Experimental Neurology 317 (2019) 10–21

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review Article

Membrane transporters in traumatic brain injury: Pathological, pharmacotherapeutic, and developmental implications

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ARTICLE INFO

Keywords:
ATP-binding cassette transporters
Blood-brain barrier
Multidrug resistance
Neurovascular unit
Solute carriers
Traumatic brain injury

ABSTRACT

Membrane transporters regulate the trafficking of endogenous and exogenous molecules across biological barriers and within the neurovascular unit. In traumatic brain injury (TBI), they moderate the dynamic movement of therapeutic drugs and injury mediators among neurons, endothelial cells and glial cells, thereby becoming important determinants of pathogenesis and effective pharmacotherapy after TBI. There are three ways transporters may impact outcomes in TBI. First, transporters likely play a key role in the clearance of injury mediators. Second, genetic association studies suggest transporters may be important in the transition of TBI from acute brain injury to a chronic neurological disease. Third, transporters dynamically control the brain penetration and efflux of many drugs and their distribution within and elimination from the brain, contributing to pharmacoresistance and possibly in some cases pharmacosensitivity. Understanding the nature of drugs or candidate drugs in development with respect to whether they are a transporter substrate or inhibitor is relevant to understand whether they distribute to their target in sufficient concentrations. Emerging data provide evidence of altered expression and function of transporters in humans after TBI. Genetic variability in expression and/or function of key transporters adds an additional dynamic, as shown in recent clinical studies. In this review, evidence supporting the role of individual membrane transporters in TBI are discussed as well as novel strategies for their modulation as possible therapeutic targets. Since data specifically targeting pediatric TBI are sparse, this review relies mainly on experimental studies using adult animals and clinical studies in adult patients.

1. Novel therapeutic strategies are needed for pediatric traumatic brain injury

Traumatic brain injury (TBI) is a major public health problem in the United States with an annual incidence of approximately 1.7 million, of which 50,000 injuries result in death (Coronado et al., 2015; Faul and Coronado, 2015), and is the leading cause of death and injury in children and older adolescents. TBI also has been associated with increased risk of neurodegenerative diseases such as Alzheimer's disease later in life (Gavett et al., 2010), and this aspect may be particularly impactful when injury occurs in a developing brain. Significant progress has been made in understanding the cellular and molecular processes involved in

Abbreviations: ABC, ATP-Binding Cassette; Aβ, amyloid β; ANLS, astrocyte-neuron lactate shuttle; BCRP, breast cancer resistance protein; CCI, controlled cortical impact; CERP, cholesterol efflux regulatory protein; CNT, concentrative nucleoside transporter; EAAT, excitatory amino acid transporter; ENT, equilibrative nucleoside transporter; FPI, fluid percussion injury; GLAST, glutamate-aspartate transporter; GLT, glutamate transporter; GOS, Glasgow Outcome Scale score; GSH, glutathione; MATE, multidrug and toxin extruder; MCT, monocarboxylate transporter; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; NAC, *N*-acetylcysteine; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OCTN, organic cation transporter novel type; PACAP, cyclase-activating polypeptide; SLC, Solute Carrier; SNP, single nucleotide polymorphism; SUR1, sulfonylurea receptor 1; TBI, traumatic brain Injury; vGLUT, vesicular glutamate transporter

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https://doi.org/10.1016/j.expneurol.2019.02.011
Received 15 December 2018; Received in revised form 12 February 2019; Accepted 20 February 2019
Available online 21 February 2019
0014-4886/

the pathogenesis of TBI. This includes potentially reversible and/or preventable secondary injury mechanisms such as excitotoxicity, oxidative stress, and inflammation that follow the irreversible primary injury phase of TBI which consists of shearing and tearing of the brain tissue (Werner and Engelhard, 2007). Although several pharmacological agents have been studied in clinical trials of TBI, none of them have shown positive results in terms of survival or relevant clinical outcomes (Diaz-Arrastia et al., 2014; Kochanek et al., 2015; Hall, 2016). It is important to note that all clinical trials evaluating outcome after TBI have been done in adult patients and have not included children.

There has been a growing interest in incorporating the neurovascular unit in the understanding of pathological mechanisms of TBI, similar to the framework developed for stroke (Lok et al., 2015). This framework emphasizes the dynamic interaction among the different cells that constitute the neurovascular unit, i.e., neurons, endothelial cells and glial cells. A central component of this paradigm is the communication between the different cells that is mediated by proteins, lipids, hormones, amino acids, metabolites, and neurotransmitters (Lok et al., 2015). Since many of these signaling molecules are polar in physiologic conditions, they require transporters –transmembrane proteins which regulate the trafficking of molecules across the lipid bilayer of cellular membranes – to enter the intracellular environment or cross compartmental barriers such as the blood-brain barrier (BBB) (Lee et al., 2001; Ahn and Nigam, 2009; Wu et al., 2011; Piehler et al., 2012; Nigam, 2015; Stieger and Gao, 2015). Thus, membrane transporters are important contributors to the dynamic interplay within the neurovascular unit to regain homeostasis after TBI. Furthermore, lack of understanding of the nature and extent of the distribution of drugs into the brain and within the neurovascular unit, and even more so the developing brain and developing neurovascular unit, represents an unaddressed area of TBI research that has been linked to the failure of clinical drug trials in TBI (Loane and Faden, 2010). Since transporters regulate the distribution of drugs into and within the brain, they could importantly influence the outcome of therapeutics used in TBI.

Transporters are important targets for devising new therapies, optimizing existing therapies, and helping understand the toxicities of certain drugs. Given the well-documented role of transporters in restricting the distribution of drugs, modulating the relevant transporters could enhance the permeation of drugs to the brain to clinically relevant levels (Lee et al., 2001). Understanding how drugs commonly used in the management of acute brain injuries affect the function of transporters could determine how and to what extent those drugs should be utilized clinically (Nigam, 2015; Nigam et al., 2015a). In a similar way as drug-drug interactions, transporter-mediated drug-metabolite interactions and drug-nutrient interactions could provide new targets for intervention and/or optimize the way we use existing therapies (Nigam, 2015). This review examines membrane transporters as regulators of the movement of injury mediators, signaling molecules, and therapeutic drugs between cells and across biological barriers in the context of TBI. Also, specific issues in children such as transporter ontology and therapeutic strategies in TBI that target membrane transporters will be highlighted. Currently, the amount known about transporters in TBI and in particular pediatric TBI—as will be shown—is limited, and thus this represents a potentially important gap for future investigation.

