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Transporters as Drug Targets in Neurological Diseases

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Abstract

Membrane transport proteins have central physiological function in maintaining cerebral homeostasis. These transporters are expressed in almost all cerebral cells where they regulate the movement of wide range of solutes including endogenous substrates, xenobiotic and therapeutic drugs. Altered activity/expression of CNS transporters has been implicated in the onset and progression of multiple neurological diseases. Neurological diseases are heterogeneous diseases that involve complex pathological alterations with only a few treatment options; therefore there is a great need for the development of novel therapeutic treatments. To that end, transporters have emerged recently to be promising therapeutic targets to halt or slow the course of neurological diseases. The objective of this review is to discuss implications of transporters in neurological diseases and summarize available evidence for targeting transporters as decent therapeutic approach in the treatment of neurological diseases.

Over the last couple of decades, neurological disorders have become an increasingly urgent area of unmet medical need because of limited or absent availability of successful therapeutic interventions and the progressively increasing number of afflicted individuals. Moreover, our still-limited knowledge concerning the function of the central nervous system (CNS; the brain and spinal cord) makes the identification of potential therapeutic strategies to fight neurological diseases, and subsequent creation of means to exploit them, a great challenge. However, extensive research has improved our knowledge about the pathogenesis of neurological diseases, and paved the road for some successful therapeutic interventions. Despite these advances, there remains an urgent need for the identification of CNS targets that could be modulated to effectively interrupt disease pathogenesis or to improve the efficacy of CNS-active drugs. To this end, transporters have been suggested to be promising therapeutic targets, for several key reasons. Firstly, transporters are expressed in a diversity of cellular components of the CNS, wherein they play essential roles in maintaining normal brain homeostasis, or in defense (protective) functions. Secondly, altered expression or situational dysfunction of transporters can induce an array of neuropathological alterations, as has been implicated in a variety of neurological diseases.¹ Finally, transporters have been

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CONFLICT OF INTEREST

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found to affect the brain disposition (entry or clearance) of many CNS-active drugs. In consideration of the clear importance of CNS-associated transporters, the present review will briefly outline their identified or suspected roles in the pathogenesis of neurological diseases, as well as the potential of these transporters as therapeutic targets.

TRANSPORTERS OF THE CNS

Transporters are membrane proteins that are expressed in all cells, tissues, and organs of the human body. About 7% of the total number of human genes encode for transporters.² In the brain, transporters are expressed in almost all cellular components, including (but not only in) neurons, astrocytes, and endothelial cells of brain capillaries.¹ However, because the exchange of solutes between the brain interstitial fluid (ISF) and periphery is regulated mainly by the blood-brain barrier (BBB), transporters expressed by brain capillary endothelial cells (BCECs) of the BBB contribute the greatest effects on drug disposition into and out of the brain. Direct involvement of BCEC transporters has also been implicated in the pathology of various neurological diseases.

The BBB resides within the neurovascular unit (NVU) of the brain (Figure 1), and consists functionally as a complex set of interactions between a network of multiple component cells/types, mainly endothelial cells, pericytes, astrocytes and neurons, that collectively maintain and stringently coordinate CNS functions.³ Within the NVU, endothelial cells of the BBB are connected by strong tight junctions that greatly restrict paracellular permeability of the BBB, and exhibit distinct luminal and abluminal membranes and asymmetrical distributions of proteins, including transporters (Figure 1).³ Transporters expressed by endothelial cells form a very important barrier against the free exchange of solutes between CNS tissues and blood, and hence contribute very significantly to the overall barrier characteristic of the BBB.³ Besides their roles in maintaining barrier functions of the BBB, it has been convincingly proven that transporters contribute to the development, physiological activities, and maintenance of other component cells of the CNS, including neurons and astrocytes.⁴

Multiple members of the two major transporter superfamilies, ABC (ATP binding cassette) and SLC (solute carrier), have been identified in the CNS.^{3, 4} Within the ABC superfamily, drug efflux transporters, including ABCB1 (P-gp), ABCG2 (BCRP), ABCCs (MRPs) and ABCAs, are expressed in endothelial cells, astrocytes, and neurons.¹ At present, the most clinically relevant known roles of ABC membrane transporters is their direct contributions to pumping their substrates to the luminal side of the BBB (i.e., back into the bloodstream), thereby bringing about the multidrug resistance (MDR) phenomenon.¹ This phenomenon has been known and widely recognized for many years as a major impediment in cancer treatment, but has also been observed in association with a variety of neurological diseases. SLC proteins, on the other hand, are responsible for the transmembrane transport of a wide variety of endogenous substrates, such as inorganic ions, amino acids, neurotransmitters and sugars, that are essential for normal physiological functioning.⁴ SLC proteins known to be expressed in cells of the CNS include members of SLC2A (glucose transporters, GLUTs), SLC1A (high-affinity glutamate transporters, EAATs), SLC7A (large neutral amino acid, LAT), SLC16A (monocarboxylic acid transporters, MCTs), SLCO (organic anion polypeptide transporters, OATPs), SLC22A (organic anion and cation transporters, OCTs

and OATs), and SLC15 (peptide transporters).³ Moreover, SLCs have been reported to interact with large number of drugs, and are implicated in the pathogenesis of an array of neurological diseases.

In addition to ABC and SLC transporter superfamilies, various receptor-mediated transport systems (RMTs) have been identified in the CNS. For instance, low-density lipoprotein receptor-1 (LRP1) and receptor for advanced glycation end-products (RAGE) are expressed in the endothelial cells of the BBB as well as in other component brain cells/cell types. RMTs mediate transport of certain neuroactive peptides, regulatory proteins, hormones, and growth factors, and of neuronal by-products such as amyloid- β .³ Table 1 provides a short list of the major transporters in the CNS along with their function, example drugs and endogenous substrates and affected diseases.

