating the biological activity of numerous hormones and neurotransmitters. In humans, the sulfotransferase SULT1A1 has been identified at the CP in the fetal brain (152). While the enzyme is also present in other brain structures, the highest activity was observed in the CP (152). Several clinically used drugs, including acetaminophen and minoxidil, are substrates of SULT1A. SULT1A1 is also known to catalyze the sulfation of dietary carcinogens such as the heterocyclic aromatic amines.

PHARMACOKINETIC RELATIONSHIP BETWEEN CSF AND BRAIN ISF

The relationship between CSF and brain ISF has been the subject of great interest, particularly with respect to the effective drug concentration in the brain. Based on the free drug hypothesis, the free or unbound drug concentration is assumed to be responsible for its pharmacological effect. The unbound brain ISF drug concentrations thus reflect the pharmacologically active drug concentrations and are used as a measure to model the pharmacokinetic and pharmacodynamic relationship of CNS-acting drugs (153, 154). Knowledge of the unbound brain ISF drug concentrations can be used to predict efficacy and toxicity of CNS drugs during drug development, which can better inform the design of the first-in-human doses. However, the brain ISF site cannot typically be sampled directly in humans, due to its inaccessibility and the invasiveness in sampling techniques. In contrast, CSF collection through lumbar puncture provides an alternative to direct CNS sampling. Protein concentrations in the CSF are several orders of magnitude lower than plasma protein, so it is typically assumed that drug in the CSF remains unbound (155). Predictive modeling techniques based on unbound partition coefficients (Kp_{uu}) between CSF and plasma in preclinical animal species and surrogate CSF measurements in humans are used to estimate unbound brain drug concentrations in humans (155–157).

Our understanding of how CSF and brain ISF drug concentrations relate to one another is still evolving. Transport and metabolism processes at both the BBB and the BCSFB can alter drug concentrations in the ISF and CSF. In addition, physiological processes, such as CSF and ISF bulk flow and glymphatic exchange, will contribute to drug distribution and

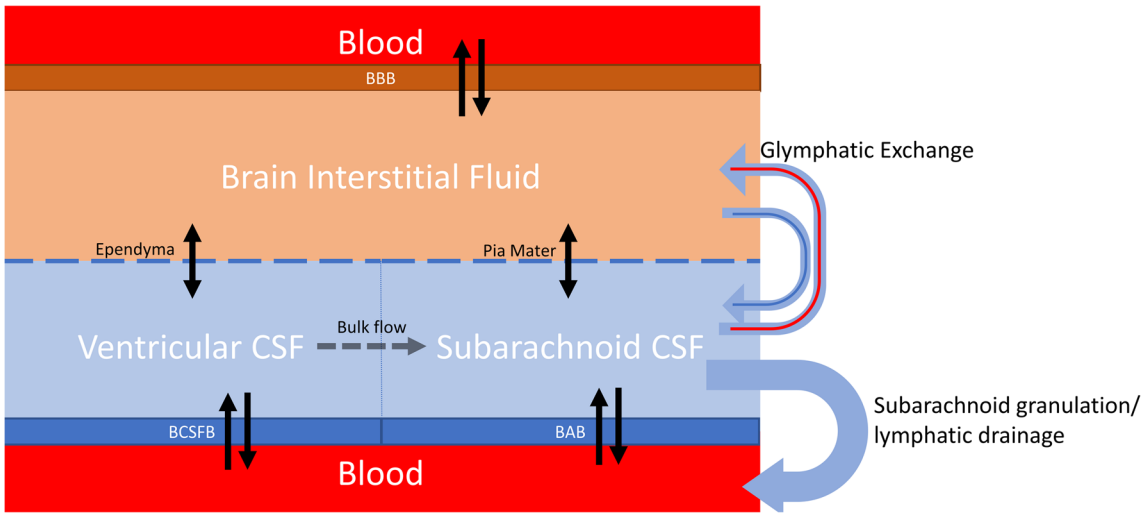

Fig. 3. Simple schematic diagram showing exchange pathways between cerebral blood, brain interstitial fluid, ventricular CSF, and subarachnoid CSF. BBB, blood-brain barrier; BCSFB, blood-CSF barrier; BAB, blood-arachnoid barrier

clearance. However, it is important to note that the CSF should not be considered a uniform compartment and that the processes of blood, CSF, and brain ISF exchange are compartmentalized and may differ between the different regions of the CSF compartment. Although there is no physical divide between ventricular and subarachnoid CSF, the slow turnover rate of CSF enables uneven drug distribution within the CSF (155). Consequently, it is important to consider that different CSF sampling sites can yield different CSF drug concentrations, and the CSF-ISF relationship can vary based on the specific region of CSF sampled (*i.e.*, ventricular, cisterna magna, lumbar) (155, 158). Drugs present in ventricular CSF can diffuse through the leaky ependyma into the brain ISF, efflux or undergo metabolism by the BCSFB, or otherwise distribute to the subarachnoid space through the cisterna magna via bulk flow (Fig. 3). Drug present in subarachnoid CSF can alternatively enter the brain ISF by glymphatic exchange or by diffusion through the pia mater. Reverse flow of CSF back into the cisterna magna and ventricles can occur during sleep. Drug in the subarachnoid CSF is primarily cleared by bulk CSF flow through arachnoid granulations or lymphatic drainage to venous blood. Additionally, there is some evidence of transporter-mediated clearance at the blood-arachnoid barrier, which may contribute to the subarachnoid CSF clearance of organic anions (159).

