Blood–Brain Barrier: An Impediment to Neuropharmaceuticals

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The blood–brain barrier (BBB) serves as a highly selective barrier separating the central nervous system from the systemic circulation. Although contributing to neurological health, the BBB restricts the ability of drugs to reach their site of action and thus presents a major challenge to the treatment of neurological disorders. Advances in our understanding of the complexity of the BBB have fostered development of novel pharmacometric models and drug delivery strategies to better predict and improve therapeutic access.

More than a century ago, Paul Ehrlich and Edmond Goldman's studies demonstrating that systemically administered dyes failed to stain brain tissue put forward the concept of a barrier between blood and brain. The dynamic divide known as the blood–brain barrier (BBB) shelters the majority of the central nervous system (CNS) from several features of the systemic circulation. Dysfunction of the BBB has been shown to be involved in the pathogenesis and progression of many neurodegenerative diseases including Alzheimer's and Parkinson's diseases.[1,2] Compromise of BBB integrity occurs as a result of several physiologic or pathophysiologic states and is a well-recognized feature of exposure to several chemotherapeutic agents, ionizing radiation and vascular toxicants which promote formation of reactive oxygen species. Alternatively this barrier poses a considerable therapeutic challenge to many aspects of clinical pharmacology. The presence of an intact BBB limits the ability for therapeutic entities to enter the brain. A deeper understanding of the molecular and cellular features regulating BBB function is, therefore, crucial to the development of more efficient strategies to selectively access neurovascular units of the CNS for therapeutic ends.

Transport across the blood–brain barrier is tightly controlled by the interaction of several cell types comprising the brain microvasculature. The primary constituent of the BBB consists of a specialized array of capillary endothelia containing tight and adherens junctions whose cell–cell interface lack intercellular fenestrations thus reducing paracellular flow. Such junctions are operant within the arachnoid (blood/subarachnoid fluid boundary), cerebrovascular (blood/interstitial fluid), and choroid plexus (blood/ventricular cerebrospinal fluid) interfaces of the CNS. Exceptions to these tight junctions are seen within the circumventricular organs of the CNS mediating neuroendocrine functions and response to systemic toxicants.[3] The unique properties of the BBB are maintained through

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homotypic interactions of a variety of transmembrane proteins including claudins, occludins, and junctional and endothelial selective adhesion molecules.[3] Claudin regulation in particular is important in the maintenance of tight junction integrity. These connections are stabilized within the cell through interaction with zonula occluden adaptors that mediate binding to catenins, vinculin, and ultimately the actin cytoskeleton. Within neighboring adherens junctions cell contacts are further stabilized through hemophilic interaction of vascular endothelial cadherin and platelet–endothelial cell adhesion molecule along with catenin and p120 binding.[4,5]

Endothelial cells of the cerebrovasculature are assisted in maintaining BBB selectivity through the cognate action of surrounding pericytes within the basal lamine and astrocytic end feet mediating neuronal nutrient exchange.[1] Coordinate regulation of such interactions is evidenced from studies of TGF-beta, endothelin-1, bFGF, and GDNF astrocyte/endothelial signaling as well as endothelial-derived PDGFb and VEGF.[3–5] Together the neurovascular unit mediates controlled access of the CNS to essential ions, nutrients, and hormones, while stimulating removal of waste products. Consistent with this, several ATP-dependent efflux transporters which are highly expressed in capillary endothelial cells oversee removal of a broad range of diverse substrates thus limiting CNS access of many clinically important drugs. Of these, P-glycoprotein (P-gp/MDR1) is the most prominent and best characterized together with breast cancer resistance protein (BCRP/ABCG2).[6] As reviewed by Miller in this issue of *Clinical Pharmacology & Therapeutics,*[7] these transporters are regulated by numerous environmental and pathophysiological factors and changes to their expression and activity has implications to both the progression and treatment of several CNS diseases.

While targeting drugs to the CNS is essential for the treatment of many neurological disorders, restrictions imposed by the BBB present a major obstacle to successful implementation. Whether an agent crosses the BBB is dependent upon both its size and physiochemical profile. In the absence of a specific transporter, lower molecular weight entities with higher lipid solubility are favored for BBB transit. The inverse of the square of drug molecular weight is often cited to approximate the influence of size on relative BBB penetration, with an upper cut-off of 400–600 daltons. However, other factors including the degree of plasma protein binding, charge distribution, affinity for uptake or efflux transporters and lipophilicity also significantly influence the rate of CNS accumulation. For instance, neuropeptides and proteins of >600 daltons have been shown to cross the BBB in sufficient concentrations to affect CNS function.[6] Systematic pharmaceutical analysis of a variety of CNS agents together with *in silico* modeling generally support the concept that relative lipophilicity is the biggest single, though not sole, factor in determining BBB penetration.[6–8] However, few computational methods are fully predictive and often compounds chosen on this basis are ill suited for CNS delivery. In particular, several lipophilic compounds exhibit high passive cellular permeability yet exhibit lower than expected accumulation in the CNS as a result of active drug efflux via transporters. While strategies to suppress the activity of efflux transporters at the BBB have been frequently proposed to enhance delivery of therapeutic entities to brain, recent BBB work has also focused on understanding the role of *in vivo* modulators controlling the expression of tight junction constituents such as ZO-1, occludin and claudin-5, as well as the role of ZO and occludin phosphorylation in modulating BBB permeability.[3,4]

