



Strategies to advance translational research into brain barriers

Edward Neuwelt, N Joan Abbott, Lauren Abrey, William A Banks, Brian Blakley, Thomas Davis, Britta Engelhardt, Paula Grammas, Maiken Nedergaard, John Nutt, William Pardridge, Gary A Rosenberg, Quentin Smith, Lester R Drewes

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Oregon Health and Science University, Department of Neurology, Veterans Administration Medical Center, Portland, OR, USA (E Neuwelt MD); King's College London, Pharmaceutical Science Research Division, BBB Group, London, UK (J Abbott PhD); Memorial Sloan-Kettering Cancer Center, Department of Neurology, New York City, NY, USA (L Abrey MD); VA Medical Center, Geriatrics Research, Educational, and Clinical Center and Saint Louis University, Department of Internal Medicine, Division of Geriatrics, St Louis, MO, USA (W A Banks MD); University of Manitoba, Department of Otolaryngology, Winnipeg, Canada (B Blakley MD); University of Arizona, Pharmacology Department, Tucson, AZ, USA (T Davis PhD); University of Bern, Theodor Kocher Institute, Bern, Switzerland (B Engelhardt PhD); Texas Tech University Health Sciences Center, Garrison Institute on Aging, TX, USA (P Grammas PhD); University of Rochester, Department of Neurosurgery, 575 Elmwood Avenue, Box 645 Rochester, NY, USA (M Nedergaard PhD); Oregon Health and Science University, Department of Neurology, Portland, OR, USA (J Nutt MD); University of California Los Angeles, Warren Hall 13-164, Los Angeles, CA, USA (W Pardridge MD); University of New Mexico, Department of Neurology, Albuquerque, NM, USA (G A Rosenberg MD); Texas Tech University Health Sciences Center, Pharmaceutical Sciences, TX, USA (Q Smith PhD); and University of Minnesota, Department of Biochemistry and Molecular Biology, Duluth, MN, USA (L R Drewes PhD)

Correspondence to: Edward Neuwelt, 3181 SW Sam Jackson Park Road, L603, Portland, OR 97239, USA neuwelte@ohsu.edu

There is a paucity of therapies for most neurological disorders—from rare lysosomal storage diseases to major public health concerns such as stroke and Alzheimer's disease. Advances in the targeting of drugs to the CNS are essential for the future success of neurotherapeutics; however, the delivery of many potentially therapeutic and diagnostic compounds to specific areas of the brain is restricted by the blood–brain barrier, the blood–CSF barrier, or other specialised CNS barriers. These brain barriers are now recognised as a major obstacle to the treatment of most brain disorders. The challenge to deliver therapies to the CNS is formidable, and the solution will require concerted international efforts among academia, government, and industry. At a recent meeting of expert panels, essential and high-priority recommendations to propel brain barrier research forward in six topical areas were developed and these recommendations are presented here.

Introduction

The difficulty in delivering therapeutic drugs to the CNS is deemed by many researchers to contribute to the limited success of neurotherapeutics. In their neuroprotective role, the blood–brain barrier (BBB), the blood–CSF barrier, and the other specialised brain barriers hinder the delivery of many potentially important diagnostic and therapeutic drugs to the CNS. The effective delivery of molecules and genes to the CNS is problematic and will require a concerted effort among academia, governments, and industry. The aims of this Review are to outline current research in the field of brain barriers, the main advances made since 2000, the barriers to progress, and to recommend research priorities and the resources needed to advance the field.

Inflammation and brain barriers

The BBB and the neurovascular unit (NVU) are involved in various neuroinflammatory processes, and the pathophysiology at most brain barriers is affected by neuroinflammation.¹ Inflammatory events probably occur at the blood–retinal barrier and other specialised barriers.^{2,3} One of the unifying concepts in brain barrier research is the neuroinflammatory interactions with the BBB; for example, the involvement of brain barriers in neurodegenerative diseases, CNS injury, neuroendocrine secretions, and drug delivery. Important examples of BBB–neuroinflammatory interactions include alterations in BBB permeability (eg, disruption of, and alterations to, tight junction architecture and dysregulation of transporters), enhanced movement of immune cells across the BBB, secretion and transport of neuroimmune substances by the cells that comprise the various brain barriers, developmental changes in the BBB induced by perinatal inflammatory events, and the traffic of pathogens across the BBB. As the BBB develops, there is evidence of inflammation-induced changes in barrier function and subsequent neurological and behavioural development.^{4,5} Proinflammatory

interactions with the BBB are at the basis of various diseases, including multiple sclerosis, posterior uveitis, CNS vasculitides, stroke, Alzheimer's disease, neuroAIDS, insulin resistance and obesity, and cerebral malaria. The concept of the NVU emphasises the dynamic and continuous cross-talk among the cellular elements of the NVU (eg, endothelial cells, pericytes, astrocytes, and microglia) and is easily expanded to include communication with cells on the blood side of the BBB (eg, immune cells, tissue macrophages, and dendritic cells); much of this cross talk is mediated by substances with neuroimmune activity (figure 1).