2. Membrane transporters in the CNS

Membrane transporters are divided into two major super-families: the solute carrier (SLC) transporters which rely on electrochemical (passive or created by secondary-active transporters) or ionic gradients to facilitate translocation of substrates across barriers, and the adenosine triphosphate (ATP)-binding cassette (ABC) transporters which utilize ATP to traffic various substrates across membranes (Moitra and Dean, 2011; Hediger et al., 2013). Because of the critical role transporters play in determining the pharmacokinetics of drug molecules and the implication in the efficacy and safety of drugs, an understanding of drug-transporter interactions is required in the FDA drug approval process and is an area of intense research (Giacomini et al., 2010, Nigam, 2015). However, the role of transporters in mediating physiological and pathological processes related to endogenous substrates has remained relatively understudied. A growing body of evidence is revealing the importance of transporters in regulating the movement of endogenous molecules including metabolites, nutrients, signaling molecules, and biosynthesis and degradation by-products between cells, tissues and body fluid compartments (Loscher and Potschka, 2005b; Loscher and Potschka, 2005a; Moitra and Dean, 2011; Cesar-Razquin et al., 2015; Nigam, 2015; Nigam et al., 2015b). Transporters have been described as forming a “remote signaling and sensing system” to regulate the communication between organs, tissues and cells (Ahn and Nigam, 2009; Wu et al., 2011; Nigam et al., 2015a; Nigam et al., 2015b). This role makes transporters significant players not only in maintaining homeostasis but also in the initiation, progression, and resolution of pathological processes. For example, it is estimated that one in four of the SLC transporters is implicated in Mendelian diseases (Lin et al., 2015). Numerous studies including several genome wide association studies have demonstrated the association of genetic variants in SLC and ABC transporters with complex human diseases including diabetes, neurodegenerative diseases, liver diseases, cardiovascular diseases and cancer (Pedersen, 2007; Zutz et al., 2009; Kubitz et al., 2012; Hediger et al., 2013; Cesar-Razquin et al., 2015; Lin et al., 2015; Qosa et al., 2015; Schaedler et al., 2015). There are also several examples of FDA-approved drugs and drugs in development that target transporters directly or indirectly (Cesar-Razquin et al., 2015). Interactions with transporters have also been described as the mechanism behind the toxicity of some commonly used drugs such as simvastatin. In some cases, transporter-mediated toxicity has led to the discontinuation of clinical development of drugs (Link et al., 2008; Zhang et al., 2014).

Entry into CNS is tightly regulated by highly dynamic barriers, namely, the BBB, the blood-CSF barrier, and the arachnoid epithelium (Abbott et al., 2010; Yasuda et al., 2013). Similar barriers also exist within the CNS including the ependymal barrier as well as the cellular membrane barriers of the neurons and glia (Cavanagh and Warren, 1985; Lee et al., 2001; Whish et al., 2015). In addition to the physical and cellular structures, the prominent feature of all these barriers are numerous efflux and uptake transporters regulating the bidirectional movement of mediators of physiologic and pathologic processes as well as drugs within the CNS, and between the CNS and the periphery. The significance of transporters on these barriers in health, disease, and pharmacotherapy of the CNS is well-established; years of study have been dedicated to understanding and characterizing the expression, distribution, localization, regulation and nature of substrates of transporters in the CNS. These have been reviewed extensively in both health (Lee et al., 2001; Kusuhara and Sugiyama, 2005; Loscher and Potschka, 2005b; Neuwelt et al., 2011; Chan et al., 2013; Geier et al., 2013; Qosa et al., 2015; Stieger and Gao, 2015; Mahringer and Fricker, 2016) and CNS disease including epilepsy, stroke and ischemic injury, brain tumors and multiple sclerosis, as well as chronic neurodegenerative diseases such as Alzheimer's Disease, amyotrophic lateral sclerosis, and Parkinson's Disease (Ronaldson, 2014; Henderson and Piquette-Miller, 2015; Qosa et al., 2015).

3. Membrane transporters in TBI

A summary of membrane transporters investigated for their role in TBI is presented in Table 1 and Table 2. There are three ways transporters may impact outcomes in TBI. First, transporters play a key role in the clearance of endogenous biological mediators following TBI. Second, genetic association studies suggest transporters may be important in the transition of TBI from acute brain injury to a chronic

Table 1
Summary of solute carrier (SLC) family membrane transporters investigated for their role in TBI (studies examining pediatric TBI in bold font).

Transporter	TBI model or type of clinical study	Major findings	Reference
Excitatory amino acid transporters (EAATs)			
SLC1A1 (EAAT3)	Genetic association, time to first seizure and seizure risks in adults after severe TBI	GG genotype at *SLC1A1* rs10974620 was associated with increased posttraumatic seizures risk. TT genotype at *SLC1A1* rs7858819 was associated with increased early and later posttraumatic seizures risk	(Ritter et al., 2016)
SLC1A2 (EAAT2; GLT-1)	Adult rats, CCI	Decreased RNA and protein expression of EAAT2 at 6–72 h after injury	(Rao et al., 1998)
	Adult rats, FPI	Decreased Vmax of EAAT2 in cortex and hippocampus 5 min to 2 h after injury	(Karklin Fontana et al., 2016)
	Adult rats, CCI	Decreased RNA and protein expression of EAAT2 in hippocampus 24–72 h post injury; administration of antisense oligodeoxynucleotides targeting EAAT2 resulted in exacerbated hippocampal neuronal death and increased mortality	(Rao et al., 2001b)
	Adult rats, weight drop injury	Ceftriaxone reduced glutamate levels, attenuated cerebral edema and neuronal death, and improved cognitive function	(Wei et al., 2012, Cui et al., 2014)
	Adult rats, FPI	Ceftriaxone prevented the injury-related down expression of EAAT2 and reduced cumulative post-traumatic seizure duration	(Goodrich et al., 2013)
SLC17A7 (vGLUT1)	Genetic association, concussion severity and duration in athletes	Subjects carrying the minor allele at *SLC17A7* rs7417284 were more likely to experience prolonged recovery	(Madura et al., 2016)
Monocarboxylate transporters (MCTs)			
SLC16A (MCT1, MCT2, MCT4)	**Juvenile (PND 35) and rats, CCI**	**Increased protein expression of MCT2 in the ipsilateral cerebral vasculature**	**(Prins and Giza, 2006)**
	Epidural compression in adult rats	Increased *Mct1* mRNA expression in ipsilateral and contralateral cortex, and ipsilateral hippocampus. Increased *Mct2* mRNA expression in contralateral hippocampus	(Moreira et al., 2009)
Equilibrative nucleoside transporters (ENTs)			
SLC29A1 (ENT1)	Genetic association, post-traumatic epilepsy risk in adults with severe TBI	No association between *SLC29A1* SNPs tested and post-traumatic epilepsy	(Diamond et al., 2015)

Other abbreviations: CCI = controlled cortical impact; FPI = fluid percussion injury; glutamate transporters (GLT); PND = postnatal day; SNP = single nucleotide polymorphism; vesicular glutamate transporters (vGLUT).

neurological disease. Finally, transporters dynamically control the brain penetration of many drugs and their distribution within the brain, contributing to pharmacoresistance and possibly in some cases pharmacosensitivity. Understanding the nature of drugs or candidate drugs in development for TBI with respect to whether they are a transporter substrate is relevant to understanding whether they distribute to therapeutic targets in sufficient concentrations. Evidence supporting the role of particular membrane transporters in each of these processes is presented below.

3.1. Trafficking of injury mediators early after injury

Immediately after TBI, transporters may be involved in helping the brain manage the aftermath of injury, for example, by clearing injury mediators. This is exemplified by glutamate transporters that remove excess glutamate from synapses to prevent excitotoxicity, and by multidrug resistance-associated protein (MRP) transporters that efflux out potentially damaging by-products such as 4-hydroxynonenal after they are conjugated with glutathione (GSH). Additionally, early after injury transporters also play a critical role in directing the movement of substrates that would be needed in higher amounts in neurons in the context of injury. For example, monocarboxylate transporters (MCT) 1, 2 and 4 work in close coordination to direct lactate from the periphery or astrocytes to neurons to be used as an energy source. Each of these transporters and their role in TBI will be discussed below.

