Targeting Regulation of ABC Transporters at the Blood-Brain Barrier to Improve Pharmacotherapy of CNS Disorders

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Abstract

The blood-brain barrier is the capillary endothelium between blood and brain that controls what goes in and comes out of the CNS. One major factor contributing to barrier function is a group of ATP-binding cassette (ABC) drug efflux transporters that restrict brain uptake of xenobiotics including a large number of CNS therapeutics. Recent research has focused on the regulation of these transporters with the goal of finding the molecular switches to selectively modulate transporter expression and function for improving brain drug delivery. The findings of this research are summarized in this short review.

Introduction

At the molecular level, the primary elements that underlie blood-brain barrier function are tight junctions, influx transporters, and efflux transporters. Tight junctions seal the paracellular spaces between adjacent endothelial cells to restrict solute diffusion from blood to brain, and thus, represent a passive physical barrier. To supply the brain with nutrients, highly specific influx transporters facilitate brain uptake of glucose, amino acids, and ions. ATP-binding cassette (ABC) efflux transporters utilize ATP to actively extrude metabolic wastes from the brain into the blood and to limit xenobiotics, including toxins and a large number of therapeutic drugs, from entering the brain. These drug efflux transporters represent the selective, active component of barrier function and pose a tremendous challenge for delivering drugs into the brain and for successfully treating CNS disorders.

ABC Transporters at the Blood-Brain Barrier

Three ABC drug efflux transporters, P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), and breast cancer resistance protein (BCRP), are major contributors to active, selective blood-brain barrier function. This is based on four characteristics that these transporters share [1]. First, P-gp, MRP, and BCRP are highly expressed in the brain capillary endothelium. Second, localization in the luminal plasma membrane of brain capillary endothelial cells, right at the interface between blood and CNS, is an ideal location for these transporters to prevent xenobiotics from entering the brain. Third, P-gp, MRP, and BCRP are potent ATP-driven efflux transporters that have the capability of pumping their substrates out of the brain capillary endothelial cells into the blood even against a concentration gradient. Fourth, all three transporters have remarkably broad, and in part, overlapping substrate spectra covering structurally diverse compounds that range from small drugs (e.g., morphine, 285 Da; verapamil, 450 Da) to mid-size molecules (e.g., lapatinib, 580 Da; leukotriene C4, 625 Da) and even larger molecules such as peptides (e.g., cyclosporin A, 1200 Da; amyloid-beta, 4200 Da). Drugs handled by P-gp, MRP, and/or BCRP are from various classes including chemotherapeutics, HIV protease inhibitors, opioids, antibiotics,

TABLE 1

Drug Class	P-glycoprotein	BCRP	MRPs
Chemotherapeutics	Taxanes Vinca-Alkaloids Etoposide and Analogues Anthracyclines Lanafarnib Imatinib Topotecan	Doxorubicin Daunorubicin Epirubicin Mitoxantrone Imatinib Topotecan Irinotecan	Vincristine Etoposide Methotrexate Daunorubicin Cisplatin
HIV Drugs	Ritonavir Indinavir Saquinavir Amprenavir Nelfinavir Lopinavir	Zidovudine Lamivudine	Ritonavir Indinavir Saquinavir Nelfinavir Zidovudine
Opioids	Morphine-6-glucuronide Methadone Loperamide Fentanyl Asimadoline		
Antibiotics	Erythromycin Rifampicin	Ciprofloxacin Ofloxacin Norfloxacin	Azithromycin Ciprofloxacin

ABC drug efflux transporter substrates

and many more (Table 1). All of these characteristics combined make P-gp, MRP, and BCRP ideal "gatekeepers" that protect the brain from potentially harmful toxins but at the same time also restrict therapeutic drugs from crossing the blood-brain barrier and entering the CNS.

Overcoming Blood-Brain Barrier ABC Efflux Transporters to Improve CNS Drug Delivery

One possibility to overcome efflux transporter-mediated barrier function is by utilizing transporter inhibitors, and this approach showed promise in rodent models [2]. However, clinical trials were disappointing because translation of this therapeutic strategy to humans proved difficult due to the side effects of the inhibitors [3]. Therefore, recent research has focused on the intracellular signaling pathways that control ABC efflux transporters at the blood-brain barrier. The rationale for such an approach is that finding the molecular switches of these transporters will allow selectively modulating transporter function and thereby improving CNS drug delivery. For example, briefly turning off blood-brain barrier drug efflux transporters could potentially be used to provide brain access to normally non-penetrating CNS drugs. Vice versa, targeting activation of efflux transporters could help increase barrier function and provide enhanced brain protection to minimize side effects from the treatment of peripheral diseases. Moreover, studies show that ABC efflux transporters at the blood-brain barrier contribute to and are affected by various CNS diseases [1,4]. Therefore, understanding the regulation of blood-brain barrier ABC transporters holds the promise of finding novel approaches to protect the brain and new, innovative therapeutic strategies to treat CNS disorders.