Transport proteins are recognized as a key determinant of brain disposition of CNS-active drugs, and become increasingly important in CNS drug development because they provide insight into the mechanisms of treatment failure, adverse drug reactions, and individual differences in the management and overall clinical outcome of CNS therapeutics. Once in brain ISF, a drug is available to interact with a cell-surface target, which is usually a protein (the “target protein”), and in sufficient concentrations, able to exert its therapeutic effects. On the other hand, most unwanted effects of the drug (side-effects or toxicities) occur through interaction with other proteins (““off-target” proteins”). Therefore, finely tuning ISF levels of a CNS-active drug is crucially important to obtain the desired therapeutic efficacy while averting complications. Transporters, mainly at BBB, significantly affect the ISF levels of many (perhaps most) established or prospective CNS drugs. Efflux transporters at the BBB limit penetration of CNS drugs into the brain ISF and may further impact disposition within the brain parenchyma, whereas influx transports at the BBB will act to potentially enhance the accumulation of drugs in the ISF.

To recapitulate: transporters of the CNS represent multiple families, contribute extensively to normal CNS functioning; control access to, distribution within, and clearance from the brain parenchyma of endogenous and exogenous substances; and are likely involved in (neuro)pathological conditions of the CNS.

IMPLICATION OF CNS TRANSPORTERS IN NEUROLOGICAL DISEASE

Alzheimer's disease (AD)

AD is a progressive neurodegenerative disorder (more accurately, as we now understand, an array of closely related disorders). All varieties of AD in aggregate constitute the most common form of age-related dementia, accounting for about 60% of all dementias. AD begins with memory loss and progresses to include severe cognitive impairment.⁵ The neuropathology of AD is characterized by two pathogenic hallmarks: neurofibrillary tangles (NFTs) and amyloid plaque.⁵

The involvement of ABC transporters in AD pathology was first introduced by Lam et al., who reported that amyloid- β ($A\beta$) peptide, a major hallmark found in the brains of AD patients, is a substrate of ABCB1 (P-glycoprotein; P-gp).⁶ Since then, roles for ABCB1, and

for other ABC transporters, such as ABCC1, ABCG2, and ABCA1, in the pathology of AD have been proved (Figure 2). In-vitro and in-vivo preclinical studies further confirmed an Abcb1-associated contribution to the clearance of A β across the BBB, and down-regulation of this transporter's mRNA and protein expression was shown to increase brain A β levels and accumulation over time in mice and rats.^{7, 8} Consistent with its role in the clearance of A β , an inverse relationship between A β and ABCB1 levels has been observed in postmortem brain tissues of AD patients.⁹ Moreover, findings from positron emission tomography (PET) studies to assess ABCB1 function in patients with AD compared to control healthy subjects revealed the transport function of ABCB1 to be compromised, again suggesting that decreased ABCB1 protein expression could be linked to A β accumulation and thus, to the pathogenesis of AD.¹⁰

Besides ABCB1, it has been suggested that ABCG2 may act together with ABCB1 to protect the brain from peripheral A β influx, as suggested by Tia and colleagues using a human in-vitro BBB model.¹¹ Furthermore, in addition to its role in A β clearance, ABCG2 is reported to transport glutathione (GSH), a major antioxidant in the CNS, and regulate oxidative stress where the genetic deletion of *Abcg2* in a mouse model of AD augmented oxidative stress and cognitive deficits when compared to *Abcg2*-expressing animals.¹²

ABCC1 is localized at the luminal and abluminal sides of the BBB endothelium, and is also expressed by glial cells. Recent evidence suggests that this transporter is also involved in A β transport and accumulation in mice.¹³ Deficiency of *Abcc1* in an aggressive mouse model of AD stimulated the accumulation of A β in brain parenchyma and along the perivascular drainage channels involved in A β clearance.¹³ In addition, *Abcc1* was found to mediate A β -induced GSH release from astrocytes in culture.¹⁴ It appears that at early stages of AD pathogenesis, increased levels of monomeric A β induce GSH release from astrocytes through up-regulation of *Abcc1*, so as to provide temporary protection from oxidative stress in 5xFAD, an AD mouse model.¹⁴

ABCA1 is also implicated in AD; however, it does not directly transport A β , but instead mediates A β clearance by degradation. ABCA1 is highly expressed in astrocytes and microglia, where it mediates lipidation of apolipoprotein E (ApoE), an important lipoprotein whose genotype largely influences the risk for AD.¹⁵ ApoE is a chaperone for A β , and much evidence indicates that the presence (especially when homozygous) and expression of the gene coding for the ApoE4 isoform has a profound effect on A β deposition and conformation.¹⁵ Mice lacking *Abca1* were found to have low levels of lipidated ApoE in the CNS.^{15, 16} PDAPP transgenic mice (an AD model) with *Abca1*^{-/-} had significantly higher levels of vascular and hippocampal A β compared to PDAPP *Abca1*^{+/+} mice.¹⁶ With respect to different ApoE isoforms (i.e., ApoE2, ApoE3, and ApoE4, homozygously and heterozygously present and expressed), such observations have been replicated in many AD mouse models, further lending support for the hypothesis that ABCA1 plays a role in mediating A β brain deposition in AD.¹⁵

Recently, multiple genome-wide association studies (GWAS) have identified *ABCA7* variants as a genetic susceptibility factor for AD. The loss-of-function variants in *ABCA7* were seen to be associated with a significantly increased AD risk.¹⁷ On the other hand,

carrying *ABCA7*rs3764650T is protective against AD, likely by increasing this protein's expression level in the brain.¹⁸ *ABCA7* is expressed in microglia and neurons. Although the role of *ABCA7* in the brain is not well understood, reduced expression/activity of *ABCA7* is predicted to contribute to AD progression. Mechanistic studies to investigate the effects of *Abca7* deletion in AD pathology demonstrated that *Abca7* deletion facilitated the processing of A β precursor protein (APP), and A β production, by inducing β -secretase 1 levels in primary neurons and in mice brains, without affecting the A β clearance rate.¹⁹ Further studies are necessary to better understand the functional aspects for *ABCA7* in the brain, both to explain this protein's contribution to disease pathology, and with respect to its potential as a therapeutic target.

