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Targeting the neurovascular unit for treatment of neurological disorders

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Abstract

Drug discovery for CNS disorders has been restricted by the inability for therapeutic agents to cross the blood-brain barrier (BBB). Moreover, current drugs aim to correct neuron cell signaling, thereby neglecting pathophysiological changes affecting other cell types of the neurovascular unit (NVU). Components of the NVU (pericytes, microglia, astrocytes, and neurons, and basal lamina) act as an intricate network to maintain the neuronal homeostatic microenvironment. Consequently, disruptions to this intricate cell network lead to neuron malfunction and symptoms characteristic of CNS diseases. A lack of understanding in NVU signaling cascades may explain why current treatments for CNS diseases are not curative. Current therapies treat symptoms by maintaining neuron function. Refocusing drug discovery to sustain NVU function may provide a better method of treatment by promoting neuron survival. In this review, we will examine current therapeutics for common CNS diseases, describe the importance of the NVU in cerebral homeostasis and discuss new possible drug targets and technologies that aim to improve treatment and drug delivery to the diseased brain.

Keywords

blood-brain barrier; Alzheimer's disease; stroke; astrocyte; drug delivery

1. Difficulties with Treating CNS Diseases

1.1 Limited Therapeutic Options

Complex central nervous system (CNS) diseases, such as stroke and Alzheimer's disease (AD), are among the leading causes of disability and death in the developed world. A majority of modern pharmacological therapies provide symptomatic relief, but are commonly associated with adverse side effects and often do not halt disease progression.

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Moreover, patients afflicted with complex CNS diseases typically require life-long medication with a marginal improvement in the quality of life. While therapeutic and neurosurgical approaches have improved the outcomes for some neurological disorders (i.e. hydrocephalus, benign tumors, and epilepsy), certain CNS diseases, such as amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS), are predominantly unresponsive to current treatment. An explanation for the lack of effective therapies is that current drugs aim to improve neuron function, but fail to address the underlying disease pathology. Given the disappointment with recent neuroprotective agents, future studies must emphasize the importance of transport across the blood brain barrier (BBB) and consider the use of drugs with both neuroprotective and regenerative properties to achieve long lasting effects on functional outcome (Savitz & Schabitz, 2008).

1.2 Limited Drug Delivery to the Brain

An estimated 98% of small molecule drugs and 100% of large molecule drugs on the market do not enter brain or cannot achieve concentrations needed for therapeutic benefit (Pardridge, 1998a). Pharmacokinetic properties (e.g. protein binding, rapid clearance or metabolism) contribute to limited drug bioavailability in the periphery. However, the BBB has metabolic and physical barriers that further restrict passage of molecules from peripheral circulation into cerebral circulation. Difficulty with developing drugs that cross the BBB is illustrated by a study characterizing over 6,000 marketed medications; from which, only 6% of the drugs were capable of entering into the brain (Ghose, Viswanadhan, & Wendoloski, 1999). Chemical properties that favor passage across the BBB and into the brain include: a lipophilic nature, a size no larger than 600 Da or affinity for an endogenous transport system (i.e. transport proteins, receptor-mediated, or absorptive transcytosis) (Pardridge, 2006). The heterogeneous nature and variability in brain region vascularization contribute to the complexities facing CNS drug delivery and must be further explored. Very few CNS drugs currently in development will cross the BBB due to size, polarity, or lack of endogenous transport pathways (Pardridge, 2006). Current research has elucidated limitations of crossing the BBB, therefore CNS drug development should concentrate on small, lipophilic chemical structures or moieties that possess an endogenous transport system.

2. Existing Pharmacological Therapies for CNS Disorders

2.1 Parkinson's Disease

Degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) is classified as the movement disorder, Parkinson's disease (PD). First line therapy for moderate to severe PD includes a combination therapy of L-DOPA [(–)-3-(3,4-dihydroxyphenyl)-L-arginine] and carbidopa [(2S)-3-(3,4-dihydroxyphenyl)-2-hydrazine-2-methylpropanoic acid], which provides symptomatic relief by increasing central dopamine levels. L-DOPA, a metabolic precursor to dopamine, crosses the BBB via the large neutral amino acid transporter and undergoes decarboxylation to dopamine in the brain. Since peripheral dopamine can not enter the brain, adjuvant therapy with carbidopa, a peripheral DOPA decarboxylase inhibitor, is necessary to increase L-DOPA bioavailability and prevent dopamine conversion in the periphery. These two medications supplement neuron function by providing a precursor to the neurotransmitter dopamine. However, efflux pumps limit bioavailability of an oral dosing with less than 1% entering the brain (Hawkins, Mokashi, & Simpson, 2005) and long-term L-DOPA-carbidopa treatment leads to drug tolerance and reemergence of symptoms (Vaamonde, Flores, Weisser, Ibanez, & Obeso, 2009). The medication maintains neuron function for a short time, but ultimately selective dopaminergic neuron degeneration in the SNpc ensues.