Targeting ABC Transporter Regulation to Improve CNS Drug Delivery

Several signaling pathways and regulatory networks have been identified in the past years. Four of those have been described in detail and will be discussed in the following section:

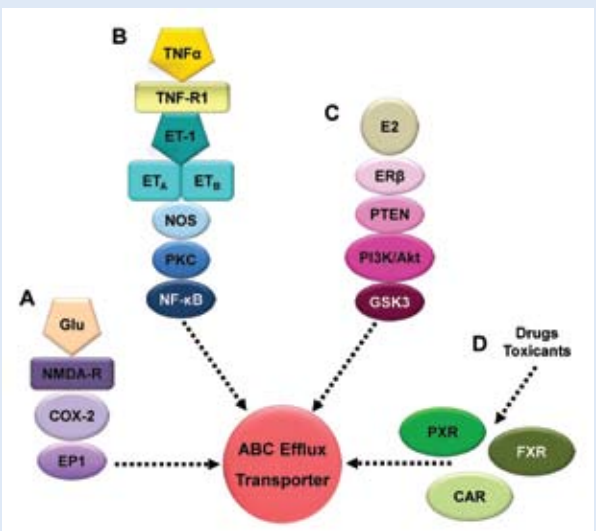
Glutamate – NMDAR – COX-2 – EP1 Signaling Pathway in Epilepsy

The drug transporter hypothesis of refractory epilepsy postulates that up-regulation of ABC efflux transporters at the blood-brain barrier underlies drug resistance in patients not responding to treatment with antiepileptic drugs [5]. Thus, unraveling the mechanism(s) that lead(s) to transporter up-regulation could provide new targets to reduce antiepileptic drug resistance in refractory epilepsy. Recent research shows that the neurotransmitter glutamate, which is released during seizures, up-regulates P-gp in cell cultures, isolated brain capillaries, and in brain capillaries *in vivo* [6,7]. Glutamate signals P-gp up-regulation through a signaling pathway that is summarized in Figure 1A. Glutamate signaling involves the NMDA receptor (NMDAR, [7,8]), the inflammatory enzyme cyclooxygenase-2 (COX-2, [7,9,10]), and the prostaglandin E receptor EP1 [11]. Blocking glutamate signaling in seizure animal models by inhibiting NMDAR, COX-2, or EP1 completely abolished P-gp up-regulation at the blood-brain barrier [7,11]. Importantly, COX-2 inhibition in a phenobarbital-resistant epilepsy rat model reduced seizure occurrence [9]. Whether the above mentioned approaches can be used in humans remains to be seen. Although NMDAR inhibition has never been studied in patients with refractory epilepsy, previous clinical trials testing NMDAR antagonists for their use in epilepsy were unsuccessful due to the drugs' narrow therapeutic windows. Considering the potential spectrum of side effects, COX-2 inhibition may also not be a realistic therapeutic approach in patients with refractory epilepsy. No drugs are currently available to specifically target EP1 and whether this could be used as a novel target to prevent transporter up-regulation remains unclear. Future research may well identify other, potentially better, therapeutic targets to improve antiepileptic drug delivery in refractory epilepsy.

TNF-α Signaling Pathway in Brain Inflammation

Most CNS disorders are accompanied by brain inflammation, and inflammatory mediators such as tumor necrosis factor-α (TNF-α) have been shown to regulate ABC efflux transporters in various tissues including the brain [12]. TNF-α plays a key role in the regulation of P-gp in brain capillaries during inflammation. Figure 1B shows that in this pathway, TNF-α signals through the TNF receptor 1 (TNFR1), which triggers release of the peptide endothelin-1 (ET-1). ET-1 in turn acts through the endothelin receptors ET_A and ET_B, and activates a cascade involving nitric oxide synthase (NOS) and protein kinase C (PKC). In the short-term (minutes), TNF-α signaling reduces P-gp transport activity, suggesting that targeting this pathway could be used to rapidly down-regulate transporter activity for brain drug delivery [13]. Over the long-term (hours), however, TNF-α signaling involves the transcription factor nuclear factor-κB (NF-κB), transcription, and translation, which leads to up-regulation of P-gp in brain capillaries [14]. Recent studies confirmed these findings and demonstrated involvement of the PKC isoform β_1 in TNF-α signaling [15]. TNF-α has also been shown to up-regulate vascular endothelial growth factor (VEGF, [16]), which acutely decreases P-gp transport activity in brain capillaries by acting through the VEGF receptor flk-1 (fetal liver kinase 1) and the tyrosine kinase Src [17]. This implies that P-gp activity is reduced in disease states associated with increased brain VEGF expression and that this new pathway could also potentially be used to reduce transporter function for brain drug delivery.