The glucose transporter subtype 1 (GLUT1), expressed at the capillary endothelium of the BBB and neurons (Figure 2), is also implicated in AD. It has been reported that glucose uptake from blood to brain is reduced in AD subjects due to a reduction in GLUT1 protein expression and activity at the BBB.²⁰ It is not clear, however, whether decreased GLUT1 expression and glucose uptake is due to decreased glucose demand by dysfunctional brain or caused after a critical level of A β accumulation inside the brain.

In addition, certain receptor-mediated transporter systems (RMTs) have been implicated in the pathogenesis of AD. For instance, LRP1 is expressed on the abluminal side of endothelial cells of the BBB, and is involved in transport not only of free A β , but also of A β bound to chaperone molecules such as ApoE, across the BBB, via transcytosis.⁵ Brain levels of LRP1 decrease in elderly and AD patients, and this decline becomes more pronounced as AD progresses, suggesting that reduction in LRP1 function might contribute to A β deposition and cognitive decline.²⁰ On the other hand, increased protein expression of RAGE in a transgenic mouse model of AD in which the mice exhibit a high brain A β load accelerates and accentuates pathologic, biochemical, and behavioral abnormalities as compared with mice overexpressing only mutant APP.²¹ RAGE opposes the clearance of A β and favors its entrance from the peripheral circulation and accumulation inside the brain. In addition, interaction of A β with RAGE expressed at neurons and astrocytes was seen to induce a cascade of inflammatory events that lead to cell death. Thus, overexpression of RAGE may increase AD pathology through several cellular molecular mechanisms.²¹ Figure 2 demonstrates transporters implicated in AD and their contribution to the disease pathology.

Amyotrophic Lateral Sclerosis (ALS)

ALS, also called Lou Gehrig's disease, is a slowly progressive, but ultimately fatal neurodegenerative disorder characterized by gradual degeneration and death of motor neurons of the CNS.²² ALS is another neurodegenerative disorder that shows changes in the expression of transporters suggestive of direct contributions to its pathology. ALS is characterized by decreased expression levels of the glial glutamate transporter SLC1A2 (EAAT2), which contributes to the increase in glutamate ISF levels, and consequent excitotoxicity.²² Excitotoxicity is caused by excessive stimulation of glutamate receptors, which is also implicated in a number of other neurodegenerative diseases (e.g., Huntington's disease, epilepsy, and the sequelae of ischemic insults, for example from a stroke). Normally at synapses, excitotoxicity is prevented by rapid binding and clearance of synaptic released

glutamate by high-affinity, Na⁺-dependent glutamate transporters. An elevation of synaptic glutamate concentration can be caused by defects to the glutamate transporter and receptor systems,²³ and the consequent excessive stimulation of the glutamate receptors in turn increases intracellular concentrations of Ca⁺⁺ ions, which can ultimately lead to neuronal death. Loss of the glial glutamate transporters Eaata1 or Eaata2 in mice is linked with elevated extracellular glutamate levels, which can lead to excitotoxicity-mediated neurodegeneration and progressive paralysis.²⁴ Accordingly, changes in glutamate transporters expressed by astrocytes appear to play an important role in the development of ALS.

Another alteration of transporters expression observed in ALS is the up-regulation of efflux ABC transporters. A very recent study showed significant disease-driven overexpression of Abcb1 and Abcg2 at the mRNA and protein levels in the brain capillaries of SOD1-G93A mouse model of ALS, and in postmortem human brain samples.²⁵ Also, spinal cord tissue samples from ALS patients exhibited higher ABCB1 and ABCG2 proteins expression specifically at the capillary endothelium of the blood spinal cord barrier (BSCB), when compared to healthy individuals.²⁵ In addition, immunostaining of Abcb1 in the lumbar spinal cord of SOD1-G93A mice revealed higher protein expression of Abcb1 in astrocytes of symptomatic compared to pre-symptomatic mice.²⁵

Lewy Body Dementia and Parkinson's Disease (PD)

PD is the second-most-common neurodegenerative disease, and is characterized by progressive loss of dopaminergic neurons in the brain's nigrostriatal pathway. Alpha-synuclein (α -Syn), a small protein with multiple physiological and pathological functions, is one of the dominant proteins found in Lewy Bodies (LB), a pathological hallmark of Lewy body disorders, including PD.²⁶ Formation of LB in the brain, and pathological alterations associated with their accumulation, has been linked to brain accumulation of neurotoxins such as pesticides.²⁶ Implication of transporters in the pathogenesis of PD has been emerging, with clinical reports identifying several genetic variants of the *MDR1* gene that are associated with lowered expression of ABCB1, correlated with increased risk of PD.²⁷ Moreover, positron emission tomography of PD patients' brains, as well as biochemical analysis of ABCB1 expression in brain endothelium of postmortem PD patients, revealed significant reductions in ABCB1 activity as determined by the significantly elevated uptake of ¹¹C-verapamil, which is normally extruded from the brain by ABCB1, in the midbrain of PD patients relative to controls.²⁸ Very limited evidence is available with regard to any possible contribution of other ABC transporters to the pathology of PD; however, very recently Matsuo et al. reported a dysfunctional *ABCG2* variant to be associated with lower risk of PD.²⁹ ABCG2 dysfunction in the BBB plays an important direct role with respect to regulating uric acid levels in the CNS. Diminished activity of this transporter at the BBB in conjunction with concomitant increases in serum uric acid levels caused by ABCG2 dysfunction in the gut and kidneys results in increased brain levels of uric acid. Therefore, authors of this report proposed that the reduced PD risk might be attributable to uric acid's antioxidant activity playing a protective role against onset and development of PD.