2.2 Alzheimer's Disease

Neuropathological changes of Alzheimer's disease (AD) are characterized by the presence of cortical atrophy, neurofibrillary tangles, and neuritic plaques (Bell & Zlokovic, 2009). However, there is an undefined role for these neuroanatomical changes in AD progression and no consensus exists for the causality (Silvestrelli et al. 2006). Altered behavior and cognition in AD, associated with the loss of cholinergic input (Bartus, 1982), is a consequence of the pathological changes in brain anatomy. Much like PD treatment, current AD therapy aims to replace depleting levels of a neurotransmitter to treat symptoms. Donepezil, an acetylcholinesterase inhibitor, improves cognition in patients with mild to moderate AD (Brousseau et al., 2007) by enhancing acetylcholine bioavailability. Donepezil crosses the BBB through the organic cation transporter (Kim et al., 2010); however, P-glycoprotein (P-gp)-mediated efflux limits therapeutic concentrations of donepezil and other acetylcholinesterase inhibitor in the brain (Ishiwata, 2007). There is a threshold for treatment, in that most patients only tolerate low dose therapy secondary to adverse peripheral side effects from increased cholinergic activity. Once AD progression is beyond a "moderate" stage, patients are unresponsive to therapy because the number of neurons and neuron synapses are limited (Scheff et al., 2006).

2.3 Huntington's Disease

Huntington's disease is an autosomal dominant neurodegenerative disease that affects movement and cognition. The glutamine repeat extension of the Huntingtin gene has been well documented in association with HD, yet Huntingtin protein function remains unknown. Treating HD is particularly challenging due to the lack of understanding in disease etiology. This gap in knowledge limits current treatment options to focusing on symptomatic relief of depression, psychosis, and chorea (involuntary writhing movement). Chorea, being the most difficult symptom to treat, has recently been managed with tetrabenazine (TBZ), an antipsychotic medication (Fasano et al., 2008; (Frank et al., 2008). TBZ promotes neurotransmitter degradation by preventing reuptake of monoamine neurotransmitters into presynaptic vesicles (Ondo, Hanna, & Jankovic, 1999). Even though TBZ is one of the few drugs available for treating chorea and has fewer side effects than the analogous drug reserpine (Kenney, 2007), neuron-focused medications do not halt disease progression.

2.4 Epilepsy

Epilepsy is characterized by spontaneous, recurrent seizing episodes due to uncontrolled, repetitive neuron firing. Antiepileptic drugs (AED) provide symptomatic relief by modifying neurotransmission through three different mechanisms: increasing GABAergic (inhibitory) activity, decreasing glutaminergic (excitatory) activity or modifying ion conductance. When a patient no longer responds to AED treatment, the disease state is categorized as refractory epilepsy. The only treatment options for patients with refractory epilepsy are invasive procedures such as vagal nerve stimulation (Murphy, 2003), hemispherectomy (Obrador & Larramendi, 1953; Chandra et al., 2008), or complete removal of affected areas (resection) (Penfield & Baldwin, 1952; Schramm & Clusmann, 2008). Studies have shown refractory epilepsy coincides with increased expression of P-glycoprotein or multi-drug resistance efflux pumps, which may limit therapeutic concentrations of AEDs from entering the brain (Tishler et al., 1995; Lazarowski et al., 2007). Until the pathology of refractory epilepsy is better understood, invasive procedures remain the only treatment options.

2.5 Brain Cancer

Malignant gliomas are the most common brain tumor and have the worst prognosis. Treating brain cancer has been particularly challenging because chemotherapy drugs (e.g. carboplatin, methotrexate, leucovorin, cyclophosphamide, etc.) have limited BBB

permeability (Bart et al., 2000). Intra-arterial delivery of chemotoxic drugs has been used for temporary osmotic opening of the BBB to increase drug delivery to the tumor. Osmotic opening of the BBB can increase drug delivery to the tumor between 10-fold and 100-fold (Kroll et al., 1998). However, disrupting the BBB using this invasive procedure has been associated with transient neurological deficits and increased risk of seizure (Haluska & Anthony, 2004).