The pia mater and ventricular ependyma are porous membranes, and diffusion across these membranes is limited by molecular size. The brain ISF is not a well-stirred compartment. Diffusion in the ISF is complex and influenced by physicochemical properties of the drug and its interaction with the extracellular matrix. The extracellular matrix in the brain ISF carries a negative charge, which can affect diffusion of charged molecules (160). Glymphatic exchange may play a more significant role for large molecule biologics for ISF distribution due to their larger size. However, there is some evidence of a "sieving" effect on larger macromolecules exiting the perivascular space into the parenchyma during glymphatic flow (42, 46, 161). Further research is needed to clarify how molecular weight can influence the extent of glymphatic exchange.

Physiologically based pharmacokinetic models can take the advantage of integrating these processes to predict drug pharmacokinetic behaviors in the brain. A detailed understanding of the transport, metabolism, and CSF flow in the CP-CSF system can improve the predictive capacities of these models. Notably, most models of the CNS do not consider active transport by the BCSFB, and transport processes at the BCSFB remain understudied. Several transporters shown to be expressed at the BCSFB have yet to be functionally characterized. Additional study of these transporters could clarify their role in CSF clearance, improving our predictions of CSF and ISF drug concentrations. Furthermore, we may expect considerations of glymphatic exchange in physiologically based models as its role on drug distribution is further clarified. Indeed, Chang *et al.* recently developed a physiologically based pharmacokinetic model incorporating glymphatic flow to describe monoclonal antibody disposition in the brain (162).

CONCLUSIONS

The CP-CSF system plays a significant role in clearing waste products and xenobiotics from the brain, critical for healthy brain homeostasis. Understanding the underlying mechanisms that drive drug concentrations in the CSF can improve our capacity to predict drug concentrations in the CNS, a key determinant for local drug efficacy and toxicity. It is well established that drug transporters and drug-metabolizing enzymes are key determinants for systemic absorption, distribution, and elimination characteristics. In the CSF, BCSFB transporters and metabolic enzymes act alongside physiological processes (*i.e.*, CSF bulk flow, exchange between brain ISF and CSF) to impact drug concentrations in the CSF. While the significance of these processes is well recognized, the underlying mechanisms that ultimately determine drug concentrations in the CSF are still not fully understood. With recent technological advances and a renewed interest in studying the CP-CSF system, further investigations should shed new insights on the complex interplay of CSF flow, CSF-ISF exchange, and BCSFB transport and metabolism. Furthermore, the CP-CSF system is known to show marked morphological and functional changes during aging or in neurodegenerative diseases. Understanding how these physiological and pathophysiological processes affect drug transporters, metabolizing enzymes, and CSF dynamics may help to

better predict changes in CSF and CNS pharmacokinetics in the affected populations.

CONTRIBUTION BY AUTHORS AS WOMEN SCIENTISTS

Joanne Wang is currently a Professor of Pharmaceutics at the University of Washington and an affiliate investigator at the Fred Hutchinson Cancer Research Center in Seattle, Washington. Dr. Wang obtained her B.S. in Biochemistry from Peking University and Ph.D. in Pharmaceutical Chemistry from the University of California, San Francisco. Her research focuses on understanding the biological and pharmacological functions of membrane transporters and their roles in drug disposition, tissue-specific targeting, drug-drug interactions, and drug-induced organ toxicities. Dr. Wang's laboratory first cloned and characterized the plasma membrane monoamine transporter (PMAT) and established its function in monoamine neurotransmitter uptake and in organic cation transport at the blood-CSF barrier. Her group also demonstrated organic cation transporter 3 (OCT3) in mediating salivary glands and placental transport of organic cation drugs and contributed to the understanding of the roles of OCTs and MATEs in renal handling of antihypertensive and anticancer drugs. Dr. Wang has mentored numerous graduate students, postdoctoral fellows, visiting scientists, undergraduate, and professional students from diverse backgrounds, including women and underrepresented minorities. Dr. Wang has served on numerous NIH study sections and special emphasis panels. She is a longstanding member of AAPS and ASPET, and currently serves as the Chair-elect of the Division for Drug Metabolism and Disposition at ASPET. She was an editorial board member of *The AAPS Journal* and currently serves on the editorial advisory boards of *Molecular Pharmacology, Drug Metabolism and Disposition,* and *Biopharmaceutics and Drug Disposition.*

FUNDING

This work was supported in part by NIH grants R01 GM066233 and funding from the Elmer M. Plein Endowment Research Fund.