Efflux transporters have for many years been known to alter the efficacies of various PD therapeutic agents, but some more-recent studies have also pointed at disturbances in uptake

transporter expression or function as impacting responses to anti-Parkinsonian drugs. Treatment of wild type C57BL/6 mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) for 7 days to induce PD exhibited significantly reduced expression of mRNA encoding the Slc7a5 transporter (L-type amino acid transporter 1, Lat1) in the brain capillaries.³⁰ While the implication of LAT1 in the pathogenesis of PD is unknown, this reduction in LAT1 expression could affect the disposition of levodopa, an effective treatment for PD and a substrate of LAT1 carrier.³⁰ Similarly, based on a population-based study it was reported that the presence of the rs622342 minor C variant allele in the *SLC22A1* gene, encoding OCT1, was associated (in patients with either AC or CC genotype, to differing degrees) with higher prescribed doses and shorter survival time after start of drugs known to be OCT1 substrates, such as amantadine and selegiline.³¹ This variant allele is associated with decreased transport of these anti-Parkinsonian drugs to the brain.³¹

Epilepsy

Epilepsy is an array of neurological disorders characterized in common by recurrent seizures. A number of antiepileptic drugs (AEDs) are in clinical use, with many new additions in recent years, yet about 40% of the patients still fail to respond adequately to AED treatment, due in numerous instances to the development of pharmacoresistance.³² A possible cause for this resistance is the overexpression of efflux drug transporters at the BBB, as numerous in-vivo and in-vitro studies have shown many AEDs (e.g. phenytoin, phenobarbital, carbamazepine, lamotrigine, gabapentine, felbamate, topiramate, levetiracetam; Table 1) to be substrates for ABC efflux transporters.^{32, 33} ABCB1 recognizes a wide range of AEDs, and is thought to play a major role in their extrusion from the brain and development of pharmacoresistance. The first evidence of a role for ABCB1 in AED pharmacoresistance was provided by Tishler et al., who detected high protein expression of ABCB1 in the capillary endothelial cells isolated from the brain tissue of refractory epileptic patients.³⁴ Since then, overexpression of ABCB1 and other ABC efflux transporters, such as ABCG2 and ABCCs, have been confirmed by in-vitro and in-vivo preclinical, and clinical studies.³⁵ ABCB1 overexpression in the brain of epileptic patients may be a consequence of seizure flares, induction brought about by certain anticonvulsants such as carbamazepine, or genetic variation.³⁶ Various regulatory mechanisms that underlie the overexpression of ABC efflux transporters in epilepsy have been studied, and recent reports suggest a high degree of mechanistic complexity and involving multiple elements of pathological alterations observed in epilepsy, such as inflammatory responses, oxidative stress, responses mediated by ligand-activated nuclear receptors, and glutamate-driven processes.³⁶ ABCG2 and ABCCs proteins expressions also increased in epileptic patients' brains, but in contrast to ABCB1, both transporters seem to have a low affinity for AEDs.³⁷

Early evidence obtained from epileptic patients undergoing cortical resection, demonstrated abnormal expression of the glucose transporter SLC2A1 (GLUT1). Immunogold imaging of BBB GLUT1 performed on tissue resected from patients with seizures provided evidence that GLUT1 is downregulated in the endothelial cells in regions within and around the seizure focus.³⁸ Additionally, in a previous study the distribution of the SLC transporter monocarboxylate transporter 1 (MCT1, SLC16A1) in brain tissues extracted from epileptic patients was assessed. A marked reduction was observed in cerebrovascular MCT1, which is

an important transporter for essential metabolic fuels, including lactate, pyruvate and ketone bodies (and possibly acidic drugs such as valproic acid) across the BBB.³⁹

Multiple Sclerosis (MS)

MS is a chronic inflammatory disease that affects the myelin sheath of nerve axons, halting the ability of different CNS regions to properly communicate.⁴⁰ A number of efflux transporters have been studied at the BBB of MS brain. While ABCB1 has been observed to decrease at the BBB of MS patients, ABCG2 and ABCCs levels did not change.⁴¹ Loss of vascular ABCB1 function during MS neuroinflammation may disturb brain homeostasis and thereby aggravate disease progression via exposure of vulnerable CNS cells to detrimental compounds. Using MS animal model, a previous study identified a crucial role for activated CD4⁺ T-cells in endothelial Abcb1 down-regulation via intracellular adhesion molecule-1 and NF- κ B signaling.⁴¹ In addition to vascular changes in efflux transporters, Kooji et al showed that in MS patients both active and inactive MS lesions were associated with an increase in astrocytic ABCB1, ABCC1 and ABCC2 but not ABCG2 proteins expression. The authors suggested that, toll like receptor-3 (TLR-3) activation of astrocytes by TNF- α led to increased ABCB1 and ABCC activities.⁴⁰ Available studies also support role of BBB efflux transporters in affecting patients' response to MS drugs.⁴² Genetic variants in ABC-transporter genes (such as *ABCB1* and *ABCG2*) have been suggested to serve as pharmacogenetic markers to predict clinical response to mitoxantrone (a substrate for ABCB1 and ABCG2; Table 1) therapy in MS where specific polymorphisms were linked with reduced function increased the clinical response rate to mitoxantrone in MS patients.⁴²

Stroke

Stroke, the second leading cause of death worldwide, occurs when blood flow to specific brain region is stopped because of a blocked blood vessel or bleeding in the brain. Stroke has complex pathophysiological alterations that triggers a cascade of biochemical and molecular events leading to dysfunctional BBB.⁴³ Several studies reported that ABC efflux transporters at the BBB to exhibit profound expressional changes in the brain capillaries of the ischaemic brain that have functional implications. Shortly after induction of stroke in rat, Abcb1 expression and activity were elevated and persisted up to 24 h independent of the severity of the ischaemic event, and newly generated capillaries were observed 14 days post-stroke.⁴⁴ Moreover, Lazarowski et al. observed an increase in the expression of Abcb1 in astrocytes and neurons of rat brain after induction of focal experimental hypoxia by local injection of cobalt chloride. The authors of this study suggested that the observed increase in Abcb1 protein expression is related to oxygen deprivation.⁴⁵ Similar to Abcb1, Dazert et al. showed an increase in Abcg2 and Oatp2 (Slc21a5) mRNA and protein levels 3 to 14 days post-ischemic injury in rats.⁴⁶