Focused ultra-sound is a less invasive mechanism for reversible barrier opening to promote localized drug delivery. This method shows promise for controlling dose delivery to the affected brain region (Yang et al., 2010). However, like osmotic opening, preclinical studies indicate post-treatment damage as indicated by ubiquitinated neurons (Alonso et al. 2010a), reorganization of tight junction proteins (Alonso et al., 2010b) and deposited astrocyte and neuron gap junction plaques (Alonso et al., 2010b). Ultra-sound barrier opening may offer a better prognosis (Liu et al., 2010) for patients with malignant CNS-related cancers than no treatment, but further studies are needed to assess the risk for permanent brain damage.

2.6 Stroke

Aging effects vascular function and basal level inflammation (Rosen et al., 2005; Persky, 2010; Wassertheil-Smoller, 2010), which leaves the aged NVU more vulnerable to insult. Advanced age is a primary risk factor for stroke and other neurodegenerative diseases (PD, AD, etc) and is a factor that should not be ignored in animal models for studying disease. Ignoring age in preclinical studies may account largely for the failed neuroprotectants in clinical trials for stroke and other neurodegenerative diseases (Koziol & Feng, 2006; Lohle & Reichmann, 2010). However, only recently has a clinically-relevant aged animal model for stroke been developed (Sahakyan et al., 2010; Baccarelli et al., 2010). Specifically for stroke, it is well-established that risk factors (e.g. age, diabetes, hypertension, dyslipidemia, etc.) correlate with increased circulating inflammatory mediators. Inflammatory peripheral markers may play a role in cerebrovascular disease (Tuttolomondo et al., 2009), thus mediating stroke occurrence. A well-substantiated association between specific peripheral mediators (biomarkers) and stroke risk or occurrence does not yet exist. Identifying and understanding the function of peripheral inflammatory mediators on the BBB will give insight to better clinical diagnosis for stroke. Moreover, innovative neuroprotectants may emerge from better understanding of the heterogenous nature of stroke pathology and brain region variability (e.g. vascularization, blood flow, type of neuron, pro-longed neuroinflammation, etc).

3. The Importance of the Neurovascular Unit in CNS Homeostasis and Disease

3.1 The Rationale for Pharmacological Targeting of the NVU

NVU components (i.e. the BBB, astrocytes, pericytes, microglia, and neurons) work together in a coordinated fashion to maintain the homeostatic neuron microenvironment (Figure 1a) (Pardridge, 1998b; Hawkins & Davis, 2005). Regulation of intracellular signaling cascades is a key component to maintaining the metabolic homeostasis of the neuron. Dysregulation of this intracellular signaling network may be the initial step in leading to NVU pathology and neuron dysfunction (Figure 1b). Intracellular signaling between cell types can alter the passage of substances into the brain by modifying BBB receptor expression or BBB functional integrity (Abbott, 2002). In many CNS diseases, decreased BBB functional integrity precedes neuron damage (DiNapoli et al., 2008; Abraham et al., 2002; Lee et al., 2007; Kermode et al., 1990; Katz et al., 1993; Ujiiie, Dickstein, Carlow, & Jefferies, 2003), thus indicating a role NVU dysfunction in BBB dysfunction. Current drug development efforts often overlook the contribution of non-

neuronal cells to the development and progression of disease states. Pathophysiological signaling cascades between NVU cells must be better understood before targeted therapies are developed to restore the neuron microenvironment.

3.2 The Cerebral Endothelium in CNS Disease

The cerebral endothelium possesses both metabolic and physical barriers that limit molecular passage into the brain (Figure 2). Endothelial enzymes (e.g. CYP450, xanthine oxidase, MAO) metabolize molecules, which may decrease bioactivity of CNS drugs. Efflux pumps (e.g. P-gP, MDR, MRP, etc.) on the abluminal surface extrude molecules back into peripheral circulation. In AD (Wijesuriya et al., 2010) and epilepsy (Luna-Tortos et al., 2008), over expression of efflux pumps may contribute to unresponsiveness to drug therapy. Efflux pump inhibition (Rigor et al., 2010) may provide a new mechanistic target for improving drug delivery in pharmacoresistant disease states.