DECLARATIONS

Disclaimer The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

REFERENCES

1. Lun MP, Monuki ES, Lehtinen MK. Development and functions of the choroid plexus-cerebrospinal fluid system. Nat Rev Neurosci. 2015;16(8):445–57.
2. Fame RM, Cortés-Campos C, Sive HL. Brain ventricular system and cerebrospinal fluid development and function: light at the end of the tube: a primer with latest insights. Bioessays. 2020;42(3):e1900186.
3. Betts JG, Young KA, Wise JA, Johnson E, Poe B, Kruse DH, et al. Circulation and the central nervous system. In: Anatomy and physiology. OpenStax: Houston; 2013.
4. Spector R, Keep RF, Snodgrass SR, Smith QR, Johanson CE. A balanced view of choroid plexus structure and function: focus on adult humans. Exp Neurol. 2015;267:78–86.
5. Damkier HH, Brown PD, Praetorius J. Epithelial pathways in choroid plexus electrolyte transport. Physiology. 2010;25(4):239–49.
6. Davson H, Purvis C. Cryoscopic apparatus suitable for studies on aqueous humour and cerebro-spinal fluid. J Physiol. 1954;124:1–47 12P.
7. Welch K, Sadler K. Electrical potentials of choroid plexus of the rabbit. J Neurosurg. 1965;22(4):344–51.
8. Wolburg H, Paulus W. Choroid plexus: biology and pathology. Acta Neuropathol. 2010;119(1):75–88.
9. Prinz M, Priller J. The role of peripheral immune cells in the CNS in steady state and disease. Nat Neurosci. 2017;20(2):136–44.
10. Prinz M, Priller J, Sisodia SS, Ransohoff RM. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. Nat Neurosci. 2011;14(10):1227–35.
11. Schwartz M, Baruch K. The resolution of neuroinflammation in neurodegeneration: leukocyte recruitment via the choroid plexus. EMBO J. 2014;33(1):7–22.
12. Davson H, Welch K, Segal MB. Physiology and pathophysiology of the cerebrospinal fluid. Edinburgh–London: Churchill Livingstone; 1987.
13. Davson H. Segal MB. Physiology of the CSF and blood-brain barriers. Boca Raton, FL: CRC press; 1996.
14. Spector R, Robert Snodgrass S, Johanson CE. A balanced view of the cerebrospinal fluid composition and functions: focus on adult humans. Exp Neurol. 2015;273:57–68.
15. Dawson RMC, Freinkel N. The distribution of free mesoinositol in mammalian tissues, including some observations on the lactating rat. Biochem J. 1961;78(3):606–10.
16. de Rougemont J, Ames A, Nesbett FB, Hofmann HF. Fluid formed by choroid plexus: a technique for its collection and a comparison of its electrolyte composition with serum and cisternal fluids. J Neurophysiol. 1960;23(5):485–95.
17. Johanson CE, Stopa EG, McMillan PN. The Blood-Cerebrospinal Fluid Barrier: Structure and Functional Significance. In: Nag S, editor. The Blood-Brain and Other Neural Barriers. New York: Springer; 2011. p. 101–31.
18. Herndon RM, Brumback RA. The Cerebrospinal Fluid. Boston/Dordrecht/London: Kluwer Academic Publishers; 2012.
19. Thompson EJ, The CSF. Proteins: A Biochemical Approach. Amsterdam: Elsevier; 1988. p. 9–26.
20. Sakka L, Coll G, Chazal J. Anatomy and physiology of cerebrospinal fluid. Eur Ann Otorhinolaryngol Head Neck Dis. 2011;128(6):309–16.
21. Nilsson C, Stahlberg F, Thomsen C, Henriksen O, Herning M, Owman C. Circadian variation in human cerebrospinal fluid production measured by magnetic resonance imaging. Am J Physiol Integr Comp Physiol. 1992;262(1):R20–4.
22. Dreha-Kulaczewski S, Joseph AA, Merboldt K-D, Ludwig H-C, Gärtner J, Frahm J. Inspiration is the major regulator of human CSF flow. J Neurosci. 2015;35(6):2485–91.
23. Bateman GA, Brown KM. The measurement of CSF flow through the aqueduct in normal and hydrocephalic children: from where does it come, to where does it go? Childs Nerv Syst. 2012;28(1):55–63.
24. Rubin RC, Henderson ES, Ommaya AK, Walker MD, Rall DP. The production of cerebrospinal fluid in man and its modification by acetazolamide. J Neurosurg. 1966;25(4):430–6.
25. Cutler RW, Page L, Galicich J, Watters GV. Formation and absorption of cerebrospinal fluid in man. Brain. 1968;91(4):707–20.
26. Lorenzo AV, Page LK, Watters GV. Relationship between cerebrospinal fluid formation, absorption and pressure in human hydrocephalus. Brain. 1970;93(4):679–92.
27. Preston JE. Ageing choroid plexus-cerebrospinal fluid system. Microsc Res Tech. 2001;52(1):31–7.