3.1.1. Glutamate transporters

Glutamate transporters are responsible for the trafficking of glutamate, the most predominant excitatory neurotransmitter in the mammalian brain, from the extracellular space into cells. Although essential

Table 2
Summary of ATP-binding cassette (ABC) family membrane transporters investigated for their role in TBI (studies examining pediatric TBI in bold font).

Transporter	TBI model or type of clinical study	Major finding	Reference
ABCA1 (CERP)	Adult mice, CCI	Administration of LXR agonist increased expression of Abca1 leading to increased clearance of Aβ	(Loane et al., 2011)
ABCB1 (P-glycoprotein; MDR1)	**Adult rats injured on PND 17, CCI**	**Reduction in Abcb1 in brains from adult rats injured 2 months earlier**	**(Pop et al., 2013)**
	Genetic association, outcome in adults with severe TBI	Patients homozygous for the T allele at *ABCB1* rs1045642 were less likely to be assigned poor outcome vs. those possessing the C allele	(Cousar et al., 2013)
	Protein expression study of brain tissue from adult patients with severe TBI	No change in relative expression of ABCB1 in injured brain tissue vs. control, proteolysis of ABCB1 into peptide fragments	(Willyerd et al., 2016)
ABCC1 (MRP1)	Genetic association, outcome in adults with severe TBI	Patients homozygous for the G allele at *ABCC1* rs4148382 were less likely to be assigned poor outcome vs. those possessing the A allele	(Cousar et al., 2013)
	Protein expression study of brain tissue from adult patients with severe TBI	Relative expression of ABCC1 was increased in injured brain tissue in vs. control	(Willyerd et al., 2016)
ABCC8 (SUR1)	Genetic association, cerebral edema in adults with severe TBI	Multiple *ABCC8* SNPs associated with an increased risk of cerebral edema	(Jha et al., 2017a, Jha et al., 2017b)
ABCG2 (BCRP)	Genetic association, outcome in adults with severe TBI	*ABCG2* c.421C > A was associated with outcome after severe TBI in age-dependent manner	(Adams et al., 2018a)

Other abbreviations: BCRP = breast cancer resistance protein; CCI = controlled cortical impact; CERP = cholesterol efflux regulatory protein; FPI = fluid percussion injury; MDR = multidrug resistance protein; MRP = multidrug resistance-associated protein; PND = postnatal day; SNP = single nucleotide polymorphism; SUR1 = sulfonylurea receptor 1.

to basic brain functions such as cognition, memory, and learning (McEntee and Crook, 1993; Lopez-Bayghen and Ortega, 2011), when present in excessive amounts glutamate can be become neurotoxic due to overstimulation of post-synaptic neurons (Choi, 1987). Because of this, glutamate levels are tightly regulated through close coordination between its release and clearance, which depends on glutamate transporters (Beart and O'Shea, 2007).

In TBI, it has been shown, both in preclinical models and in humans, that there is a massive release of glutamate into the synapses early after injury (Faden et al., 1989; Bullock et al., 1995). This increase in extracellular glutamate causes excessive stimulation of *N*-methyl-D-aspartate glutamate receptor and triggers several cellular processes such as Ca^{++} and Na^{+} overload, activation of phospholipases, endonucleases and proteases, and generation of reactive oxidative species that eventually lead to neuronal injury and death (Rothman and Olney, 1995; Ganel and Rothstein, 1999; Raghupathi, 2004). This glutamate-mediated excitotoxicity is a well-established injury mechanism in TBI that can lead to neurodegeneration and unfavorable neurological outcomes. Indeed, in pediatric TBI elevated levels of glutamate are associated with poor outcome (Ruppel et al., 2001). Owing to their prominence in modulation of extracellular glutamate, glutamate transporters have been a subject of significant investigation in TBI (Fontana, 2015).

In the mammalian brain, glutamate transporters include five sodium-dependent and high-affinity subtypes (Danbolt, 2001): the excitatory amino acid transporter (EAAT) 1 (also called glutamate-aspartate transporter or GLAST), EAAT2 (also called glutamate transporter-1; GLT-1), EAAT3/EAAC1, EAAT4, and EAAT5. EAAT2, expressed throughout the brain and spinal cord is responsible for > 90% of total glutamate uptake which makes it an important glutamate transporter (Maragakis et al., 2004; Maragakis and Rothstein, 2004). Solute carriers that transport glutamate include the vesicular glutamate transporters (vGLUTs) vGLUT1, vGLUT2 and vGLUT3 (SLC17A7, SLC17A6, and SLC17A8, respectively) (Reimer, 2013). These are technically not a type of membrane transporter but are involved in trafficking glutamate intracellularly and are expressed only in neurons (Li et al., 2013).

With a few exceptions, studies examining the role of glutamate transporters in TBI are mainly focused on EAAT2. In a controlled cortical impact (CCI) model of TBI in rats, Rao et al., found that the expression of Glt-1 and Eaat1 was decreased 6–72 h after injury and was accompanied by reduced D-[3H]-aspartate binding activity (Rao et al., 1998). Another study found that after fluid percussion injury (FPI) in rats, Glt-1 activity in the cortex and hippocampus was reduced as early as 5 min and up to 2 h post injury compared with sham (Karklin Fontana et al., 2016). Rao et al., also demonstrated that RNA and protein abundance of Glt-1 was decreased at 24–72 h in the ipsilateral hippocampus after CCI in rats vs. the contralateral hippocampus or sham-injured rats, suggesting that loss of function was related to a reduction in Glt-1 abundance. Additionally, administration of antisense oligodeoxynucleotides specific for *Glt-1* resulted in exacerbated hippocampal neuronal death and increased mortality in the rat CCI model compared with control rats infused with random oligodeoxynucleotides (Rao et al., 2001a). Landeghem et al., reported that both Eaat1 and Glt-1 were down-regulated after CCI in rats with lowest expression 24 to 72 h post injury (van Landeghem et al., 2001). This was accompanied by increased glutamate levels in CSF that reached a maximum value at 48 h post injury. Yi et al., found that Glt1v, but not Glt1a, Eaat1 or Eaac-1 were acutely downregulated following FPI in rats (Yi and Hazell, 2006).

Two studies in human have examined the role of glutamate transporters in TBI using genomic approaches. Ritter et al. found that genetic variations in *SLC1A1*, but not *SLC1A6* were associated with reduced time to first seizure and increased seizure risk in adult TBI patients (Ritter et al., 2016). Specifically, the *SLC1A1* single nucleotide polymorphism (SNP) rs10974620 was associated with increased risk of posttraumatic seizures up to 3 years post-injury, and rs7858819 was associated with increased risk of early and late posttraumatic seizures from day 2 to 3 years post-injury. In the second human study, Madura et al. found that the SNP rs7417284 in the promoter region of the *SLC17A7* gene was associated with severity and duration of sport-related concussion symptoms (Madura et al., 2016). Specifically, those carrying the minor allele were 6-times more likely to experience prolonged recovery rates.