Physical characteristics of the BBB differ from peripheral capillary beds due to the absence of fenestrations, presence of tight junctions (claudin-5, occluding, zonula occludens, JAM junctions), limited pinocytotic transport, and close apposition to astrocytes, neurons, and microglia (Huber et al., 2001). Tight junctions provide a physical and electrical barrier restricting molecular passage. Altered expression of tight junction proteins and decreased functional integrity are characteristic pathophysiological changes to the BBB during CNS disease. Such changes have been implicated in diseases including AD (Bowman et al., 2007), PD (Curran & Becker, 1991), ALS (Zhong et al., 2008), and epilepsy (Marchi et al., 2007). Restoring BBB integrity may provide a method for saving existing neurons by retaining a homeostatic microenvironment. Small molecules and signaling cascades alter the neuronal microenvironment and induce functional changes in BBB permeability during disease onset. BBB functional integrity may worsen with time by allowing the passage of molecules normally restricted from cerebral circulation (Huber et al., 2006), into the brain. Studies have shown that BBB integrity can be induced experimentally with transforming growth factor- β (TGF- β), IL-6, bFGF, and GDNF stimulation (Abbott, 2002). Likewise, regional differences in vascularization and vascular permeability may allow for passages of targeted therapies that would typically be excluded from the brain. Therapies designed to restore and maintain an intact BBB may prolong onset of CNS diseases (e.g. BBB permeability precedes AD diagnosis) (Starr et al., 2009).

3.3 Contributions of Pericyte Processes in Disease

Pericytes send out cellular projections that penetrate the basal lamina and ensheath approximately 20% to 30% of the microvessel circumference (Dore-Duffy & Lamanna, 2007). This close interaction supports the idea that pericytes communicate with endothelial cells and other pericytes through gap junctions and cell adhesion contacts (Allt & Lawrenson, 2001). CNS pericytes lack the contractile protein alpha actin, unlike peripheral pericytes. However, evidence suggests that CNS pericytes do help regulate cerebral blood flow (Krueger & Bechmann, 2010).

Proposed roles for pericytes in the brain include maintaining BBB integrity, angiogenesis and maturation of endothelial cells with BBB phenotype (Dohgu et al., 2005; Kutcher & Herman, 2009). For example, co-cultures of primary rat brain pericytes and cerebral endothelial cells induce tight junction formation; conversely, inhibition of pericyte-secreted TGF- β leads reduces tight junction formation and BBB integrity (Hellstrom et al., 2001). Other factors (e.g. PDGF, FGF, EPO, MMPs) released by brain pericytes may contribute to microvasculature membrane remodeling as observed in the periphery (Huang et al., 2010). Consequently, a lack of pericyte derived factors may contribute to pathological changes in the brain. Detachment of pericytes from the vasculature or pericyte apoptosis (*i.e.* pericyte

ghosts, as observed in diabetic retinopathy) leads to pathological changes such as aberrant vasculogenesis and endothelial hyperplasia (McCarty, 2009) in the brain. NG2 proteoglycan is an essential contact adhesion protein that stabilizes pericytes-endothelium interactions in the retina and the brain. This pathway may be a potential drug target for decreasing vasculogenesis (Kimelberg, 2010) to tumors or helping to maintain capillary function in incidences of stroke and other small vessel diseases (Nagelhus et al., 2004).

3.4 Astrocytes in CNS Pathology

Astrocytes are specialized glia cells that regulate metabolic factors (e.g. glucose, neurotransmitters, ions, blood flow, etc.) affecting neuron function (Neuhaus 1991) and have end feet that envelope 99% of the brain microvasculature. Gap and adherens junctions at the astrocytic end feet provide a mode of intercellular communication with the endothelium. Aquaporin 4 (AQP4), the primary water channel located in the end feet, contributes to endothelial cell polarity and brain water volume (Wolburg et al., 1994). Astrocyte secreted factors induce the BBB phenotype in endothelial cell cultures (Croitoru-Lamoury et al., 2003; Didier et al., 2003), VEGF and fibroblast growth factor-2 (FGF-2) promote angiogenesis and regulate BBB transport (Benarroch, 2009) under a physiological state.

Under a pathophysiological state, astrocytes respond to inflammation and trauma (Cacheaux et al., 2009) by releasing proinflammatory cytokines (Dauchy et al., 2009) and altering BBB chemokine receptor expression (Song & Wang, 2010). Pathological changes due to neuroinflammation may feature astrocytes as a novel drug target. Astrocyte dysfunction lays a key role in epilepsy pointing to altered potassium glutamate homeostasis (Chakraborty et al., 2010) and enhanced TGF- β signaling (Gao & Ji, 2010) and enhanced local drug metabolism (Garden & Moller, 2006). Likewise, evidence of activated astrocytes is a characteristic pathological change in brain diseases from psychiatric disorders like depression (Qian, Flood, & Hong, 2010) to neurodegenerative diseases like HD and PD (Lee & Landreth, 2010) or neuropathic pain (Persidsky, 2006). A pathological role for astrocytes during CNS disease is emerging, which may soon lead to astrocyte-targeted therapy.