28. May C, Kaye JA, Atack JR, Schapiro MB, Friedland RP, Rapoport SI. Cerebrospinal fluid production is reduced in healthy aging. Neurology. 1990;40(3 Part 1):500 LP – 500.
29. Chen CPC, Chen RL, Preston JE. The influence of cerebrospinal fluid turnover on age-related changes in cerebrospinal fluid protein concentrations. Neurosci Lett. 2010;476(3):138–41.
30. Chiu C, Miller MC, Caralopoulos IN, Worden MS, Brinker T, Gordon ZN, et al. Temporal course of cerebrospinal fluid dynamics and amyloid accumulation in the aging rat brain from three to thirty months. Fluids Barriers CNS. 2012;9(1):3.
31. Silverberg GD, Heit G, Huhn S, Jaffe RA, Chang SD. Bronte–Stewart H, et al. The cerebrospinal fluid production rate is reduced in dementia of the Alzheimer's type. Neurology. 2001;57(10):1763–6.
32. de Leon MJ, Li Y, Okamura N, Tsui WH, Saint-Louis LA, Glodzik L, et al. Cerebrospinal fluid clearance in alzheimer disease measured with dynamic PET. J Nucl Med. 2017;58(9):1471–6.
33. Schubert JJ, Veronese M, Marchitelli L, Bodini B, Tonietto M, Stankoff B, et al. Dynamic 11C-PiB PET shows cerebrospinal fluid flow alterations in Alzheimer disease and multiple sclerosis. J Nucl Med. 2019;60(10):1452–60.
34. Alperin N, Lee SH, Sivaramakrishnan A, Hushek SG. Quantifying the effect of posture on intracranial physiology in humans by MRI flow studies. J Magn Reson Imaging. 2005;22(5):591–6.
35. Fultz NE, Bonmassar G, Setsompop K, Stickgold RA, Rosen BR, Polimeni JR, et al. Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. Science (80-). 2019;366(6465):628–31.
36. Grubb S, Lauritzen M. Deep sleep drives brain fluid oscillations. Science. 2019;366(6465):572–3.
37. Abbott NJ. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. Neurochem Int. 2004;45(4):545–52.
38. Jessen NA, Munk ASF, Lundgaard I, Nedergaard M. The glymphatic system: a beginner's guide. Neurochem Res. 2015;40(12):2583–99.
39. Pardridge WM. CSF, blood-brain barrier, and brain drug delivery. Expert Opin Drug Deliv. 2016;13(7):963–75.
40. Davson H, Segal MB. The effects of some inhibitors and accelerators of sodium transport on the turnover of 22Na in the cerebrospinal fluid and the brain. J Physiol. 1970;209(1):131–53.
41. Oldendorf WH, Davson H. brain extracellular space and the sink action of cerebrospinal fluid: measurement of rabbit brain extracellular space using sucrose labeled with carbon 14. Arch Neurol. 1967;17(2):196–205.
42. Abbott NJ, Pizzo ME, Preston JE, Janigro D, Thorne RG. The role of brain barriers in fluid movement in the CNS: is there a "glymphatic" system? Acta Neuropathol. 2018;135(3):387–407.
43. Cserr HF, Cooper DN, Suri PK, Patlak CS. Efflux of radiolabeled polyethylene glycols and albumin from rat brain. Am J Physiol Physiol. 1981;240(4):F319–28.
44. Cserr HF, Cooper DN, Milhorat TH. Flow of cerebral interstitial fluid as indicated by the removal of extracellular markers from rat caudate nucleus. Exp Eye Res. 1977;25:461–73.
45. Plog BA, Nedergaard M. The glymphatic system in central nervous system health and disease: past, present, and future. Annu Rev Pathol Mech Dis. 2018;13(1):379–94.
46. Wang M, Liao Y, Venkataraman A, Plog BA, Nedergaard M, Deane R, et al. Cerebral arterial pulsation drives paravascular CSF-interstitial fluid exchange in the murine brain. J Neurosci. 2013;33(46):18190–9.
47. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, et al. Sleep drives metabolite clearance from the adult brain. Science (80-). 2013;342(6156):373–7.
48. Smith AJ, Yao X, Dix JA, Jin B-J, Verkman AS. Test of the "glymphatic" hypothesis demonstrates diffusive and aquaporin-4-independent solute transport in rodent brain parenchyma. Huguenard J, editor. Elife. 2017;6:e27679.
49. Mestre H, Hablitz LM, Xavier ALR, Feng W, Zou W, Pu T, et al. Aquaporin-4-dependent glymphatic solute transport in the rodent brain. Kleinfeld D, Morrison SJ, editors. Elife. 2018;7:e40070.
50. Wolak DJ, Thorne RG. Diffusion of macromolecules in the brain: implications for drug delivery. Mol Pharm. 2013;10(5):1492–504.
51. Holter KE, Kehlet B, Devor A, Sejnowski TJ, Dale AM, Omholt SW, et al. Interstitial solute transport in 3D reconstructed neuropil occurs by diffusion rather than bulk flow. Proc Natl Acad Sci. 2017;114(37):9894–9.
52. Giacomini KM, Huang S-M, Tweedie DJ, Benet LZ, Brouwer KLR, Chu X, et al. Membrane transporters in drug development. Nat Rev Drug Discov. 2010;9(3):215–36.
53. Wagner DJ, Hu T, Wang J. Polyspecific organic cation transporters and their impact on drug intracellular levels and pharmacodynamics. Pharmacol Res. 2016;111:237–46.
54. Giacomini KM, Huang S-M, Tweedie DJ, Benet LZ, Brouwer KLR, Chu X, et al. Membrane transporters in drug development. Nat Rev Drug Discov. 2010;9(3):215–36.
55. Lin L, Yee SW, Kim RB, Giacomini KM. SLC transporters as therapeutic targets: emerging opportunities. Nat Rev Drug Discov. 2015;14(8):543–60.
56. Dahlin A, Royall J, Hohmann JG, Wang J. Expression profiling of the solute carrier gene family in the mouse brain. J Pharmacol Exp Ther. 2009;329(2):558–70.
57. Ho HTB, Dahlin A, Wang J. Expression Profiling of Solute Carrier Gene Families at the Blood-CSF Barrier. Front Pharmacol. 2012;3:154.
58. Choudhuri S, Cherrington NJ, Li N, Klaassen CD. Constitutive expression of various xenobiotic and endobiotic transporter mRNAs in the choroid plexus of rats. Drug Metab Dispos. 2003;31(11):1337–45.
59. Uchida Y, Zhang Z, Tachikawa M, Terasaki T. Quantitative targeted absolute proteomics of rat blood-cerebrospinal fluid barrier transporters: comparison with a human specimen. J Neurochem. 2015;134(6):1104–15.
60. Braun C, Sakamoto A, Fuchs H, Ishiguro N, Suzuki S, Cui Y, et al. Quantification of transporter and receptor proteins in dog brain capillaries and choroid plexus: relevance for the distribution in brain and CSF of selected BCRP and P-gp substrates. Mol Pharm. 2017;14(10):3436–47.
61. Uchida Y, Goto R, Takeuchi H, Łuczak M, Usui T, Tachikawa M, et al. Abundant expression of OCT2, MATE1, OAT1, OAT3, PEPT2, BCRP, MDR1, and xCT transporters in blood-arachnoid barrier of pig and polarized localizations at CSF- and blood-facing plasma membranes. Drug Metab Dispos. 2020;48(2):135–45.
62. Akanuma S, Kubo Y, Hosoya K. Techniques for Evaluating Efflux Transport of Radiolabeled Drugs and Compounds from the Cerebrospinal Fluid Across the Blood-Cerebrospinal Fluid Barrier. In: Barichello T, editor. Blood-Brain Barrier. New York, NY: Springer New York; 2019. p. 231–48.
63. Kläs J, Wolburg H, Terasaki T, Fricker G, Reichel V. Characterization of immortalized choroid plexus epithelial cell lines for studies of transport processes across the blood-cerebrospinal fluid barrier. Cerebrospinal Fluid Res. 2010;7(1):11.
64. Pellegrini L, Bonfio C, Chadwick J, Begum F, Skehel M, Lancaster MA. Human CNS barrier-forming organoids with cerebrospinal fluid production. Science (80-). 2020;369(6500):eaaz5626.
65. Rao VV, Dahlheimer JL, Bardgett ME, Snyder AZ, Finch RA, Sartorelli AC, 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. 1999;96(7):3900–5.
66. Gao B, Stieger B, Noé B, Fritschy J-M, Meier PJ. Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. J Histochem Cytochem. 1999;47(10):1255–63.
67. Gazzin S, Strazielle N, Schmitt C, Fevre-Montange M, Ostrow JD, Tiribelli C, et al. Differential expression of the multidrug resistance-related proteins ABCb1 and ABCc1 between blood-brain interfaces. J Comp Neurol. 2008;510(5):497–507.
68. Morris ME, Rodriguez-Cruz V, Felmlee MA. SLC and ABC Transporters: Expression, Localization, and Species