Because of the critical role of glutamate transporters in countering the effect of the excessive release of glutamate, glutamate transporters, especially EAAT2, have been investigated as a therapeutic target. This approach has also gained traction since glutamate receptor antagonists have shown unacceptable toxicity (Ginsberg, 2008; Fontana, 2015). Ceftriaxone, a β-lactam antimicrobial, is a transcriptional inducer of EAAT2 expression and has been investigated preclinically in diverse animal models of glutamate excitotoxicity and clinically in amyotrophic lateral sclerosis and stroke patients (Nederkoorn et al., 2011; Berry et al., 2013; Cudkowicz et al., 2014; Vermeij et al., 2016). Goodrich et al. found that ceftriaxone prevented injury-related down regulation of Glt-1 after FPI observed in placebo-treated rats (Goodrich et al., 2013). Ceftriaxone also mitigated injury-associated astrogliosis and cumulative post-traumatic seizure duration. Two studies reported that ceftriaxone reduced glutamate levels in the brain, attenuated cerebral edema and neuronal death, and improved cognitive function after weight drop injury in adult rats (Wei et al., 2012 and Cui et al., 2014).

Pituitary adenylate cyclase-activating polypeptide (PACAP), an endogenous peptide with neuromodulatory activity, has demonstrated GLT-1 expression enhancing properties. In a weight-drop model of TBI in rats, exogenously administered PACAP significantly improved motor and cognitive function, attenuated neuronal apoptosis, and decreased brain edema (Mao et al., 2012). Additionally, in a rat model of diffuse axonal injury induced by impact acceleration, PACAP was shown to reduce the density of damaged, β-amyloid precursor protein-immunoreactive axons in the corticospinal tract. However, it is worth mentioning that the beneficial effects of PACAP in TBI may be, at least in part, mediated by other mechanisms (Farkas et al., 2004).

There are two molecules with GLT-1 activity modulating capacity that have been studied in TBI, riluzole and MS-153. In a rat FPI model, riluzole was shown to improve motor (McIntosh et al., 1996) and behavioral function (Wahl et al., 1997) vs. vehicle. Similarly, MS-153 was shown to decrease neurodegeneration and attenuate calpain activation in both the cortex and the hippocampus 24 h after FPI in rats (Karklin Fontana et al., 2016). As with PACAP, the beneficial effects of both riluzole and MS-153 are at least in part attributed to mechanisms other than GLT-1 activity, such as anti-inflammatory effects and inhibition of voltage-gated Ca^{2+} channels, respectively. Translation of these therapeutic agents has not yet advanced to clinical TBI studies. However, two recent studies have reported the discovery of potent activators of EAAT2 raising the possibility of targeting EAAT2 effectively in TBI and other neurological disorders (Kong et al., 2014; Kortagere et al., 2018).

3.1.2. MCT transporters

The human *SLC16* gene family of transporters, also known as MCT family based on the encoded protein names, is comprised of 14 members identified based on sequence homology. The first four – MCT1 (*SLC16A1*), MCT2 (*SLC16A7*), MCT3 (*SLC16A8*) and MCT4 (*SLC16A3*) – have been shown to transport monocarboxylates (Halestrap and Price, 1999; Halestrap and Wilson, 2012). Monocarboxylates including pyruvate, lactate, and ketone bodies are important intermediaries in brain energy metabolism and serve as major energy sources under diverse conditions including in the developing brain, during extended starvation, and periods of high neuronal activity such as required for long-term memory formation (Cremer, 1982; Hawkins et al., 1986; Schurr et al., 1999a; Nybo and Secher, 2004; Pierre and Pellerin, 2005). Similarly, monocarboxylates, especially lactate, assume a critical role in acute brain injuries where dysfunctional energy utilization is a

hallmark and is associated with poor prognosis (Pierre and Pellerin, 2005; Vespa et al., 2005; Carre et al., 2013; Mason, 2017). In such situations, lactate could be derived from peripheral tissues (mainly the liver) or from the brain itself, specifically from glycolytic astrocytes via the astrocyte-neuron lactate shuttle (ANLS). The ANLS involves a sequence of events where synaptically released glutamate initiates neuronal activity, followed by an increased rate of glucose uptake and glycolysis by astrocytes and subsequent release of lactate that can be used as an energy substrate by neurons (Suzuki et al., 2011; Tadi et al., 2015; Dienel, 2017; Mason, 2017; Magistretti and Allaman, 2018).

Preclinical and clinical studies have shown that lactate can counteract the consequences of imbalanced excessive energy demand and inadequate supply – a phenomenon known as metabolic decoupling (Schurr et al., 1999b; Glenn et al., 2003; Vespa et al., 2005; Gallagher et al., 2009; Alessandri et al., 2012; Berthet et al., 2012; Carre et al., 2013; Jalloh et al., 2013; Sala et al., 2013; Glenn et al., 2015; Patet et al., 2016; Mason, 2017). The administration of an exogenous lactate solution in TBI has shown some promise (Glenn et al., 2003; Bouzat et al., 2014; Glenn et al., 2015; Bisri et al., 2016). Others, however, have questioned the notion that lactate could be preferentially and beneficially used as an energy source in TBI (Dienel, 2014). There are even suggestions that lactate accumulation during TBI could be detrimental (Lama et al., 2014). Understanding the specific function of MCT transporters in the context of TBI, along with the kinetics and expression patterns of these transporters including during development, may provide insight into the role of lactate after TBI.

Because monocarboxylates exist as polar, anionic species in the body they require transporters to effectively cross biological barriers. This makes MCT transporters key determinants of the trafficking from the tissues/cells where they are produced to where they are consumed. Studies in models of TBI and other brain injuries focusing on changes in expression of MCT isotypes and/or the effect of small molecule inhibitors or genetic manipulation have provided evidence on how the monocarboxylates and MCTs are essential components of the brain's adaptative mechanism to altered energy sources and demands.

Prins and Giza reported increased expression of Mct2 in cerebral blood vessels from the ipsilateral cortex in 35 day old vs. adult rats after CCI in one of the few pediatric-specific studies evaluating transporters and TBI (Prins and Giza, 2006). The developmental issue discovered may be an important and overlooked factor, given that a ketogenic diet appears to favorably alter brain metabolism in juvenile, but not adult, rats after TBI (Giza and Prins, 2006; Deng-Bryant et al., 2011). In a preliminary study, we investigated Mct1 expression, along with 26 other transporters in a rat model of pediatric CCI. *Mct1* mRNA transcript levels increased in both the ipsilateral and contralateral hemispheres 12 h after injury (Adams et al., 2018b). Moreira et al. investigated the expression of *Mct1* and *Mct2* following unilateral extradural compression in rats (Moreira et al., 2009). *Mct1* mRNA expression was increased in the ipsilateral and contralateral cortex and ipsilateral hippocampus while *Mct2* mRNA expression was enhanced in the contralateral hippocampus. They also observed strong Mct1 and Mct2 protein expression in perilesional macrophages/microglia and in an isolectin B4 + /S100β + cell population in the corpus callosum.

3.1.3. ABCG transporters

To date, there are five members of the ABCG subfamily identified in humans: *ABCG1, 2, 4, 5*, and *8* (Kusuhara and Sugiyama, 2007; Woodward et al., 2011; Alexander et al., 2017). *ABCG1,4,5,* and *8* are involved in the transport of cholesterol, plant sterols and various precursors, intermediaries of sterol biosynthesis, as well as some synthetic and semisynthetic sterols (Kusuhara and Sugiyama, 2007; Kerr et al., 2011). (Woodward et al., 2011)

ABCG2, first identified as a MRP in human breast cancer cell lines – hence its other name “breast cancer resistance protein” (BCRP) - is the most-studied member of the family (Doyle et al., 1998). In the CNS it is expressed in the BBB, choroid plexus, pericytes, astrocytes, microglia and neuronal progenitor and stem cells (Hartz and Bauer, 2011). Most of the studies have been focused on the BBB and choroid plexus, where ABCG2 along with ABCB1 (also known as p-glyocoprotein) are known to play a critical role in restricting access of anticancer and other drugs from permeating into the brain.