3.5 Microglia in CNS Disease

Microglia, stemming from the monocyte lineage, generates an innate and adaptive immune response within the CNS if the brain is exposed to injury, ischemia, or inflammatory stimuli. Microglia undergo a morphological change from stellate to ramified or an activated confirmation. This physical change permits the microglia to initiate an inflammatory response within the brain through cell proliferation, moving to the site of injury, and engulfing cell debris (Lai, 2005). While these processes have neuroprotective roles, over activation of inflammatory cascades leads to neurodegeneration from excessive ROS release. As with astrocytes, a prevailing theory in the field of neurodegenerative disease research points to neuroinflammation as a source of complications and potential area for treatment (Haskins et al., 1998; Martin-Padura et al., 1998).

3.6 Basement membrane in CNS disease

The basement membrane structure is made from heterogeneous protein matrix composed of actin, laminin, integrins, collagen, fibronectin and proteoglycans. This protein layer separates the brain capillary from nearby NVU cells and provides support for cell attachment and migration. Data suggests that the basal lamina also plays a role in intercellular communications and regulating cell adhesion molecules (Martin-Padura et al., 1998). Actin organization can influence tight junction rearrangement, which may contribute to enhanced BBB permeability (Fournier et al., 2009). Tight junction proteins are interconnected to transmembrane and cytosolic portions of the basal lamina. Zonula occludens belong to the membrane-associated guanylate kinase family, thus indicating a role

for signal transduction (Fournier et al., 2009). Junction adhesion molecules (JAM) belong to the immunoglobulin superfamily, which promote leukocyte transmigration when endothelial adhesion molecules are expressed (Hyong et al., 2008). Drugs designed to stabilize basal lamina interactions may protect against BBB permeability changes and subsequent neuroinflammation.

4. A Modern Approach for CNS Drug Delivery

4.1 Enhancing Drug Delivery to the Brain

Current therapies aimed at correcting neuron function have had limited success in treating CNS disease. Managing symptoms with medication in the early stages of disease can be effective. Yet, disease progression often leads to the use of higher doses of medication with unwanted side effects or the medication may no longer be helpful for symptom management. A flow chart depicting NVU dysfunction and clinical diagnosis can be viewed in Figure 3. Targeting the NVU, which has primarily focused upon the vasculature, has had some success with treating or limiting CNS disease progression (Egleton et al., 2000; Gynther et al., 2009). However, any proposed drug targets are ineffective without drug transport across the BBB. The remainder of this review will discuss advances in strategies for drug delivery to the CNS.

4.2 Pro-drugs

Pro-drugs are defined as therapeutically inactive agents that can be enzymatically converted to the active metabolite. Pro-drugs enhance bioavailability because drug metabolism occurs in the brain and not in the periphery. While the BBB possesses enzymes that maybe useful for pro-drug conversion, pro-drug have not been well characterized. A better understood mechanism involves circulating molecules entering the CNS by interacting with endogenous transport proteins on the capillary endothelium. Carrier mediated transporters (CMT) facilitate passage of molecules essential for brain metabolism. Utilizing CMT passage has enhanced CNS drug delivery for L-DOPA and other drug conjugates (Gynther et al., 2008). L-DOPA is a pro-drug that uses the LAT1 transporter to enter the brain. In the brain, L-DOPA is converted to dopamine, the active compound.

One study demonstrated the endogenous transport of a water-soluble NSAID, ketoprofen, across the BBB when conjugated to L-tyrosine, a LAT1 substrate (Begley, 2004). *In situ* brain perfusion confirmed that the ketoprofen-L-tyrosine hybrid entered the brain using LAT1 in a concentration-dependent manner. The results of this study are novel, but the pharmacokinetics of the drug complex in the brain parenchyma remains unclear. While targeting an endogenous transport protein is ideal for drug delivery, this method has limitations. Modifying a chemical compound to favor passage into the brain may result in loss of drug function or bioactivity may be altered if the drug is not converted to the active form. Current usage as in PD and AD show efficacy for disease treatment, but may be best used in conjunction with other treatments that stabilize NVU function.