Differences at the Blood-Brain and the Blood-Cerebrospinal Fluid Barriers. AAPS J. 2017;19(5):1317–31.
69. Felmlee MA, Jones RS, Rodriguez-Cruz V, Follman KE, Morris ME. Monocarboxylate transporters (SLC16): function, regulation, and role in health and disease. Daws LC, editor. Pharmacol Rev. 2020;72(2):466–85.
70. Pritchard JB. Intracellular alpha-ketoglutarate controls the efficacy of renal organic anion transport. J Pharmacol Exp Ther. 1995;274(3):1278–84.
71. Nigam SK, Bush KT, Martovetsky G, Ahn S-Y, Liu HC, Richard E, et al. The organic anion transporter (OAT) family: a systems biology perspective. Physiol Rev. 2015;95(1):83–123.
72. Nagata Y, Kusuhara H, Endou H, Sugiyama Y. Expression and functional characterization of rat organic anion transporter 3 (rOat3) in the choroid plexus. Mol Pharmacol. 2002;61(5):982–8.
73. Cha SH, Sekine T, Fukushima J, Kanai Y, Kobayashi Y, Goya T, et al. Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. Mol Pharmacol. 2001;59(5):1277–86.
74. Matsumoto S, Yoshida K, Ishiguro N, Maeda T, Tamai I. Involvement of rat and human organic anion transporter 3 in the renal tubular secretion of topotecan [(S)-9-dimethylaminomethyl-10-hydroxy-camptothecin hydrochloride]. J Pharmacol Exp Ther. 2007;322(3):1246–52.
75. Takeda M, Khamdang S, Narikawa S, Kimura H, Kobayashi Y, Yamamoto T, et al. Human organic anion transporters and human organic cation transporters mediate renal antiviral transport. J Pharmacol Exp Ther. 2002;300(3):918–24.
76. Tahara H, Shono M, Kusuhara H, Kinoshita H, Fuse E, Takadate A, et al. Molecular cloning and functional analyses of OAT1 and OAT3 from cynomolgus monkey kidney. Pharm Res. 2005;22(4):647–60.
77. Ueo H, Motohashi H, Katsura T, Inui K. Human organic anion transporter hOAT3 is a potent transporter of cephalosporin antibiotics, in comparison with hOAT1. Biochem Pharmacol. 2005;70(7):1104–13.
78. Yin J, Wagner DJ, Prasad B, Isoherranen N, Thummel KE, Wang J. Renal secretion of hydrochlorothiazide involves organic anion transporter 1/3, organic cation transporter 2, and multidrug and toxin extrusion protein 2-K. Am J Physiol Physiol. 2019;317(4):F805–14.
79. Ebner T, Ishiguro N, Taub ME. The use of transporter probe drug cocktails for the assessment of transporter-based drug-drug interactions in a clinical setting - proposal of a four component transporter cocktail. J Pharm Sci. 2015;104(9):3220–8.
80. Duan H, Wang J. Impaired monoamine and organic cation uptake in choroid plexus in mice with targeted disruption of the plasma membrane monoamine transporter (Slc29a4) gene. J Biol Chem. 2013;288(5):3535–44.
81. Zhou M, Xia L, Wang J. Metformin transport by a newly cloned proton-stimulated organic cation transporter (plasma membrane monoamine transporter) expressed in human intestine. Drug Metab Dispos. 2007;35(10):1956–62.
82. Mimura Y, Yasujima T, Ohta K, Inoue K, Yuasa H. Functional identification of plasma membrane monoamine transporter (PMAT/SLC29A4) as an atenolol transporter sensitive to flavonoids contained in apple juice. J Pharm Sci. 2017;106(9):2592–8.
83. Shu C, Shen H, Teuscher NS, Lorenzi PJ, Keep RF, Smith DE. Role of PEPT2 in peptide/mimetic trafficking at the blood-cerebrospinal fluid barrier: studies in rat choroid plexus epithelial cells in primary culture. J Pharmacol Exp Ther. 2002;301(3):820–9.
84. Song F, Hu Y, Jiang H, Smith DE. Species differences in human and rodent PEPT2-mediated transport of glycylsarcosine and cefadroxil in pichia pastoris transformants. Drug Metab Dispos. 2017;45(2):130–6.
85. Li M, Anderson GD, Phillips BR, Kong W, Shen DD, Wang J. Interactions of amoxicillin and cefaclor with human renal organic anion and peptide transporters. Drug Metab Dispos. 2006;34(4):547–55.