With regards to ABCG2's endogenous substrates and physiological role, it was through genetic studies that its function as a urate transporter was established. Loss-of-function mutations in *ABCG2* were associated with increased serum uric acid levels and gout (Dehghan et al., 2008). Subsequently, more endogenous and exogenous substrates of ABCG2 has been identified including porphyrins, amyloid β and numerous therapeutic drugs (Robey et al., 2009; Abuznait and Kaddoumi, 2012; Feher et al., 2013). It has also been implicated in a number of pathological conditions including Parkinson's and Alzheimer's diseases (Abuznait and Kaddoumi, 2012; Feher et al., 2013; Matsuo et al., 2015).

There may also be a role for ABCG2 in humans after TBI. We reported that a genetic variant of *ABCG2*, rs2231142, was associated with outcome (Glasgow Outcome Scale (GOS) score) after severe TBI in an age-dependent manner (Adams et al., 2018a). Interestingly, in this adult study the association with outcome was only observed in patients < 34 years-of-age, raising the possibility of age-differences that should be explored in pediatric studies. Patients with this variant allele *ABCG2* c.421C > A have decreased ABCG2 protein expression concomitant with increased blood uric acid levels. Since uric acid is a potent antioxidant, it could be speculated that favorable outcome observed in patients with the variant allele may be related to increased levels of uric acid in the brain. However, other functions of ABCG2 such as clearance of toxic porphyrins (Robey et al., 2009; Sachar et al., 2016; Sachar and Ma, 2016) and its specific role in drug resistance in stem cells where it is relatively abundant cannot be discounted.

3.1.4. ABCC transporters

The ABCC family of transporters consists of 13 members, 9 of which are transporters (ABCC1–6 and ABCC10-12), one is a truncated protein with no transport capacity (ABCC13), one is an ion channel (cystic fibrosis transmembrane regulator gene), and two are cell surface receptors (Nies et al., 2004). In the CNS, ABCC1-5 are the best-characterized members of the family. ABCC7, 8, 10, 11, and 12 are expressed in the CNS but their specific functions remain unknown (Dallas et al., 2006; Hartz and Bauer, 2011). Substrates of ABCC transporters include endogenous and exogenous organic anions including a large number of drugs and their metabolites. Often, ABCC transporters work in conjunction with organic anion transporters (OATs) to mediate vectorial transport of organic anions.

ABCC1, 2, 3 generally show preference to conjugates of lipophilic compounds including glucuronate, sulfate, GSH conjugates, and bile acid conjugates (Nies et al., 2004; Dallas et al., 2006). ABCC4 and 5 substrates include cAMP, cGMP, nucleotide analogs, and glutamate conjugates (Dallas et al., 2006; Jansen et al., 2015). ABCC6 transports small peptides and GSH conjugates. ABCC1, 2 and 4 are also reported to transport reduced GSH (Rappa et al., 1997; Dallas et al., 2006).

Expression and localization of the two structurally related ABCC transproters, ABCC1 and ABCC4, are well-established in the brain (Nies et al., 2004; Hartz and Bauer, 2011). They are reported to be expressed in the BBB, choroid plexus, microglia, neurons, and astrocytes. ABCC2 is enriched in brain capillary endothelial cells and choroid plexus. Limited data also suggest the possible expression of ABCC3 in the BBB and choroid plexus. ABCC5 expression has been shown in the BBB, neurons, and glia (Jansen et al., 2015).

Our group examined changes in ABCC1 protein expression in brain tissue obtained after decompressive craniectomy in adult patients with severe TBI (Willyerd et al., 2016). The study reported increased expression of ABCC1 in blood vessels and cells that appeared morphologically to be neurons as well as glia in brain tissue from TBI patients compared with non-TBI control subjects. In a separate study by our team, Cousar et al. examined the association of *ABCC1* and *ABCC2* SNPs

with neurological outcomes in a larger cohort of adult TBI patients (Cousar et al., 2013). We reported that *ABCC1*, but not *ABCC2*, genotype was associated with neurological outcome after TBI (GOS assigned at 6 months). Studies are also underway to examine the role of ABCC4 in TBI through its role 2′,3′-cAMP efflux from the intracellular space – where it opens mitochondrial permeability transition pores – to the extracellular space where it gets converted to adenosine by CNPase (Jackson et al., 2016).

SUR1, encoded by *ABCC8*, is a nonselective cation channel. Although not strictly a transporter of small molecules as the definition of transporters is often applied, it plays a significant role in the pathogenesis of TBI and other acute brain injuries. Its expression in neurons, astrocytes and/or endothelial cells is upregulated in TBI, stroke, spinal cord injury and subarachnoid hemorrhage. Mechanistic studies in animal models of acute CNS injury and human genetic association studies have confirmed the role of ABCC8 in edema formation (Patel et al., 2010; Martinez-Valverde et al., 2015; Stokum et al., 2016; Jha et al., 2017a; Jha et al., 2017b). In a series of studies published from our group, we have shown that SNPs in *ABCC8* are associated with an increased risk of cerebral edema following TBI in humans (Jha et al., 2017a; Jha et al., 2017b), and that glyburide, an inhibitor of SUR1, decreased cerebral edema at 24 h in a murine model of TBI and hemorrhagic shock. Clinical trials testing glyburide are underway in stroke and TBI (Sheth et al., 2016; Khalili et al., 2017).

Overall, there are only a few studies examining the role of ABCC transporters in TBI, suggesting that their role may be underappreciated. Endogenous substrates of ABCC transporters, including GSH, cyclic nucleotides, and prostaglandins are all thought to play a role in resilience to or pathogenesis of TBI. GSH is an abundant intracellular antioxidant that plays a key role in countering many deleterious mechanisms elicited after TBI. In the brain, ABCC transporters in coordination with EAAT3 and cystine/glutamate exchanger, facilitate the biosynthesis, distribution, compartmentalization and recycling of GSH, GSSH (oxidized form of GSH), and GSH conjugates with toxic reactive molecules such as 4-hydroxynoneal (Renes et al., 2000; Jansen et al., 2015; Rae and Williams, 2017). Furthermore, ABCC1 and ABCC4, along with ABCB1 and ABCG2, are the major determinants of the distribution of many drugs into the brain (International Transporter, C, et al., 2010), further highlighting the relevance of this transporter family in TBI.

3.2. Transition of TBI from an acute to a chronic neurological disorder

Transporters could play a significant role in the transition of TBI from acute brain injury to a chronic neurological disease, an aspect that would be particularly impactful when injury occurs in a developing brain. Two examples will be discussed here: ABCA transporters and the risk of developing TBI-related neurodegenerative disease—currently termed chronic traumatic encephalopathy, and nucleoside transporters and the risk of post-traumatic seizures and epilepsy.