FIGURE 1



Signaling pathways that regulate ABC drug efflux transporters at the blood-brain barrier. Four main signaling pathways that control P-gp, MRP, and/or BCRP at the blood-brain barrier have been identified: A) Glutamate (Glu) – NMDA receptor (NMDAR) – cyclooxygenase-2 (COX-2) – prostanoid E receptor (EP1) signaling pathway that up-regulates P-gp in epilepsy. B) TNF-α signaling pathway that regulates P-gp through the TNF receptor (TNFR1), endothelin-1 (ET-1), endothelin receptors (ET_A, ET_B), nitric oxide synthase (NOS), protein kinase C (PKC), and nuclear factor-κB (NF-κB; only long-term signaling) in brain inflammation. C) E2 – ER – PTEN – PI3K/Akt/GSK3 signaling pathway that mediates 17β-estradiol (E2) reduction of BCRP expression and function through estrogen receptor β (ERβ), phosphatase and tensin homolog (PTEN), phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), and glycogen synthase kinase 3 (GSK3). D) Ligand-activation of pregnane X receptor (PXR), farnesoid X receptor (FXR), or constitutive androstane receptor (CAR) up-regulates ABC drug efflux transporters.

Little information is available on the regulation of other ABC efflux transporters at the blood-brain barrier in inflammation. Von Wedel-Parlow et al. showed that the inflammatory mediators TNF-α and IL-1β affect BCRP in brain capillary endothelial cells [18] and we previously demonstrated that ET-1 significantly decreases BCRP protein expression in isolated rat brain capillaries [14]. Given that brain inflammation is a major component of most CNS disorders, it is expected that future research will identify more pathways where inflammatory mediators signal changes in blood-brain barrier transporter expression and/or function that could potentially be used to improve brain drug delivery.

E2 – ER – PTEN – PI3K/Akt/ GSK3 Signaling Pathway in Brain Cancer

BCRP-mediated multidrug resistance in brain tumor cells and brain tumor stem cells is a major problem in neurooncology. Targeted modulation of BCRP could be one possibility to increase anticancer drug levels in brain tumor tissue. In this regard, 17β-estradiol (E2) has recently been demonstrated to act as a powerful modulator of blood-brain barrier BCRP [19,20]. Figure 1C shows the signaling pathway through which E2 down-regulates BCRP in brain capillaries. In this pathway, E2 signaling through the estrogen receptor β (ERβ) activates the tumor suppressor PTEN (phosphatase and tensin homolog), which in turn inactivates PI3K/Akt (phosphoinositide 3-kinase/ protein kinase B) leading to activation of GSK3 (glycogen synthase kinase 3) and proteasomal degradation of BCRP. This is a particularly interesting pathway given that a recent study demonstrated PTEN/PI3K/Akt regulation of BCRP activity in mouse and human gliomas [21]. These data suggest that E2-mediated degradation of blood-brain barrier BCRP has the potential to increase brain uptake of chemotherapeutics that are BCRP substrates.

PXR Signaling Pathway in Alzheimer's Disease

Ligand-activated nuclear receptors are major regulators of ABC efflux transporters. Ligand binding to these receptors activates transcription of their target genes including efflux transporters, resulting in up-regulation of transporter protein expression and activity. At the blood-brain barrier, several nuclear receptors have been detected including the pregnane X receptor (PXR), the farnesoid X receptor (FXR), and the constitutive androstane receptor (CAR). Activation of these receptors up-regulates protein expression and transport function of P-gp, MRP, and/or BCRP (Figure 1D), suggesting a tightening of the barrier [22-24]. Thus, activation of nuclear receptors to selectively up-regulate transporters could help protect the brain. Such a scenario would be useful during pharmacotherapy of diseases in the periphery to prevent CNS side effects or in brain disorders where transporter expression and function are diminished. An example of the latter is Alzheimer's disease, where blood-brain barrier P-gp is substantially reduced [25]. A recent study used PXR activation as an approach to restore P-gp at the blood-brain barrier of an Alzheimer's disease mouse model [26]. Importantly, restoring P-gp through PXR activation substantially lowered brain levels of amyloid-beta, the neurotoxic peptide that accumulates in the brain and contributes toAlzheimer's disease pathology. This finding implies that targeting intracellular signals that up-regulate blood-brain barrier P-gp in early stages of Alzheimer's disease has the potential to reduce amyloid-beta brain accumulation, which suggests a novel and innovative therapeutic strategy in AD to delay onset and slow progression of the disease.

Conclusions

Over the last years, the CNS drug delivery field has evolved from directly inhibiting blood-brain barrier ABC drug efflux transporters to finding and targeting the intracellular signaling pathways that regulate these transporters. The picture that is emerging from recent research shows a complex regulatory network that controls expression and activity of several brain capillary efflux transporters. Understanding these signaling networks under physiological and pathophysiological conditions holds the promise of identifying valid therapeutic targets and designing effective therapeutic strategies to improve drug delivery to the brain in CNS disorders or prevent disease onset and progression. Future research has to provide further proof-of-principle that targeting transporter signaling is beneficial and can be a valid therapeutic strategy in the clinic to improve brain drug delivery in patients with CNS disorders.

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Footnotes

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