As reviewed by Parrish *et al*. in this issue of *Clinical Pharmacology & Therapeutics,*[9] the poor prognosis of primary and metastatic brain tumors stem in part from the difficulties in achieving sufficiently high concentrations of therapeutics at the tumor site. While small molecule agents may be able to penetrate across brain capillary many are efflux transporter substrates; and thereby effectively removed from brain into blood. Moreover novel molecularly targeted therapeutics such as monoclonal antibodies are unable to cross an intact BBB. Likewise difficulties arise in targeting diagnostics to tumor cells shielded by the BBB. Therefore, despite the extraordinary efforts spent in developing novel therapeutics, overall there has been limited success in improving patient survival. In the context of these outcomes, this emphasizes the importance of developing new strategies to overcome the blood–brain barrier.

Many delivery tactics have been examined over the past decade such as localized drug delivery or modification of drug entities to either reduce efflux potential or exploit influx processes across the BBB. Inhibition of drug efflux transporters has been proposed as a simple mechanism to increase delivery of therapeutic agents to the brain. While preclinical studies support this approach, demonstrating 2- to 100-fold increases in CNS drug exposure, appreciable changes are not seen in humans. Therefore, clinical outcomes with transporter inhibitors have been largely unsuccessful. Based on review of clinical evidence, the International Transporter Consortium has recently concluded that very few drugs that are potent inhibitors *in vitro* reach sufficiently high unbound systemic concentrations or are sufficiently tolerable to impose clinically significant inhibition at the BBB in patients.[10] While more extensive inhibition could potentially be achieved in patients, this would often require doses that are associated with unacceptable adverse effects.

More recently, research designed to promote the transfer of CNS drugs and biologics across the BBB have focused on strategies modifying aspects of absorptive or receptor-mediated transcytosis or by nanoparticle-mediated lipophilic transfer. In this issue of *Clinical Pharmacology & Therapeutics*, Pardridge discusses the molecular Trojan horse platform developed by ArmaGen which uses monoclonal antibodies targeting receptors on the BBB to promote receptor-mediated penetration of large macromolecules.[11] Using this method, re-engineering of several biological agents as a fusion protein with antibodies directed to the insulin or transferrin receptors increased brain uptake in preclinical animal models. Future work will examine whether these preclinical findings can be translated to an improvement of therapeutic outcomes in the clinic.

Other strategies proposed to increase CNS exposure to pharmaceuticals include temporary disruption of the BBB. However, challenges remain at present to dynamically regulate BBB function in a therapeutically useful manner and information on the long term consequences of these transient changes is unknown. Key among these are gaps in our current understanding of human BBB signaling *in vivo* as part of an integrated neurovascular unit.[12] With respect to molecular assessments of BBB function, significant progress has been made in the identification of biomarkers relevant to endothelial injury, however, none yet described exhibit sufficiently high specificity with respect to monitoring insult to cerebrovascular tissues. Concerning reversible modulation of BBB integrity, studies have at present identified several mediators capable of either enhancing (IL-1β, interferon-gamma, tumor necrosis factor-α, VEGF, and histamine) or reducing (steroids, erythropoietin and calcium channel blockers) endothelial permeability through distinct signaling pathways.[3–5] Intriguing advances

in noninvasive CNS delivery have also been made recently in the area of intranasal applications.[13]

The challenge is to gain sufficient understanding of these mechanism to use them in a productive manner as BBB opening needs to be kept as brief as is practical to reduce potential edema and other side effects.

Technological advances in neuroimaging have made it possible to noninvasively examine the pathology of neurological diseases, obtain information on drug distribution to brain regions as well as assess biomarkers reflective of efficacy. Moreover, the advent of combining multiple imaging modalities allows for the functional imaging of distinct physiological mechanisms involved in brain function and disease. Current means of assessing BBB integrity in humans focus on either magnetic resonance imaging (MRI) or computed tomography (CT) due to favorable spatial resolution. The MRI and CT modalities are also most frequently used as diagnostic tools to assess brain tumors. Functional MRI imaging is frequently superior to CT due to its far greater resolution of soft tissue changes, however, CT continues to be widely used in urgent clinical settings due to its greater speed (5 vs. 30 minutes) and perceived lower cost. Though both gadolinium (MRI) and iodine (CT) based contrast agents exhibit some propensity for allergic reactions, incidence is lower for MRI contrast agents and their use does not necessitate exposure to ionizing radiation. Under circumstances where no contrast agent can be used greater detail is obtained using MRI vs. CT.[14] These features may be of particular significance in cases of multiple, longitudinal assessments. This is important given the fact that BBB disruption may be an early precursor to some forms of neurodegeneration and many neurodegenerative conditions exhibit a significant vascular component (i.e., stroke, dementia, epilepsy, multiple sclerosis). On the other hand, many contrast agents do not cross an intact BBB, therefore, much assessment of CNS neurodegeneration is still performed through neuroanatomic means with MRI (volumetric comparisons, vascular atrophy etc.).[14] The use of manganese contrast agents has also been investigated for purposes of functional MRI neural imaging in recent years due to their ability to cross the BBB and enter calcium channels. While radio labeled small molecules have been used for CT, positron emission tomography (PET) and single positron emission computed tomography (SPECT) analyses; the lower relative spatial resolution of these methods continues to impose a significant challenge.