3.2.1. ABCA transporters

The ABCA family of transporters consists of 12 members, ABCA1–10, 12, and 13 (Piehler et al., 2012). All members except ABCA4 and ABCA13 show some degree of expression in the CNS (Hartz and Bauer, 2011). ABCA transporters mediate the trafficking of cholesterol, phospholipid, apolipoproteins and several other physiologic lipid compounds (Kaminski et al., 2006). Mutations in four members of the family are known to cause monogenic diseases in humans: *ABCA1* (Tangier disease), *ABCA3* (neonatal surfactant deficiency), *ABCA4* (autosomal recessive macular dystrophies) and *ABCA12* (hereditary keratinization disorders) (Kaminski et al., 2006). All of these disorders are related to dysregulation of lipid homeostasis. Additionally, ABCA transporters are involved in complex diseases where lipids are part of the central mechanism including Alzheimer's disease (Piehler et al., 2012).

Thus far, one study has evaluated the role of the ABCA family of transporters in TBI. Using a mouse model of TBI, Loane et al., showed that the accumulation of amyloid beta (Aβ) was attenuated by administration of a liver X receptor (LXR) agonist that induced the expression of Abca1 (also known as cholesterol efflux regulatory protein; CERP) and enhanced the clearance of Aβ (Loane et al., 2011). This was accompanied by improved functional recovery.

Furthermore, the substrates of the ABCA family transporters, specifically, apolipoprotein E (ApoE) and cholesterol byproducts, are implicated in the pathogenesis of TBI and a transition from acute injury to chronic neurodegenerative disease. Evidence from genetic-association studies, animal models, and in isolated neuronal and astrocyte systems have shown that ABCA1 plays a critical role in facilitating cholesterol and phospholipid loading onto apolipoproteins (Piehler et al., 2012). In the CNS, ABCA1 mediates the lipidation of ApoE (the most abundant lipoprotein in the CNS), trafficking of cholesterol to and from neurons, astrocytes and endothelial cells, and Aβ deposition. Separately, the *APOE ε4* variant which is a strong genetic risk factor for Alzheimer's disease, dramatically increases the risk of later developing chronic traumatic encephalopathy (Mayeux et al., 1995). It is also associated with increased mortality and worse functional outcomes in severe, moderate and repetitive TBI through a mechanism that is thought to involve dysregulation of lipid homeostasis (Ariza et al., 2006; Stern et al., 2013; Cao et al., 2017). Given this strong, mechanistically definable, interaction between members of the ABCA family, *APOE*, and Alzheimer's disease on one hand, and the association of *APOE*, TBI, and chronic traumatic encephalopathy, on the other hand, close examination of ABCA family transporters as a possible link between TBI and chronic traumatic encephalopathy appears warranted.

3.2.2. Nucleoside transporters

There are two families of transporters that are responsible for trafficking nucleosides, nucleoside analogs and nucleoside-like drugs, the concentrative nucleoside transporters (CNTs) of the SLC28 family and the equilibrative nucleoside transporters (ENTs) of the SLC29 family (Young et al., 2013). By mediating the uptake of nucleosides, these transporters play a key role in salvage pathways of nucleotide biosynthesis. Critically, these transporters determine the intracellular and extracellular levels of purine and pyrimidine nucleosides, cyclic nucleosides, adenosine and other endogenous and exogenous nucleosides. Several studies have examined the changes in levels and effects of different types of nucleosides after TBI (Jackson et al., 2016). Adenosine in particular has been extensively studied in TBI owing to its anticonvulsant effects (Kochanek et al., 2006). Diamond et al. studied the association of a variant of the gene *SLC19A1*, as part of the adenosine signaling regulatory cycle, with epileptogenesis or post-traumatic epilepsy (Diamond et al., 2015). This was based on the role of SCL29A1 in maintaining intracellular and extracellular levels of adenosine. The study reported a significant association of epileptogenesis or post-traumatic epilepsy with other genes of the adenosine signaling regulatory cycle, but not *SLC29A1*. Given the increasing interest in understanding the role of nucleosides in TBI (Jackson et al., 2016), further evaluation of the SLC28 and SLC29 families of transporters and brain distribution of nucleosides appears warranted.

3.3. Distribution of drugs into brain, pharmacoresistance, and pharmacosensitivity

Transporters dynamically control brain uptake, distribution, and efflux of many drugs. Understanding the nature of drugs or candidate drugs in development with respect to whether they are substrates or inhibitors of transporters becomes highly relevant as a drug that does not reach its target (in this case injured brain) in sufficient quantities or is rapidly eliminated cannot be effective. The most thoroughly studied drug transporter is ABCB1, which will be discussed below along with the concept of pharmacoresistance/pharmacosensitivity due to ABC transporters. Some SLC transporters are also involved in facilitating or

limiting permeation of drugs into the brain and will also be discussed briefly. Obviously, transporters in tissues outside the brain such as the gut and kidney significantly impact systemic bioavailability of drug substrates and consequently brain bioavailability, with elimination and uptake impacting pharmacoresistance and pharmacosensitivity, respectively (Rives et al., 2017; Momper and Nigam, 2018). The important role of non-CNS membrane transporters in TBI is beyond the scope of this review.

3.3.1. ABCB transporters

This subfamily of transporters has 11 members, ABCB1–11 (Alexander et al., 2017). ABCB1 (also known as P-glycoprotein, P-GP, or multidrug resistance protein 1) is the most studied transporter in the CNS or otherwise, as it is involved in efflux of numerous drugs and other xenobiotic molecules from cells and compartments. It plays a paramount role in altering the pharmacokinetic properties and permeation to the CNS of numerous drugs (Schinkel, 1999; Hartz and Bauer, 2011). Also, Aβ is a major endogenous substrate of ABCB1, and decreased clearance of Aβ due to downregulation of ABCB1 has been implicated in Alzheimer's disease (Urakami et al., 1994; Cascorbi et al., 2013). More recently, it has been shown to mediate the transport of several other endogenous molecules including opioid peptides, although the implication in health and disease remains to be established (Oude Elferink and Zadina, 2001). Other members of the ABCB family include ABCB11 (bile salt export pump), the mitochondrial transporters (ABCB6, ABCB7, ABCB8 and ABCB10), ABCB4 and ABCB2. To date, only mRNA expression of *ABCB2-11* has been detected in various brain structures and the discussion here will be limited to ABCB1.

In our single-center study in adult TBI patients, we found that *ABCB1* genotype was associated with neurological outcome (GOS assigned at 6 months) (Cousar et al., 2013). The specific mechanism underlying this association remains unknown; however, one could speculate that it may be related to the transport of drug or endogenous substrates. We also reported that although the relative abundance of ABCB1 was not altered in brain tissue obtained after decompressive craniectomy in patients with severe TBI, proteolysis of ABCB1 into peptide fragments was observed, perhaps impacting transporter function (Willyerd et al., 2016). ABCB1 was primarily seen in cerebral blood vessels.

ABCB1 is well-known for its effects on brain permeation of therapeutic drugs, however, this aspect is not yet fully explored after TBI in adults or children. However, in a model of focal ischemic brain injury, Spudich et al. demonstrated that inhibiting ABCB1 increases brain accumulation of two potentially neuroprotective drugs, FK506 (tacrolimus) and rifampicin (Spudich et al., 2006). This study, in addition to numerous studies conducted in various CNS diseases showing a profound effect of ABCB1 on drug distribution into the brain, suggest that consideration of whether drugs administrated in the management of TBI are substrates of ABCB1 is warranted.