Stroke increases brain energy demand due to changes in brain energy metabolism.⁴⁷ Therefore, glucose transport activity at the BBB may adapt to ensure the delivery of glucose to affected brain region. It has been observed that glucose transporters (Glut1 and 3) increased significantly in mice brains microvascular endothelial cells after short exposure to ischemic injury.⁴⁷ Similarly, the expression of Mct1 increased in rat brain endothelial cells 120 h after exposure to ischemic injury.⁴⁸

TARGETING TRANSPORTERS IN THE TREATMENT OF NEUROLOGICAL DISEASES

Along with advances in molecular understanding of the role of transporters in neurological diseases, targeting these transporters to modify the disease course or to enhance efficacy of CNS-active drugs have started to emerge. Moreover, there have been fruitful recent instances for the exploitation of drug transporters as therapeutic targets in many disease areas. For example, the gliflozin class of anti-diabetic drugs (canagliflozin, dapagliflozin, and empagliflozin) was developed to inhibit the renal reabsorption and increase clearance of glucose via targeting the sodium-glucose co-transporter 2 (SGLT2) for the treatment of type 2 diabetes.⁴⁹ This in addition to other successful examples provides feasibility of targeting intestinal, hepatic and renal transport proteins for therapeutic purposes. However, until now targeting CNS transporters as therapeutic strategy remains under-developed. In this section, we will review available studies that utilized CNS transporters to develop a therapeutic approach for the treatment of neurological diseases or to improve the efficacy of CNS-active drugs in clinical use.

AD and PD are heterogeneous diseases that lack effective treatments. As discussed above, available evidence supports down-regulation of transporters that are necessary to efflux endogenous neurotoxins (e.g. A β ; Figure 2) and to limit brain access to toxic neurotoxins (e.g. A β and pesticides), which could implicate the etiology by contributing to dysfunctional BBB observed in both diseases and other neurological diseases. Thus, targeting these transporters to upregulate their function to enhance the clearance and limit access of neurotoxins is considered innovative and necessitates more attention and efforts toward the development of effective and preventive therapeutic strategies.

Consistent with this premise, findings from our laboratory support targeting clearance pathways of A β efflux across the BBB to reduce A β brain burden.^{8, 50–52} Wild-type and AD mice models treatment with Abcb1 and Lrp1 inducers, including rifampicin,⁵⁰ acetylcholine esterase inhibitors,^{8, 51} and oleocanthal⁵² were able to enhance A β brain clearance across the BBB and reduce its brain deposition. Interestingly, this increase in A β clearance from mice brain was also associated with increased hepatic clearance of A β caused by up-regulation of Abcb1 and Lrp1 proteins localized at the liver canaliculi, which reduced plasma-A β by creating peripheral sink necessary to maintain A β brain homeostasis.^{8, 53} ABCB1 up-regulation could be achieved by activation of one or more of the nuclear receptors that regulate ABCB1 expression including Pregnane X Receptor (PXR), Retinoid X Receptor (RXR), and Liver X Receptor (LXR).¹ For example, activation of RXR with pregnenolone-16 α -carbonitrile restored Abcb1 protein expression and function in brain capillaries and significantly reduced A β brain level in Tg2576 mice, a model of AD.⁵⁴ Moreover, a new approach with potential to increase ABCB1 expression has recently been proposed to enhance Abcb1 expression by decreasing its proteasomal degradation by ubiquitination, a process that could be activated by A β .⁵⁵

In addition to ABCB1 and LRP1, the receptor-mediated transport protein RAGE could be a possible therapeutic target at the BBB and/or within brain of AD patients. The infusion of soluble RAGE (s-RAGE) decreased A β brain accumulation and improved learning/memory

and synaptic function, in a murine transgenic model of AD.⁵⁶ s-RAGE interfered with A β -RAGE interaction by decreasing the transport of peripheral A β to the brain.⁵⁶ Azeliragon (PF-04494700), a small molecule that antagonizes RAGE, was successful in reducing brain A β load in transgenic mice, and improved mice performance in behavioral assays.⁵⁷ In phase I and II clinical trials, azeliragon was safe and well tolerated in patients with mild-to-moderate AD indicating the feasibility of a larger long-term efficacy trial.⁵⁷ A phase III clinical trial was launched in April 2015 for azeliragon at three sites at the United States and Toronto, which sat to run till 2018.

Therapeutic targeting of ABCA1 in AD has also been reported. Treatment of APP transgenic mice with LXR and RXR agonists ameliorated AD phenotype in animal models of the disease. Four months treatment of Tg2576 mice with LXR agonist, GW3965, induced the expression of both ABCA1 and ApoE and reduced A β deposition by more than 50% and improved contextual memory.⁵⁸ Furthermore, activation of RXR by the anti-cancer drug bexarotene induced the expression of ABCA1 and promoted cholesterol efflux and consequent A β clearance by the ApoE clearance pathway.⁵⁹ Induction of ABCA1 can also be achieved by activation of peroxisome proliferator-activated receptor γ (PPAR γ). The PPAR γ agonists rosiglitazone and pioglitazone, FDA approved drugs for diabetes, were tested as potential therapeutics for AD. Multiple clinical trials were accomplished to determine rosiglitazone and pioglitazone safety, tolerability, and efficacy in AD patients, yet none of these studies recommended the use of either drug to treat AD.⁶⁰ An extension phase III trial testing the safety and efficacy of pioglitazone is currently running with the objective to slow cognitive decline in patients with mild cognitive impairment (ClinicalTrials.gov Identifier: NCT02284906).