86. Zaman GJ, Flens MJ, van Leusden MR, de Haas M, Mülder HS, Lankelma J, et al. The human multidrug resistance-associated protein MRP is a plasma membrane drug-efflux pump. Proc Natl Acad Sci. 1994;91(19):8822–6.
87. Zeng H, Chen Z-S, Belinsky MG, Rea PA, Kruh GD. Transport of methotrexate (MTX) and folates by multidrug resistance protein (MRP) 3 and MRP1. Cancer Res. 2001;61(19):7225–32.
88. Knauer MJ, Urquhart BL, Meyer zu Schwabedissen HE, Schwarz UI, Lemke CJ, Leake BF, et al. Human skeletal muscle drug transporters determine local exposure and toxicity of statins. Circ Res. 2010;106(2):297–306.
89. van der Sandt IC, Vos CM, Nabulsi L, Blom-Roosemalen MC, Voorwinden HH, de Boer AG, et al. Assessment of active transport of HIV protease inhibitors in various cell lines and the in vitro blood-brain barrier. AIDS. 2001;15(4):483–91.
90. de Waart DR, van de Wetering K, Kunne C, Duijst S, Paulusma CC, Oude Elferink RPJ. Oral availability of cefadroxil depends on ABCC3 and ABCC4. Drug Metab Dispos. 2012;40(3):515–21.
91. Leggas M, Adachi M, Scheffer GL, Sun D, Wielinga P, Du G, et al. Mrp4 confers resistance to topotecan and protects the brain from chemotherapy. Mol Cell Biol. 2004;24(17):7612–21.
92. Chen Z-S, Lee K, Walther S, Raftogianis RB, Kuwano M, Zeng H, et al. Analysis of methotrexate and folate transport by multidrug resistance protein 4 (ABCC4): MRP4 is a component of the methotrexate efflux system. Cancer Res. 62(11):3144–50.
93. Tian Q, Zhang J, Chan SY. Chin Tan, Theresa M., Duan W, et al. Topotecan is a substrate for multidrug resistance associated protein 4. Curr Drug Metab. 2006;7(1):105–18.
94. Chen ZS, Lee K, Kruh GD. Transport of cyclic nucleotides and estradiol 17-beta-D-glucuronide by multidrug resistance protein 4. Resistance to 6-mercaptopurine and 6-thioguanine. J Biol Chem. 2001;276(36):33747–54.
95. Imaoka T, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal elimination of the antiviral drugs adefovir and tenofovir. Mol Pharmacol. 2007;71(2):619–27.
96. Ci L, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Involvement of MRP4 (ABCC4) in the luminal efflux of ceftizoxime and cefazolin in the kidney. Mol Pharmacol. 2007;71(6):1591–7.
97. Hasegawa Maki, Kusuhara Hiroyuki, Adachi Masashi, Schuetz John D, Takeuchi Kenji, Sugiyama Yuichi. Multidrug resistance-associated protein 4 is involved in the urinary excretion of hydrochlorothiazide and furosemide. J Am Soc Nephrol. 2007;18(1):37–45.
98. VanWert AL, Bailey RM, Sweet DH. Organic anion transporter 3 (Oat3/Slc22a8) knockout mice exhibit altered clearance and distribution of penicillin G. Am J Physiol Physiol. 2007;293(4):F1332–41.
99. VanWert AL, Sweet DH. Impaired Clearance of methotrexate in organic anion transporter 3 (Slc22a8) knockout mice: a gender specific impact of reduced folates. Pharm Res. 2008;25(2):453–62.
100. Pappenheimer JR, Heisey SR, Jordan EF. Active transport of diodrast and phenolsulfonphthalein from cerebrospinal fluid to blood. Am J Physiol Content. 1961;200(1):1–10.
101. Fishman RA. Blood-brain and CSF barriers to penicillin and related organic acids. Arch Neurol. 1966;15(2):113–24.
102. Sweet DH, Wolff NA, Pritchard JB. Expression cloning and characterization of ROAT1: the basolateral organic anion transporter in rat kidney. J Biol Chem. 1997;272(48):30088–95.
103. Sekine T, Watanabe N, Hosoyamada M, Kanai Y, Endou H. Expression cloning and characterization of a novel multispecific organic anion transporter. J Biol Chem. 1997;272(30):18526–9.
104. Bode U, Magrath IT, Bleyer WA, Poplack DG, Glaubiger DL. Active Transport of Methotrexate from Cerebrospinal Fluid in Humans. Cancer Res. 1980;40(7):2184–7.
105. Sweet DH, Miller DS, Pritchard JB, Fujiwara Y, Beier DR, Nigam SK. Impaired Organic Anion Transport in Kidney and Choroid Plexus of Organic Anion Transporter 3 (Oat3 (Slc22a8)) Knockout Mice. J Biol Chem. 2002;277(30):26934–43.