4.3 Trojan Horses

Molecular Trojan horses permit non-invasive delivery of large molecules to the brain using RMT (Bell and Zlokovic 2009). Larger molecular weight peptides such as insulin, transferrin, and leptin, are transported across the BBB through receptor mediated transcytosis (RMT) and peptidomimetic monoclonal antibodies (MAb) follow the same path to enter the brain. Receptor specific MABs should bind exofacial epitopes on the BBB that differ from the endogenous ligand in order to not interfere with endogenous ligand transport (Pardridge, 2002). Both the insulin and transferrin systems have been used for mAB research. Proof of concept has been shown by conjugating peptides such as BDNF or FGF-2 to the

TfR (Pardridge, 1998). However, the insulin receptor comparatively is the better candidate for RMT drug delivery with the human insulin mAb (HImAb) being 900% more active and 10 times more effective than any human TfR (Boado et al., 2010; Ulbrich et al., 2010). Successful pre-clinical trials using HImAb include transport of neuroprotective peptides like BDNF, which may have therapeutic benefit in diseases like stroke (Marini et al., 2008), memory disorders (Longo et al., 2007), or depression (Yulug et al., 2009); reintroducing an essential enzyme like tyrosine hydroxylase in PD (Zhang et al., 2003); decoy receptor for TNF β to reduce neuroinflammation (Zhang et al., 2003); or antibodies to A β fibrils to ferry them out of the brain (Boado et al., 2010). The latter being the most novel finding showing the possibility for bidirectional drug transport across the BBB. The primary concern with the delivery of larger molecule drugs is accumulation in the brain. Endothelial and astrocytic efflux pumps may have a limited ability to extrude larger molecules from the brain (Pardridge, 2002).

4.4 Liposomes

Liposomes are a self-sustainable bilayer of phospholipids or sphingolipids that form small unilamellar or multilamellar vesicles (Witt et al. 2001), which can be used for drug delivery. These lipid spheres are non-toxic, biocompatible, and can deliver lipophilic, hydrophilic or amphoteric drugs either within the sphere or on the sphere's surface. Liposomes enhance drug bioavailability by protecting the therapeutic agent from metabolism in the body, thereby prolonging a drug's pharmacokinetic profile in the circulatory system. Moreover, immunolabeled liposomes have the potential for site-specific delivery of other novel brain targeting technologies like mAb Trojan horses, cDNA, and siRNA for treating a several CNS diseases.

In the instance of cancer, enclosing a drug within the liposome increases the probability of extravasation from the tumor vasculature (Alam et al., 2010). More specifically, liposomes with modified surfaces can target the affected tissues or organ, which has been illustrated in cancer and rheumatoid arthritis treatment (Goren et al., 1996). In the treatment of cancer, multilamellar vesicles of doxorubicin are more effective than doxorubicin administered alone (Williams et al., 1987). More specifically, a doxorubicin tumor therapy study used an obligate anaerobe protein to target the hypoxic regions of the tumor, which lead to enhanced local release at the tumor (Papahadjopoulos et al., 1991). Use of conjugated liposome shows promise for targeting cancers with poor prognosis like gliomas (Cheong et al., 2006).

Advanced liposomes, such as Depofoam® and nanoshells, contain nano-sized vesicles that permit continual release of drugs. This differs from a standard liposome, which undergoes one rupturing event (Arai et al., 2010). Depofoam® technology has been used in the treatment of neoplastic meningitis and acute lymphoma. Fewer and less severe side effects were observed than compared to standard treatment (Sancho et al., 2006). A fewer number of side effects may be attributed to slower drug release and improved site-specific delivery. Liposome technology has the potential to improve pharmacodynamic properties of current CNS drug therapies to promote better treatment with lower dosings (e.g. neuropsychiatric drugs, chronic pain medication, cholinergic stimulating drugs for AD). Additionally, potential exists for enhancing site specific drug delivery with gene therapy, as describe below.

4.5 Gene Therapy

There are few options available to replace dysfunctional genes with working copies. However, using viral vectors to reintroduce cDNA of functional genes or using siRNA to silence mutant genes shows promise for clinical purposes. These methods have been

extensively used with *in vitro* studies to understand protein functionality and show potential in translating to clinical therapy.

A lack of an essential protein can lead to neuron degeneration or malignant glioma. Insertion of cDNA into HIV, adeno or herpes virus is the common method for delivering the gene to the cell, where by the vector utilizes cell replication machinery for mRNA transcription and protein translation. Retroviruses were one of the first recombinant vectors used in the clinical setting and phase I and II clinical studies for recurrent malignant glioma showed a favorable safety profile (Benesch et al., 2009). Yet, due to immunogenic safety concerns adeno, herpes and retro virus vectors are considered to have limited clinical use (Ren et al., 2003).