Molecular imaging of the brain by PET to confirm whether drugs reach their target has become an essential tool in most CNS drug development programs. As reviewed by Raaphorst *et al*. in this issue,[15] PET imaging using radiolabeled pharmaceuticals is frequently used to noninvasively assess the activity of efflux transporters at the BBB as well as determine the extent of drug uptake and target engagement within the CNS. Nevertheless, while these imaging tools have played an important role in predicting bioavailability of drug substrates into brain and development of drug resistance; difficulties remain due to shortcomings in brain uptake, transporter specificity, and metabolic profiles of current radiolabeled tracers. Identification and development of radiotracers with high affinity and specificity to transporters or other biomarkers are needed.[15,16] Given the importance of this key technology in neurological research and drug development, the FDA has provided draft guidance on Investigational New Drug Applications for Positron Emission Tomography Drugs.

One of the major challenges that has impeded advancements in CNS drug development has been the poor preclinical to clinical translatability of neuropharmaceuticals

which stems in part due to poor delivery to the CNS. While the choice of lead compounds are generally determined through a series of assessments in preclinical models, the ability of these drugs to cross the BBB and achieve therapeutic concentrations at their site of action is often not considered until later stages of development. Moreover, estimating brain-drug bioavailability is complicated by species differences or disease-induced changes in drug transporters at the BBB. To this point, it is necessary to develop pharmacometric models that define and integrate the multiple parameters which impact the pharmacokinetics of a drug within the CNS. Such models can delineate the contribution and variability of individual biological processes along with the physiochemical properties of the drug entity. As introduced in this issue by de Lange and Hammarlund-Udenaes,[17] a "Mastermind Approach" to *in silico* predictions of brain exposure combines advanced strategic preclinical research such as microdialysis and PET imaging along with extensive mathematical modeling reflecting the intricacies of CNS physiology. Considerations of unbound drug concentrations in brain as well as time-resolution have led to improvements in the prediction of CNS drug activity by these pharmacometric approaches.

Another key challenge in the development of neuropharmaceuticals stem from the difficulties in assessing efficacy in preclinical settings. There are clear deficiencies and differences in the available animal models compared with their human equivalents, particularly for neurodegenerative disorders. Over the past several decades much progress has been made in identifying and testing the functional role of key constituents within tight and adherens junctions *in vivo*, as well as the transport mechanisms operant in endothelia, astrocytes, and pericytes in health and disease. These findings have helped set the stage for many of the signal transduction studies in cultured cells and rodent slice preparations which followed. These have led to key advances in understanding the fundamental mechanics of many neurological disorders and their relation to the BBB. The challenge now is to determine with greater resolution how closely our existing animal models compare with human BBB responses *in vivo*. Though such challenges may appear daunting, recent advances in the derivation of pluripotent stem cells (iPS) from differentiated human tissues[18,19] together with progress in lineage differentiation of stem cell stems, provides an unprecedented opportunity to use these methods both alone or in conjunction with rodent models to interrogate BBB function and neurological disorders. Moreover our increased understanding of the physiological and biochemical parameters in rodents and primates allows current physiologically based pharmacokinetic modeling to extrapolate across species. Future identification and validation of plasma biomarkers which reflect disease progression will be sure to promote rapid advances in the field. Nevertheless many of our remaining gaps in understanding integrated BBB signaling, the endogenous modulation of BBB dynamism, and posttranslational control of tight junction integrity, will continue to be usefully informed from homologous studies in lower mammals.

Conquering these challenges impact the entire paradigm of drug development and will be key to future advancements in neurological health. Due to the advancing age of the population, the number of patients with neurological disease is increasing, however, there are few effective treatments for the majority of CNS disorders. Hence the field of neuropharmaceuticals is an important area of growth in global drug development. Developing a greater understanding of the cellular and molecular mechanisms which control the BBB will enable scientists to optimize the delivery of small molecules, biological therapeutics and diagnostic agents

to target sites within the CNS. The translation of basic and clinical neuropharmacology research are necessary to guide clinical trial design and provide direction for strategic expansion of preclinical programs. To this point clinical pharmacologists will play an important role in the integration and translation of *in silico*, *in vitro*, and *in vivo* research methods for the discovery, development, and clinical usage of neuropharmaceuticals.

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