106. Sykes D, Sweet DH, Lowes S, Nigam SK, Pritchard JB, Miller DS, et al. Organic anion transport in choroid plexus from wild-type and organic anion transporter 3 (Slc22a8)-null mice. Am J Physiol Ren Physiol. 2004;286(5):F972–8.
107. Liu W, Liang R, Ramamoorthy S, Fei Y-J, Ganapathy ME, Hediger MA, et al. Molecular cloning of PEPT 2, a new member of the H+/peptide cotransporter family, from human kidney. Biochim Biophys Acta Biomembr. 1995;1235(2):461–6.
108. Kamal MA, Keep RF, Smith DE. Role and relevance of PEPT2 in drug disposition, dynamics, and toxicity. Drug Metab Pharmacokinet. 2008;23(4):236–42.
109. Smith DE, Clémençon B, Hediger MA. Proton-coupled oligopeptide transporter family SLC15: physiological, pharmacological and pathological implications. Mol Asp Med. 2013;34(2):323–36.
110. Ocheltree SM, Shen H, Hu Y, Keep RF, Smith DE. Role and relevance of peptide transporter 2 (PEPT2) in the kidney and choroid plexus: in vivo studies with glycylsarcosine in wild-type and PEPT2 knockout mice. J Pharmacol Exp Ther. 2005;315(1):240–7.
111. Shen H, Ocheltree SM, Hu Y, Keep RF, Smith DE. Impact of genetic knockout of PEPT2 on cefadroxil pharmacokinetics, renal tubular reabsorption, and brain penetration in mice. Drug Metab Dispos. 2007;35(7):1209–16.
112. Kamal MA, Jiang H, Hu Y, Keep RF, Smith DE. Influence of genetic knockout of Pept2 on the in vivo disposition of endogenous and exogenous carnosine in wild-type and Pept2 null mice. Am J Physiol Integr Comp Physiol. 2009;296(4):R986–91.
113. Shen H, Smith DE, Keep RF, Brosius FC. Immunolocalization of the proton-coupled oligopeptide transporter PEPT2 in developing rat brain. Mol Pharm. 2004;1(4):248–56.
114. Shen H, Smith DE, Keep RF, Xiang J, Brosius FC. Targeted disruption of the PEPT2 gene markedly reduces dipeptide uptake in choroid plexus. J Biol Chem. 2003;278(7):4786–91.
115. Shen H, Keep RF, Hu Y, Smith DE. PEPT2 (Slc15a2)-mediated unidirectional transport of cefadroxil from cerebrospinal fluid into choroid plexus. J Pharmacol Exp Ther. 2005;315(3):1101–8.
116. Chen X, Keep RF, Liang Y, Zhu H-J, Hammarlund-Udenaes M, Hu Y, et al. Influence of peptide transporter 2 (PEPT2) on the distribution of cefadroxil in mouse brain: a microdialysis study. Biochem Pharmacol. 2017;131:89–97.
117. Engel K, Zhou M, Wang J. Identification and characterization of a novel monoamine transporter in the human brain. J Biol Chem. 2004;279(48):50042–9.
118. Wang J. The plasma memebrane monoamine transporter (PMAT): structure, function, and role in organic cation disposition. Clin Pharmacol Ther. 2016;100(5):489–99.
119. Foti RS, Swaan PW, Wang J, Duan H, Pan Y, Hu T. Potent and selective inhibition of plasma membrane monoamine transporter by HIV protease inhibitors. Drug Metab Dispos. 2015;43(11):1773–80.
120. Itagaki S, Ganapathy V, Ho HTB, Zhou M, Babu E, Wang J. Electrophysiological characterization of the polyspecific organic cation transporter plasma membrane monoamine transporter. Drug Metab Dispos. 2012;40(6):1138–43.
121. Duan H, Wang J. Selective transport of monoamine neurotransmitters by human plasma membrane monoamine transporter and organic cation transporter 3. J Pharmacol Exp Ther. 2010;335(3):743–53.
122. Xia L, Engel K, Zhou M, Wang J. Membrane localization and pH-dependent transport of a newly cloned organic cation transporter (PMAT) in kidney cells. Am J Physiol Physiol. 2007;292(2):F682–90.
123. Sweet DH, Miller DS, Pritchard JB. Ventricular choline transport: a role for organic cation transporter 2 expressed in choroid plexus. J Biol Chem. 2001;276(45):41611–9.
124. Miller DS, Villalobos AR, Pritchard JB. Organic cation transport in rat choroid plexus cells studied by fluorescence microscopy. Am J Physiol Physiol. 1999;276(4):C955–68.
125. Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, et al. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science (80-). 1992;258(5088):1650–4.
126. Cole SPC. Targeting multidrug resistance protein 1 (MRP1, ABCC1): past, present, and future. Annu Rev Pharmacol Toxicol. 2014;54(1):95–117.
127. Lu JF, Pokharel D, Bebawy M. MRP1 and its role in anticancer drug resistance. Drug Metab Rev. 2015;47(4):406–19.
128. Semsei AF, Erdelyi DJ, Ungvari I, Csagoly E, Hegyi MZ, Kiszel PS, et al. ABCC1 polymorphisms in anthracycline-induced cardiotoxicity in childhood acute lymphoblastic leukaemia. Cell Biol Int. 2012;36(1):79–86.
129. Krause MS, Oliveira LP Jr, Silveira EMS, Vianna DR, Rossato JS, Almeida BS, et al. MRP1/GS-X pump ATPase expression: is this the explanation for the cytoprotection of the heart against oxidative stress-induced redox imbalance in comparison to skeletal muscle cells? Cell Biochem Funct. 2007;25(1):23–32.
130. Leszek W, Bettina K, Markus S, Gregor S, Albrecht S, Albert R, et al. NAD(P)H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity. Circulation. 2005;112(24):3754–62.
131. Wijnholds J, de Lange ECM, Scheffer GL, van den Berg D-J, Mol CAAM, 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(3):279–85.
132. Kool M, De Haas M, Scheffer GL, Scheper RJ, Van Eijk MJT, Juijn JA, et al. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res. 1997;57(16):3537–47.
133. Russel FGM, Koenderink JB, Masereeuw R. Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. Trends Pharmacol Sci. 2008;29(4):200–7.
134. Nies AT, Jedlitschky G, König J, Herold-Mende C, Steiner HH, Schmitt H-P, et al. Expression and immunolocalization of the multidrug resistance proteins, MRP1–MRP6 (ABCC1–ABCC6), in human brain. Neuroscience. 2004;129(2):349–60.
135. Zamek-Gliszczynski MJ, Taub ME, Chothe PP, Chu X, Giacomini KM, Kim RB, et al. Transporters in drug development: 2018 ITC recommendations for transporters of emerging clinical importance. Clin Pharmacol Ther. 2018;104(5):890–9.
136. Reichel V, Kläs J, Fricker G, Masereeuw R. Fluo-cAMP is transported by multidrug resistance-associated protein isoform 4 in rat choroid plexus. J Neurochem. 2010;115(1):200–8.
137. Flores K, Manautou JE, Renfro JL. Gender-specific expression of ATP-binding cassette (Abc) transporters and cytoprotective genes in mouse choroid plexus. Toxicology. 2017;386:84–92.
138. Strazielle N, Ghersi-Egea JF. Physiology of blood–brain interfaces in relation to brain disposition of small compounds and macromolecules. Mol Pharm. 2013;10(5):1473–91.
139. Strazielle N, Khuth ST, Ghersi-Egea J-F. Detoxification systems, passive and specific transport for drugs at the blood–CSF barrier in normal and pathological situations. Adv Drug Deliv Rev. 2004;56(12):1717–40.
140. Ghersi-Egea JF, Leninger-Muller B, Suleman G, Siest G, Minn A. Localization of drug-metabolizing enzyme activities to blood-brain interfaces and circumventricular organs. J Neurochem. 1994;62(3):1089–96.
141. Kratzer I, Liddelow SA, Saunders NR, Dziegielewska KM, Strazielle N, Ghersi-Egea J-F. Developmental changes in the transcriptome of the rat choroid plexus in relation to neuroprotection. Fluids Barriers CNS. 2013;10(1):25.
142. Granberg L, Östergren A, Brandt I, Brittebo EB. CYP1A1 and CYP1B1 in blood-brain interfaces: CYP1A1-dependent bioactivation of 7,12-dimethylbenz(a)anthracene in endothelial cells. Drug Metab Dispos. 2003;31(3):259–65.
143. Janmohamed A, Hernandez D, Phillips IR, Shephard EA. Cell-, tissue-, sex- and developmental stage-specific expression of mouse flavin-containing monooxygenases (Fmos). Biochem Pharmacol. 2004;68(1):73–83.
144. Lindvall M, Hardebo JE, Owman CH. Barrier mechanisms for neurotransmitter monoamines in the choroid plexus. Acta Physiol Scand. 1980;108(3):215–21.