Silencing gene expression using siRNA may have therapeutic benefit in reducing mutant proteins as in HD (Rainov & Ren, 2003) or AD. Ion channels, neurotransmitters, transcription factors, growth factors and growth receptors are categories for potential therapeutic targets. Furthermore, siRNA shows promise for reversing pathology of *in vivo* disease models (AD, HD, ALS, neuropathic pain, anxiety depression, etc.) [114]. In addition to immunogenic concerns with viral vector delivery, short-comings of siRNA include decreased potency due to RNA instability, variable transfection efficacy, poor intracellular uptake, and high probability of off-target effects leading to decreased gene expression of *other* genes or not decreasing expression of the intended gene (Drouet et al., 2009).

Both delivery systems involve viral vectors, which can initiate cytotoxicity (Prakash et al., 2010) and immunological complications (Davis et al., 2004) within the CNS. Non-viral or naked cDNA or siRNAs may limit immuno-related complications. Using transfection reagents such as immunolabeled liposomes to deliver naked cDNA or siRNA may reduce complications of instability and off-target delivery and enhance bioactivity through potentially less frequent and lower dosings.

4.5 Peripheral Mediators

The BBB responds to peripheral and central factors, which serves as a signaling interface between the CNS and the periphery (Li et al., 2005). Several diseases (e.g. hypertension, diabetes, etc.) are associated with increased circulating inflammatory mediators (Banks, 2010; Montecucco, Pende, Quercioli, & Mach, 2010) [118;119], which alter vascular and immune function (Marsland et al., 2010). A few *in vivo* studies pinpoint the ability for peripheral mediators to influence BBB inflammatory mediated secretion (Li et al., 2005; Puddu et al., 2005). Thus, it is no surprise that peripheral inflammation influences BBB structure, function (Gosselin & Rivest, 2008; Brooks et al., 2005) and astrocyte signaling (Brooks et al., 2005). For example, circulating MMP-9 is up-regulated in co-morbid disease states (Ronaldson et al., 2009; Lewis, Jr. et al., 1983). TNF α is a key player in the microglia and astrocytes inflammatory response and peripheral levels may affect pathological neuroinflammation. However, the effect of specific cytokines on neuro-immunomodulation is not well defined.

The concept of modulating inflammatory mediators to prevent vascular events is not novel (e.g. heart attack, stroke, etc) (Barr et al. 2010). Studies have examined levels of circulating inflammatory factors (e.g. MMP-9, IL-6, ICAM-1) and found a positive correlation between specific inflammatory mediators and BBB disruption (Castillo et al., 2009) and morbidity or worsened stroke outcome in patients (Gelosa et al., 2010; McColl et al., 2008). Likewise, reducing peripheral inflammation leads to better experimental stroke outcome (Schondorf et al., 2010). While experimentally elevated pre-stroke peripheral inflammation, leads to worsened post-stroke damage coinciding with changes in tight junction rearrangement (Schondorf et al., 2010). Preventative treatment to reduce peripheral inflammation may be

an ideal target for pre-stroke prevention and adjuvant therapy for post-stroke treatment. This treatment approach may be efficacious for prevention of vascular-associated dementias or late-life cognitive impairment and increased risk for AD in patients with diabetes.

Conclusion

Understanding NVU dysfunction will lead to novel drug targets that aim to do more than supplement neuron function and provide symptomatic relief. Roles for astrocytes, microglia, pericytes and the BBB are emerging for CNS pathology, thus emphasizing the need for drug design that is not solely focused on the neuron. Of equal importance is the need for understanding how the BBB dynamically interacts with the CNS and periphery. Evidence suggests that peripheral mediators influence barrier function over time and peripheral inflammation may alter neuroinflammatory pathways. One problem with CNS drug development has been the physical and metabolic restrictions of the BBB, which selectively permits molecular passage from peripheral to cerebral circulation. However, trojan-horse and pro-drug methods have employed endogenous transport systems (i.e. CMT and RMT) to carry therapeutics into the brain and show promise for clinical use. Currently, liposomes have improved outcomes for patients with cancer by increasing drug bioavailability and reducing therapeutic dosages. Furthermore, immunolabeled liposomes have the ability to advance site specific drug delivery and make gene therapy more feasible by shielding cDNA or siRNA from peripheral degradation. With better insight to CNS disease pathology and improved drug delivery to the CNS, effective therapies are on the horizon.