In the contrary to AD and PD, in epilepsy, ALS and stroke the activity of ABC transporters are upregulated and create pharmacoresistance. Therapeutic targeting of pharmacoresistance in epilepsy has been extensively evaluated and reviewed.³³ Indeed, overall findings supported the idea that the overexpression of ABC transporters, especially ABCB1, in lowering brain drug concentrations, and reduce AEDs (such as phenytoin, phenobarbital, and carbamazepine) effects in drug-resistant epilepsy. The addition of verapamil (ABCB1 inhibitor) to the antiepileptic drug regimen of a patient with refractory epilepsy greatly improved the overall seizure control and the patient's quality of life.⁶¹ The emerging evidence that ABCB1 is overexpressed in epileptogenic brain tissue, particularly in BCECs and astrocytes contributing to BBB permeability, and the increasing evidence that various major AEDs are substrates for ABCB1, support that the overexpression of ABCB1 may contribute to epilepsy and that ABCB1, and possibly other ABC transporters, could be a viable therapeutic target.

With regard to ALS, riluzole, the only FDA approved drug for management of ALS improves survival by a few months in some patients; however, progressive pharmacoresistance has been reported. Given the fact that riluzole is an ABCB1 and ABCG2 substrate, this disease-driven increase in spinal cord capillary ABCB1 expression could attribute, at least partly, to reduction of brain penetration of riluzole and to its reduced therapeutic benefits. Several combinatorial therapeutic targeting have been suggested to overcome ALS-driven pharmacoresistance mediated by the overexpression of ABC

transporters at the BBB. Trotti and colleagues demonstrated the involvement of ABCB1 and ABCG2 in reducing brain penetration of riluzole in ALS.⁶² Treatment of SOD1 mice, a model of ALS, with elacridar, a known ABCB1 and ABCG2 inhibitor, significantly increased riluzole brain penetration. In addition, when the authors treated Abcb1 knockout mice with riluzole, higher level of the drug was delivered to the brains of these mice compared to Abcb1 expressing mice.⁶² These studies support the notion that overexpression of ABC transporters at the BBB contribute to ALS acquired pharmacoresistance and that their inhibition could provide therapeutic benefits.

Despite of the intense research, current therapeutics options for stroke are very limited, therefore it is especially important to explore transporters as potential targets against stroke. Spudich et al. examined the effect of ABCB1 inhibition on the brain levels of neuroprotective drugs.⁶³ Tacrolimus and rifampicin, known ABCB1 substrates with neuroprotective effects, were delivered to ischemic mice with and without the ABCB1 inhibitor tariquidar. In this set of experiments, the authors showed the co-administration of tariquidar along with tacrolimus or rifampicin significantly increased their brain concentrations in the ischemic brain, and exceeded those in the non-ischemic brain, with a marked improvement in neuroprotection efficacy.⁶³ These findings support the premise that following ischemia ABCB1 overexpression keeps drug levels low, even in the presence of a disrupted BBB, and suggest ABCB1 inhibition as a promising approach in stroke neuroprotection. Another attractive example to improve stroke therapy is by targeting LRP1 and LRP6. Several studies have linked the increased activity of these receptor-mediated transport proteins with increased risk of stroke and decreased response to recombinant tissue-type plasminogen activator (t-PA), the only FDA approved drug for stroke treatment. Treatment of rat autologous thromboembolic stroke model with receptor-associated protein (RAP; Lrp1 inhibitor) was beneficial where it was able to decrease vascular damage, enhance brain t-PA levels, and significantly improved neurorecovery.⁶⁴

In addition to targeting RMT and ABC transporters, promising therapeutic approaches have been developed against SLC transporters. For instance, restoring glial glutamate transporters expressed in astrocytes has been tested for ALS treatment.⁶⁵ Many beta-lactam antibiotics are potent inducers of Eaata2 expression. Administration of ceftriaxone showed to increase Eaata2 protein expression in astrocytes and prolonged survival in mutant SOD1 mouse model of ALS.⁶⁵ This positive outcome encouraged testing ceftriaxone in a multi-phase randomized trial in ALS patients, which was successful in phase I and II, however, phase III trial was negative due to severe side effects associated with the treatment.⁶⁶

CONCLUSION AND FUTURE DIRECTIONS

Roles for transporters with respect to the efficacy of CNS therapeutics have been known for several decades at least; however, mounting evidence supports direct roles of brain transporters in the onset and progression of an array of neurological diseases. As pertains to the latter, even for the few diseases for which the evidence is most extensive, we remain far from having a comprehensive understanding of the exact roles of these transporters in the pathology, or how best to exploit them for the invention of efficacious therapeutic agents. We can, however, confidently assert that transporters associated with the neurovascular unit

are essential for maintenance of healthy brain function, and that their sufficiency (expression and functioning) can be altered by multiple risk factors, such as aging, health status, environment, and genetics. Targeting brain transporters by modulating expression and/or activity could be a valid approach to prevent, halt or slow the progression of, or at least mitigate the impacts of certain neurological diseases. However, targeting transporters is clearly a challenging strategy, due in part to the important and complex physiological functions these transporters serve. In general, transporters can have two contradictory effects on the development and progression of neurological diseases: On the one hand, efflux transporters protect the CNS by inhibiting brain tissue penetrance or promoting efflux of toxicants, but they also constitute an obstacle to brain bioavailability of CNS therapeutics. On the other hand influx transporters enhance transport of nutrients and some therapeutic agents to the CNS. Overall, the many reports evaluating implication of transporter proteins in neurological diseases confirmed their effect in the pathology and treatment of these diseases, and identified numerous candidate transporters that deserve further investigation and replication. As genetic and environmental factors influence transporters expression/ functionality, which in turn could increase disease susceptibility and drug response, it is expected that their restoration, at least to their base level, would rectify their function and possibly correct the disease condition and improve response. Figure 3 summarize most studied transport proteins in the neurological diseases described in this review and their potential use as therapeutic targets by modulation. However, further evaluations are necessary to validate and confirm their modulatory effect on diseases treatment and drugs response.