145. Vitalis T, Fouquet C, Alvarez C, Seif I, Price D, Gaspar P, et al. Developmental expression of monoamine oxidases A and B in the central and peripheral nervous systems of the mouse. J Comp Neurol. 2002;442(4):331–47.
146. Gong B, Boor PJ. The role of amine oxidases in xenobiotic metabolism. Expert Opin Drug Metab Toxicol. 2006;2(4):559–71.
147. Ghersi-Egea J-F, Strazielle N, Murat A, Jouvet A, Buénerd A, Belin M-F. Brain protection at the blood–cerebrospinal fluid interface involves a glutathione-dependent metabolic barrier mechanism. J Cereb Blood Flow Metab. 2006;26(9):1165–75.
148. Kratzer I, Strazielle N, Saudrais E, Mönkkönen K, Malleval C, Blondel S, et al. Glutathione conjugation at the blood–CSF barrier efficiently prevents exposure of the developing brain fluid environment to blood-borne reactive electrophilic substances. J Neurosci. 2018;38(14):3466–79.
149. Leininger-Muller B, Ghersi-Egea JF, Siest G, Minn A. Induction and immunological characterization of the uridine diphosphate-glucuronosyltransferase conjugating 1-naphthol in the rat choroid plexus. Neurosci Lett. 1994;175(1–2):37–40.
150. Martinasevic MK, King CD, Rios GR, Tephly TR. Immunohistochemical localization of UDP-glucuronosyltransferases in rat brain during early development. Drug Metab Dispos. 1998;26(10):1039–41.
151. James MO, Ambadapadi S. Interactions of cytosolic sulfotransferases with xenobiotics. Drug Metab Rev. 2013;45(4):401–14.
152. Richard K, Hume R, Kaptein E, Stanley EL, Visser TJ, Coughtrie MW. 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(6):2734–42.
153. Hammarlund-Udenaes M. Microdialysis in CNS PKPD research: unraveling unbound concentrations BT - microdialysis in drug development. In: Müller M, editor. . New York: Springer New York; 2013. p. 83–102.
154. Watson J, Wright S, Lucas A, Clarke KL, Viggers J, Cheetham S, et al. Receptor occupancy and brain free fraction. Drug Metab Dispos. 2009;37(4):753–60.
155. Lin JH. CSF as a surrogate for assessing CNS exposure: an industrial perspective. Curr Drug Metab. 2008;9(1):46–59.
156. Hammarlund-Udenaes M. Active-site concentrations of chemicals – are they a better predictor of effect than plasma/organ/tissue concentrations? Basic Clin Pharmacol Toxicol. 2010;106(3):215–20.
157. Liu X, Smith BJ, Chen C, Callegari E, Becker SL, Chen X, et al. Evaluation of cerebrospinal fluid concentration and plasma free concentration as a surrogate measurement for brain free concentration. Drug Metab Dispos. 2006;34(9):1443–7.
158. Nagaya Y, Katayama K, Kusuhara H, Nozaki Y. Impact of P-glycoprotein–mediated active efflux on drug distribution into lumbar cerebrospinal fluid in nonhuman primates. Drug Metab Dispos. 2020;48(11):1183–90.
159. Yaguchi Y, Tachikawa M, Zhang Z, Terasaki T. Organic anion-transporting polypeptide 1a4 (Oatp1a4/Slco1a4) at the blood–arachnoid barrier is the major pathway of sulforhodamine-101 clearance from cerebrospinal fluid of rats. Mol Pharm. 2019;4:acs.molpharmaceut.9b00005.
160. Syková E, Nicholson C. Diffusion in Brain Extracellular Space. Physiol Rev. 2008;88(4):1277–340.
161. Pizzo ME, Wolak DJ, Kumar NN, Brunette E, Brunnquell CL, Hannocks M-J, et al. Intrathecal antibody distribution in the rat brain: surface diffusion, perivascular transport and osmotic enhancement of delivery. J Physiol. 2018;596(3):445–75.
162. Chang H-Y, Wu S, Meno-Tetang G, Shah DK. A translational platform PBPK model for antibody disposition in the brain. J Pharmacokinet Pharmacodyn. 2019;46(4):319–38.