3.3.2. ABC transporters and pharmacoresistance

Resistance to pharmacotherapy mediated by transporters, especially efflux transporters, is a well-established phenomenon. It is an obstacle in the treatment of brain cancer and HIV infection and has been gaining recognition in other brain disorders such as epilepsy, depression, schizophrenia, and amyotrophic lateral sclerosis (Loscher and Potschka, 2005a; Qosa et al., 2015). For example, in amyotrophic lateral sclerosis, Trotti and colleagues have shown that there is an increase in both expression and activity of ABCB1 and ABCG2 in the BBB which results in progressive resistance to the CNS penetration of rizuloze, the only drug currently approved for treatment (Jablonski et al., 2015). They also demonstrated that administrating elacridar, an inhibitor of ABCB1 and ABCG2, improved efficacy of riluzole in patients with amyotrophic lateral sclerosis (Jablonski et al., 2014). A study by Spudich et al. showed that inhibition of ABCB1 resulted in greater accumulation of the potentially neuroprotective drugs FK506 and rifampicin after focal cerebral ischemia, raising the possibility of phamacoresitance in acute brain injuries (Spudich et al., 2006). Similarly, Ibbotson et al. demonstrated that in a rat model of hypoxia-reoxygenation stress, there is an increased expression of Abcc1, Abcc2 and Abcc4 transporters in the BBB that is mediated by Nrf2 signaling (Ibbotson et al., 2017). Interestingly, Nrf2 is activated in TBI in response to oxidative stress (Yan et al., 2008).

3.3.3. Multidrug SLC transporters

There are several families of SLC transporters with individual members expressed on the BBB and/or blood-CSF barrier that play an active role in mediating the uptake and efflux of drugs. These include organic anion transporting polypeptides (OATPs encoded by *SLCO* genes), organic cation transporters (OCTs encoded by *SLC22A* genes), organic cation transporter novel type (OCTN encoded by *SLC22A* genes), CNTs (encoded by *SLC28A* genes), ENTs (encoded by *SLC29A* genes), multidrug and toxin extruder (MATEs encoded by *SLC47A* genes), and peptide transporters (PEPTs encoded *SLC15A* genes) (Kusuhara and Sugiyama, 2005; Roth et al., 2012; Stieger and Gao, 2015). It is therefore important to determine if a particular drug candidate's access to the brain is dependent on or restricted by transporters. It is also important to understand the impact of TBI on the activity and expression of these transporters. For example, in a hypoxia/reoxygenation stress model in rat brain microvessels, Thompson et al. showed increased expression of Oatp1a4 (Thompson et al., 2014). Uptake of atorvastatin, a statin and Oatp1a4 substrate and putative neuroprotectant, was also increased. Thus, one explanation for the mixed results observed with statins in acute brain injuries could be related to limited brain distribution, raising the possibility that modulating OATP1A4 could facilitate greater brain penetration.

Drug-drug interactions mediated by these transporters are also possible and could contribute to pharmacotherapy failure or disease progression. Many drugs commonly administrated to TBI patients including antibiotics such as cephalosporins and diuretics such as furosemide are known to be substrates and inhibitors, respectively, of these transporters (Shimizu et al., 2005; Roth et al., 2012; Stieger and Gao, 2015).

4. Specific considerations in pediatrics: the ontogeny of membrane transporters in the brain

A unified pattern of transporter expression with age does not exist. Brouwer and colleagues reviewed transporter ontogeny in highly metabolic organs including the intestines, liver, and kidneys. They found that among the most prominently expressed transporters, expression patterns and ontogeny vary across organs and time with no consistent pattern (Brouwer et al., 2015). Readers are directed to the review by Strazielle and colleagues for a broader discussion of brain transporter ontogeny (Strazielle and Ghersi-Egea, 2015).

Daood and colleagues studied the protein expression and localization of prominent ABC transporters (ABCB1, ABCG2, and ABCC1) in human embryos at 22 weeks gestational age, through newborns and adults. They found expression of ABCG2 and ABCB1 in capillary endothelial cells of the BBB, and ABCC1 in the choroid plexus and Purkinje cells of the cerebellum. They also reported that ABCB1 expression increases throughout gestation, and that ABCG2 and ABCC1 expression was stable postnatally (Daood et al., 2008). Multiple rat studies have shown *Abcb1* mRNA and protein expression in brain tissue and microvessels as early as postnatal day (PND) 2, with increasing expression with age (Harati et al., 2013; Soares et al., 2016; Adams et al., 2018b). Harati and colleagues found that *Abcg2* mRNA and protein increase early in rat development with stabilization by PND 21 (Harati et al., 2013). ABCC1 expression was also not found at any age on human cerebral microvessel endothelial cells (Daood et al., 2008), but *Abcc1* mRNA is expressed at consistent levels throughout the brain and brain microvessel endothelium in rats at all ages (Soares et al.,