Abbreviations

CNS	central nervous system
BBB	blood-brain barrier
NVU	neurovascular unit
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
MS	multiple sclerosis
Da	Dalton
SNpc	substantia nigra pars compacta
PD	Parkinson's disease
L-DOPA	[(−)-3-(3,4-dihydroxyphenyl)-L-arginine]
carbidopa	[(2S)-3-(3,4-dihydroxyphenyl)-2-hydrazine-2-methylpropanoic acid]
P-gp	P-glycoprotein
HD	Huntington's disease
TBZ	tetrabenazine
AED	antiepileptic drugs
GABA	γ-aminobutyric acid
rtPA	recombinant tissue plasminogen activator
JAM	junction adhesion molecule
AED	antiepileptic drugs

TGFβ	transforming growth factor-β
IL	interleukin
AQP4	aquaporin 4
MMP	matrix metalloproteinase
VEGF	vascular endothelial growth factor
FGF-2	fibroblast growth factor-2
CMT	carrier mediated transporter
RMT	receptor mediated transporter
BDNF	brain derived neurotrophic factor
GLUT1	glucose transporter-1
LAT1	L-amino acid transporter
TNFβ	tumor necrosis factor-α
ICAM-1	monoclonal adhesion molecule-1
mAB	monoclonal antibody
cDNA	complementary deoxyribonucleic acid
siRNA	small interfering ribonucleic acid

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Fig. 1a

Fig. 1b

Figure 1.

Figure 1a. Physiological NVU Cell Interactions. Neuron function is dependant upon a homeostatic microenvironment, which results from NVU cell-cell interactions. (A) Astrocytes interpret neuron signaling and modify release of factors in order to help maintain neuron metabolic needs. Astrocyte end feet have close contact with the cerebral endothelium, which helps to regulate blood flow and tight junction integrity. (B) Tight junctions are a unique phenotype to cerebral capillaries, which is distinctly differs from peripheral capillaries. Tight junctions provide an epithelial-like quality to the BBB, creating a physical and electrical barrier to prevent paracellular molecular passage. (C) Pericytes

maintain basal lamina (not depicted) structure and may regulate blood flow. Evidence suggests the basal lamina is a point of contact for NVU intracellular communications. Proper structure is needed for sending and responding to cell-cell communications. (D) Microglia are cerebral monocytes that possess a stellate shape under physiological conditions. Physiological function of microglia within the NVU is not well defined.

Figure 1b. Pathophysiological NVU Cell Interactions. Neuron distress signals are best characterized as inflammatory cytokines. Distress signals released from the neuron alter NVU function. (A) Astrocytes interpret distress signals, become activated and release inflammatory cytokines. This cascade may activate nearby astrocytes, depending on the degree of inflammation. (B) Signals released from the astrocyte end feet lead to decreased tight junction integrity. These signals may result from enzyme release (e.g. MMP-9) leading to tight junction proteins degradation, a lack of tight junction protein forming factors, or a combination of both. (C) Pericyte ghosts have not been characterized in the CNS, but are commonly described in peripheral capillary dysfunction. Dysfunction or lack of pericytes leads to basal lamina thickening, which dysregulates NVU cell signaling and hemodynamics of cerebral blood flow. (D) Microglia respond to inflammatory signals, which result in mobility and ramified shape. These cerebral macrophages phagocytize debris, release cytotoxic factors, and propagate neuro-inflammation by releasing inflammatory cytokines. A balance between the stellate and ramified states is essential for maintaining cerebral homeostasis.

Figure 2. Metabolic and Physical Barrier Schematic of Adjacent Cerebral Endothelial Cells. The BBB possess both physical and metabolic mechanisms by which discrete microenvironments form within the brain to support optimal neuron function. (A) Efflux pumps (MDR, MRP, PGP) are present on the abluminal surface and actively extrude molecules from the endothelial cell, thereby preventing passage into the brain. (B) Metabolizing enzymes (MAO, CYP450s) are present on the abluminal surface and/or within the endothelial cell. These protein degrade harmful molecules and metabolize potential drug therapy, thereby preventing drug activity. (C) Tight junctions are cell-to-cell contacts consisting of transmembrane proteins (e.g. junction adhesion molecules, occludin, and claudin). Tight junctions create a physical barrier limiting paracellular passage and an electrical barrier to repel molecules from attempting BBB transport.

Figure 3. CNS Disease State Progression. Schematic depicts the relationship between NVU dysfunction and CNS disease progression. Further understanding of pathological NVU signaling will give incite to potential drug targets, inwhich will maintain the neuron microenvironment and neuron function.