Targeting transporters for the treatment of neurological diseases have several potential challenges that should be considered while developing drugs that target transporters as a therapeutic approach. One of the most significant challenges is the development of ideal modulators that selectively target altered transporters in the CNS, which is very difficult to achieve without affecting their peripheral activity. Besides, most transporters (e.g. ABCB1 and ABCG2) have common substrates and inhibitors, which makes drugs development to specifically target one transporter challenging.¹ To add more complexity to this challenge, some transporters have multiple isoforms while only one isoform could be involved in the disease, which makes specific isoform targeting difficult; for example, MRPs are highly homologous to specifically target one isoform. Therefore, to date, most of drugs that have been developed to interfere with transporters activity demonstrated several side effects mainly due to the lack of specificity.¹ Another type of toxicity associated with transporters modulation originated from the fact that these transporters perform very important physiological functions that could be interrupted. For example, targeting ABC transporters at the BBB by inhibitors is expected to disrupt the BBB barrier function allowing for more toxicants to get access to the brain and induce neurotoxicity. In addition to loss of normal physiological function, targeting specific transporters could stimulate the activity of other transporters to compensate for activity loss of the of the targeted protein as in the case of ABCB1 and BCG2 where many studies showed both transporters to team up at the BBB and cooperate in preventing dual substrates from entering the brain.⁶⁷ In addition to this transporter/transporter interaction, several transporters/metabolizing enzymes interplay have been identified.⁶⁸ One common example of this interplay is ABCB1/CYP3A4 interplay

which suggests that modulating ABCB1 transporter activity could affect drug metabolism by CYP3A4.⁶⁸ Another important issue in targeting transporters that requires further investigation is how this approach could affect the neuropharmacokinetics of drug substrates. Indeed, the CNS complexity makes modeling drugs concentrations in the brain a challenging task. The flux of a substrate (rate of transport) across a biological membrane by transporter-mediated processes is characterized by saturability, therefore, the simplest way to describe relationship between the flux and substrate concentration in a transporter mediated process is by using the Michaelis–Menten kinetics. However, as most of the drugs enter CNS by passive diffusion, it is important to develop mixed models that account for passive diffusion and transporters in CNS drug disposition. One more layer of complexity resides in the fact that in many cases drug transport to CNS involves multiple transporters with different transport directions that complicates Michaelis–Menten kinetics application to assess the role of specific transporter(s) in determining the ultimate CNS drug level. Therefore, more sophisticated mechanistic models should be developed to overcome this issue.⁶⁹

While various preclinical models have been used to study transporters activity, these models may not be predictive for clinical outcomes for multiple key reasons. For example, substrate specificity may differ between human and other species transporters, or some human transporters do not have direct orthologs in other mammalian species.⁷⁰ In addition, the marked heterogeneity in transporters expressions and localization (reviewed in reference 71)⁷¹ makes extrapolation of preclinical results to clinical settings a challenging task and suggests the need for more studies to characterize transporters localization pattern.⁷⁰ Accordingly, several techniques have been emerged to study the activity of transporters in humans such as brain microdialysis or CSF sampling. However, the complexity of these techniques and invasive nature restricted their use for human subjects. Alternative to these invasive techniques, several non-invasive external imaging techniques including PET, single photon emission computed tomography (SPECT), nuclear magnetic resonance (NMR) and (functional) magnetic resonance imaging ((f)MRI) have been utilized to study the activity of transporters at the CNS.⁷⁰ Finally, development of advanced mathematical modeling presents a promising approach to predict and understand transporters activities in neurological diseases. Physiologically-based pharmacokinetic (PBPK) modeling approach has provided the basis for interspecies extrapolation that could enable the prediction of transporters activity in neurological diseases from in vivo data or data obtained from healthy subjects.⁷² Given the multifactorial and complex processes affecting the disposition of drugs in the brain, the use of mechanistic PBPK models can be a useful approach to more accurately predict human brain ISF concentrations, target site concentration, and receptor occupancy. Such models can also improve our understanding of complexities associated with intra-brain pharmacokinetic characteristics and relationships, brain distribution, and the roles and relative contributions of drug transporters associated with the brain. This approach can be taken a step further by connecting pharmacodynamic data during the course of the disease to the model, thereby enabling studying the effect of changes in transporter activity, up-regulation or down-regulation, on the disease state.⁷²

Various modulators have been investigated and developed to modify ABC transporters expression/activity; most, if not all, of these modulators, however, lack specificity and could

alter several other transporters and physiological processes as discussed above. In clinical trials evaluated inhibitors of ABC transporters, for example, exhibited side effects due to required high concentrations that possibly acted off-target and proved unsuccessful in improving pharmacotherapy. More specific modulators that selectively target specific transporter are, thus, necessary. While genetic manipulation of transporters cannot be performed in humans due to the existing international ethical standards, the possibility of developing siRNA or CRISPR technologies to modulate transporters in animals should be explored and optimized to understand their exact role in the pathology of neurological diseases.⁷³ Furthermore, targeting signaling cascades that control the expression of efflux transporters could be an attractive approach to decrease the activity of these transporters and improve penetration of therapeutic agent. At the same time, it has been speculated that using this approach would impart less toxicity and more specificity for disease-driven up-regulation of efflux transporters in epilepsy and ALS treatment. One example for this approach is the study by Bauer et al in which, the inhibition of activated NMDA receptor mediated Abcb1 overexpression in brain capillaries was able to attenuate seizure-induced increases in Abcb1 expression in epilepticus rat model.⁷⁴

Regulatory guidance currently proposes a general decision criterion to evaluate if a drug is a substrate or an inhibitor of drug transporters, primarily for the purpose of assessing the potential for problematic drug-drug interactions and associated severities thereof. While such guidance-driven evaluations may acceptably assess whether or not a drug is a substrate or inhibitor, they do not in general provide reasonable quantitative estimations of the relative contributions to total disposition of the affected drug (i.e., the target of the interaction) in the tissue – in this case, brain.⁷⁵ Indeed, additional studies are necessary to confirm and utilize transporters as therapeutic targets, which is achievable especially with the availability of current and emerging methods for characterizing disease biomarkers characterization (i.e. proteomics, metabolomics, genomics, and transcriptomics), coupled with significant advances in the computational and imaging tools. Such advances might enable transporters to serve as molecular biomarkers for diagnosis and assessing disease risk, and for the invention and deployment of transporter-targeted therapeutics for effective treatment and prevention.