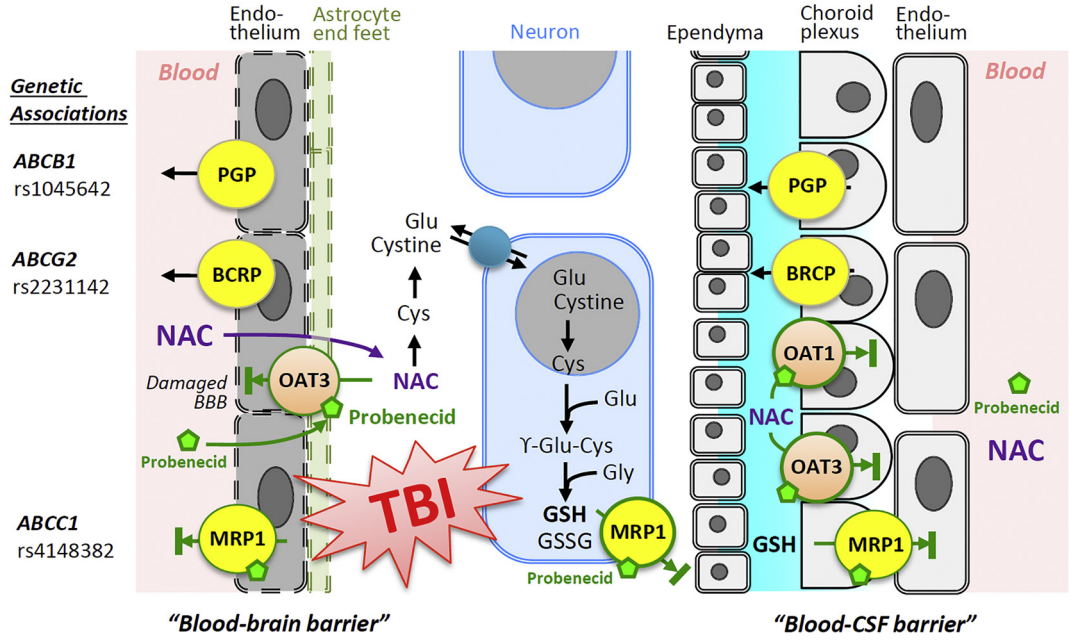

Fig. 1. Simplified schematic highlighting select membrane transporters with suspected roles in modulating brain accumulation and efflux of endogenous and drug substrates in TBI. Multidrug resistance associated proteins (MRP1; ABCC1), multidrug resistance protein (PGP; ABCB1), the breast cancer resistance protein (BCRP; ABCG2), and organic acid transporters (OATs; solute carriers) on the BBB and blood-CSF barrier, plasma and mitochondrial membranes of the various cells of the neurovascular unit function to pump drugs and endogenous substrates out of the brain. Inhibitors such as probenecid (green) act at MRP1 and OATs (and possibly others), reducing the efflux of organic acid and peptides including glutathione (GSH). Synergistic effects may be achieved by delivering cysteine (Cys) donors, such as *N*-acetylcysteine (NAC), and probenecid, to enhance both endogenous stores of GSH and provide substrate generation of GSH from cysteine (Cys), glutamate (Glu) and glycine. Genetic associations with TBI outcomes have been reported for *ABCB1*, *ABCC1* and *ABCG2*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2016; Adams et al., 2018b), and ABCC1 appears to be upregulated on brain endothelium after TBI in humans (Willyerd et al., 2016). Studies in rats have shown particular complex patterns of expression in SLC transporters that are specific to tissue (e.g. hippocampus, cortex) and the relevant barrier (e.g. blood-CSF vs. BBB) (Harati et al., 2013; Strazielle and Ghersi-Egea, 2015; Soares et al., 2016; Adams et al., 2018b). Much remains to be learned related to developmental aspects of transporter maturation and the impact of TBI on transporter expression and function. We are exploring this using a pediatric TBI model, CCI in PND 17 rats. We found a common pattern of decreased transporter mRNA expression in the first 72 h following injury, with some transporters showing a delayed increase in expression following a spike in expression of inflammatory cytokines and transcription factors including *Il-6* and *Nfe2l2* (Adams et al., 2018b). Interestingly, a reduction in Abcb1 is observed in brains from adult rats that underwent CCI two months earlier at PND 17 (Pop et al., 2013), a time of development similar to a two-year old human in terms of brain vulnerability and synaptogenesis (Harris et al., 1992; Rice and Barone Jr., 2000). A better understanding of age-dependent differences in baseline transporter expression and function, and age-dependent differences in the response to TBI, appear necessary to better estimate changes in brain exposure to xenobiotics and endogenous substrates important in the pathophysiology and treatment of pediatric TBI.

5. Membrane transporters as a novel therapeutic target in pediatric TBI

The concept of targeting membrane transporters began with identifying a neuroprotective drug and a corresponding transporter inhibitor. Somewhat serendipitously, we discovered that *N*-acetylcysteine (NAC), a cysteine donor for GSH synthesis and antioxidant, was a transporter substrate for the probenecid-inhibitable transporters SLC22A6 (OAT1) and SLC22A8 (OAT3) (Hagos et al., 2017). Using juvenile (PND 17) Sprague-Dawley rats, we showed that administration of NAC in the presence of probenecid increased the plasma and brain area under the concentration curve by 1.65- and 2.41-fold, respectively, vs. NAC administered alone. Additionally, using HEK-293 cells that overexpress SLC22A6 and SLC22A8 transporters, we showed that NAC exhibits time- and concentration-dependent uptake vs. mock-transfected cells, inhibitable by probenecid. These data provided insight regarding how NAC bidirectionally crosses biological barriers and identified a novel therapeutic strategy to increase NAC exposure (and possibly other drugs) in the brain by inhibiting membrane transporters (Fig. 1). Since this study examined membrane transporter function in the developing brain, similar studies in the mature adult brain are warranted.

The combination strategy of co-administering the transporter inhibitor, probenecid, and its recently discovered substrate, NAC, had several theoretical advantages. First, by inhibiting OATs, probenecid could increase the exposure of NAC in plasma and importantly in the brain. Second, NAC, chemically a thiol, can act as an antioxidant by reacting with and scavenging a number of radicals including ·OH, ·NO2, CO3·−, and thiyl radicals (Samuni et al., 2013). Third, by inhibiting ABCC transporters such as ABCC1 which are involved in the efflux of GSH and its conjugates from the brain, probenecid could increase the overall GSH pool available for neurons and other brain cells. Fourth, both NAC and probenecid are FDA approved drugs with favorable safety profiles, hence providing an opportunity for rapid translation to the clinic. Fifth, the combination therapy targets multiple processes vis-à-vis TBI and oxidative stress.

We verified brain exposure of systemically administered NAC and probenecid in a double-blind, placebo controlled Phase I study in children with severe TBI (Pro-NAC Trial) (Clark et al., 2017). In the Pro-NAC trial, fourteen patients (n = 7/group) received probenecid (25 mg/kg load, then 10 mg/kg/dose q6h × 11 doses) and NAC (140 mg/kg load, then 70 mg/kg/dose q4h × 17 doses), or placebos via naso/orogastric tube. Serum and CSF samples were collected for 96 h after injury. Treatment resulted in detectable CSF concentrations of

NAC and probenecid throughout the treatment period and was not associated with undesirable effects after TBI. This provided important proof-of-principle for transporter modulation as a novel therapeutic strategy for pediatric TBI.

In a follow-up study, our group utilized metabolomics, a powerful tool for comprehensively profiling metabolites and altered biochemical processes, to evaluate the CSF metabolome in children with severe TBI treated with probenecid and NAC (Hagos et al., 2018). The goal was to determine whether the combination modulated glutathione metabolism and related pathways after TBI (the purported mechanism of action) to evaluate pharmacodynamic target engagement. The CSF metabolome was analyzed at 24 h post-injury in seven Pro-NAC and five placebo treated patients as well as five control subjects. A combination of metabolomics and pathway/network analyses showed that seven glutathione-centered pathways and two glutathione-centered networks were enriched in the CSF of Pro-NAC treated vs. placebo treated TBI patients. Several pathways/networks consisting of components that are known substrates of probenecid-inhibitable membrane transporters such as prostaglandins, kynurenate and urate were also enriched, providing additional mechanistic validation. This first of its kind "neuropharmacometabolomics" assessment revealed alterations in known and previously unidentified metabolic derangements after TBI, and supported target engagement of the combination of probenecid and NAC in the treatment of severe TBI in children. The study also underscored the value of metabolomics as a tool to understand the range of substrates and functions of transporters, and in identifying markers of efficacy and toxicity for drugs that target transporters in TBI.

6. Conclusion

With regards to future pediatric TBI research, detailed investigation of the ontogeny of membrane transporters on cellular and compartmental barriers in the brain is needed. Without this fundamental understanding, isolating the effect of TBI from developmental changes would be challengingly complex. More studies of transporters and their substrates in pediatric TBI are needed to fully understand the magnitude and extent of their involvement in pathogenesis and recovery after TBI, as well as to further develop therapies that target transports and their substrates. Moreover, transporter mediated interactions with drugs commonly used in children suffering TBI need to be investigated in contemporary pediatric TBI models to avoid under or over exposure to drugs which could compromise the desired therapeutic effects.

Membrane transporters remain relatively understudied in human disease, especially TBI (Cesar-Razquin et al., 2015), and considerably less is known in terms of expression, function and substrates of membrane transporters in pediatric patients. However, their importance in maintaining brain homeostasis and modulating the brain milieu of numerous injury mediators and therapeutic drugs is emerging. Provocative genetic studies, exciting—albeit early, clinical studies including pharmacometabolomic evidence of drug transporter inhibitor target engagement, and recent studies in experimental TBI support further investigation to best define the scope of the effects of transporter manipulation and therapeutic potential.

Funding

This work was supported by NIH grant, 1TL1 TR001858-01 (FTH); R01NS069247 (RSBC, PEE, SMP, PMK), NCATS KL2 TR000146 (PEE), and U01 NS081041 (PMK); and the UPMC Children's Hospital of Pittsburgh Scientific Program and Brain Care Institute.

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