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Figure 1.

Localization of transporters at the neurovascular unit. **(A)** Schematic presentation of neurovascular unit components along with the localization of major transporters in neurons and astrocytes. Neurovascular unit is a modular structure that interconnects neurons (N) and its associated astrocytes (A) and microglia (M) with the cells forming the brain capillaries, the endothelial cells (E) and pericytes (P). **(B)** The structure of tight junction and its protein elements between two endothelial cells. Endothelial cells of the brain capillaries have several features that distinguish them from those found in the rest of the body. These features include reduced density of caveolae, increased density of mitochondria, and expression of tight and adherence junctions' proteins, such as occludin, claudins, junction adhesion molecules (JAMs), Zonulae occludin (ZO), VE-cadherin and catenin, that "glue" together the cerebral endothelial cells. **(C)** Putative localization of the major transporters on brain capillary endothelial. Due to the tightness of the endothelial barrier, paracellular transport is negligible and solutes exchanges can takes place by passive transcellular diffusion, specific transport systems or receptor-mediated transport systems. Wide range of transporters and receptors such as ABCB1, ABCG2, ABCC1, GLUTs, LATs (LAT1 and LAT2), MCTs (MCT1, MCT2, MCT8), OATPs (OATP1A4, OATP2B1, OATP1C1), OCTs (OCT1, OCT2, OCT3) and OAT3, LRP1 and RAGE have been characterized in the endothelium of the brain capillaries. Arrows indicate direction of substrate transport.

Figure 2.

Transport proteins in Alzheimer's disease. Down-regulation of ABCB1, ABCG2 and LRP1 reduce Aβ clearance across the BBB and cause Aβ accumulation in brain parenchyma. Reduced activity of ABCA1 in astrocytes and neurons decreases Aβ clearance via the ApoE clearance pathway, while ABCA7 deficiency accelerates Aβ production, which trigger formation of Aβ oligomers and amyloid plaques. Reduced glutamate uptake by astrocytes through glutamate transporters (EAAT) increases glutamate concentration in synaptic cleft. Increased expression of RAGE increases Aβ entering the brain. Glucose transporters (GLUT) are also reduced which reduce glucose cellular uptake.

Figure 3.

Transporters as potential therapeutic targets to halt or slow the course of neurological diseases.

Short list of the major transporters in the CNS along with their function, example drugs and endogenous substrates and affected neurological diseases.

Table 1

Transporter	Function	Drug substrates	Endogenous substrate	Affected diseases
ABCB1	Efflux	Amityptiline, berberine, chlorpromazine, carbamazepine, digoxin, doxepin, doxorubicin, fexofenadine, felbamate, fluphenazine, gabapentin, imipramine, irinotecan, lamotrigine, levitracetam, loperamide, mitoxantrone, morphine, nortryptiline, paclitaxel, paroxetine, phenytoin, phenobarbital, pimozone, reserpine, riluzole, seliciclib, triflupromazine, topiramate, venlafaxine, vinblastine	A β , aldosterone, phospholipids, sphingolipids	AD, ALS, PD, epilepsy, MS, stroke
ABCG2	Efflux	Anthracyclines, atorvastatin, daunorubicin, doxorubicin, fluvastatin, imatinib, irinotecan, methotrexate, mitoxantrone, pantoprazole, prazosin, riluzole, rosvastatin, topotecan, SN-38	A β , bile acid, estrones	AD, ALS, PD, epilepsy, MS, stroke
ABCC1	Efflux	Daunorubicin, doxorubicin, etoposide, melphalan, methotrexate, mitoxantrone, temiposide, vincristine	A β , glutathione	AD, epilepsy, MS
ABCA1	Efflux	α -tocopherol, cholesterol, interleukin-1 β	Cholesterol, phospholipids	AD
ABCA7	Efflux		Cholesterol, phospholipids	AD
LRP1	RMT		A β , A β -ApoE, lipoproteins, α -Synuclein	AD, PD, stroke
RAGE	RMT	Amphoterin, calprotectin, phosphatidylserine	A β	AD
EAAT2	Uptake	Ceftriaxone, diazepam	Glutamate	AD, ALS
CAT-1	Uptake	Levodopa	L-arginine, lysine	PD
GLUT1	Uptake	Glucosamine	Glucose, galactose, mannose	AD, epilepsy, stroke
LAT1	Uptake	Gabapentin, levodopa, , melphalan	tryptophan, methionine, thyroxine	PD
OATPs (OATP1A4, OATP2B1, OATP1C1)	Uptake	Atenolol, arasetan, cefiprolol, erythromycin, fexofenadine, imatinib, levofloxacin, lopinavir, methotrexate, ouabain, pitavastatin, rocuronium, rosvastatin, saquinavir, sotalol, talinolol, tebipenem		Stroke
OCTs (OCT1, OCT2, OCT3)	Uptake	Acyclovir, amantadine, amiloride, atropine, citalopram, desipramine, diphenylhydramine, memantine, metformin, oxaliplatin, ganciclovir, phenoxylbenzamine, pindolol, prazosin, procainamide, proplanolol, ranitidine, selegiline		PD
MCTs (MCT1, MCT2, MCT8)	Uptake	Bumetanide, γ -hydroxybutyrate, nateglinide, prostaglandin F $_{2\alpha}$, phenethicillin, valproic acid	Ketone bodies, lactate, pyruvate	Epilepsy, stroke