Traffic of leukocytes

Immune cells gain access to the CNS, and immune responses are thus mounted within the CNS.⁶ The unique microenvironment of the CNS strictly controls these immune reactions, which start with the tightly controlled entry of immune cells into the CNS at the endothelial barrier and, with different characteristics, at the epithelial–CSF or blood–CSF barriers in the choroid plexus. The recruitment of circulating immune cells into the CNS depends on the sequential interaction of different adhesion and signalling molecules on the leukocyte and endothelial cell surfaces. The process by which immune cells cross the brain endothelial barrier—diapedesis—is transcellular rather than paracellular for some cell types, although the molecular mechanisms remain to be characterised fully. Chemokines on endothelial cells are involved in diapedesis and are translocated from the abluminal to the luminal surface; chemokines are probably translocated from other cells of the NVU by unknown mechanisms.⁷

Research on leukocyte trafficking has been translated into the clinic, where patients with multiple sclerosis are successfully treated with natalizumab, an anti- α 4-integrin antibody. The blockade of leukocyte traffic across the BBB is now the best-validated treatment for multiple sclerosis.^{8,9}

Cytokines and immune modulators

Cytokines and other immune modulators (eg, prostaglandins, lipopolysaccharides, or opiates) have many effects on BBB function. The interactions of the BBB with cytokines, including interleukins, interferons, neurotrophic factors, smaller neurotrophic peptides, and adipokines, have been studied.¹⁰ Cytokines and immune modulators not only disrupt the BBB but can also selectively modulate saturable transport systems in the BBB. Injuries to the CNS modulate the activity of transporters, including those that carry immune-active and neurotrophic substances such as cytokines; this modulation is probably through immune mechanisms. Modulation of efflux (brain-to-blood) transport systems, such as P-glycoprotein, by neuroimmune substances and events can affect drug delivery and the microenvironment of the brain.¹¹

Because the BBB is a polarised cellular interface between the blood and the CNS parenchyma, asymmetrical responses can occur:¹² the response of the BBB can occur in the opposite compartment to the stimulus; thus, a communication pathway is formed between the peripheral and CNS tissues.

Transport of pathogens across the CNS

Parasites, bacteria, and viruses invade the CNS and have various strategies to bypass or cross the brain barriers. Some pathogens enter as free agents, often by transcytosis, whereas others, such as HIV-1, can enter the CNS inside infected immune cells by subversion of highly regulated processes, such as diapedesis and adsorptive endocytosis.^{13,14}

Pathogen-produced proteins interact directly with tight junction proteins; for example, claudin-3 and claudin-4 act as high-affinity receptors for *Clostridium perfringens* enterotoxin, which leads to barrier disruption.¹⁵

The *Neisseria meningitidis* bacterium adheres to brain endothelial cells and stimulates phosphorylation of cortactin by Rho-dependent mechanisms to help adhesion and internalisation. These mechanisms also impair leukocyte–endothelial cell interaction and, thus, diminish the normal inflammatory responses in the host cells.¹⁶

Inflammation as the BBB develops

There is evidence of systemic inflammation-induced changes in BBB function during development and subsequent neurological and behavioural changes.^{4,5} The effects of inflammation in immature organisms on brain development and behaviour in the adult might be extremely important in human beings.

Important issues for inflammation and brain barriers

The main concern with regard to inflammation and brain barriers is the role of activated BBB cells in the coordination of the neuroinflammatory axis. Brain endothelial cells and other elements of the NVU are

activated by neuroimmune stimulation. BBB cells are never inert, but are in continuous cross-communication with the cellular elements that surround them to maintain the properties of the BBB. The spectrum of neuroimmune interactions with the BBB are part of the normal physiology of the barrier, for example, during immunosurveillance. All cell types in the brain barrier (vascular, ependymal, retinal, tanycytic, and other specialised barrier cells) can be activated and can interact with other components of the NVU.

Research priorities

A research priority should be the determination of the molecular mechanisms of leukocyte trafficking through the NVU, diapedesis across the BBB, and entry into the CSF. Researchers should also investigate the endothelial cell and NVU signalling cascades in leukocyte diapedesis across the BBB.

The development of improved in vitro and in vivo BBB models that account for physiological factors such as blood flow and shear force would help to progress this topic. Researchers need to identify the components of tight junction complexes and the regulation of tight junction permeability in brain endothelial cells. Other areas of research include the mechanisms of chemokine and cytokine production, secretion, transport, localisation, and uptake at the luminal and abluminal surfaces of the BBB. Finally, the contribution of BBB endothelial cell secretion to physiology and disease is a research priority.

Barriers to progress

The lack of recognition that the BBB is not just a barrier, but is an active, regulated, and regulatory interface, with transport, secretory, and enzymatic activities, poses a barrier to the progress of research into inflammation and the brain barriers. Additional obstacles include the lack

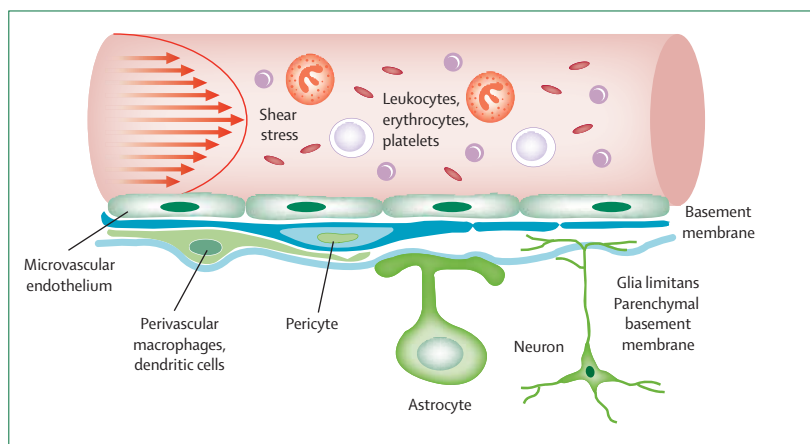


Figure 1: The neurovascular unit (NVU)

The NVU is a complex cellular system that includes circulating blood components, highly specialised endothelial cells, a high concentration of pericytes embedded in the endothelial cell basement membrane, perivascular antigen-presenting cells, astrocytic endfeet, and associated parenchymal basement membrane and neurons. Although the endothelial cells form the BBB, the continuous communication between the brain endothelium and the other cellular elements of the NVU are prerequisites for barrier function.

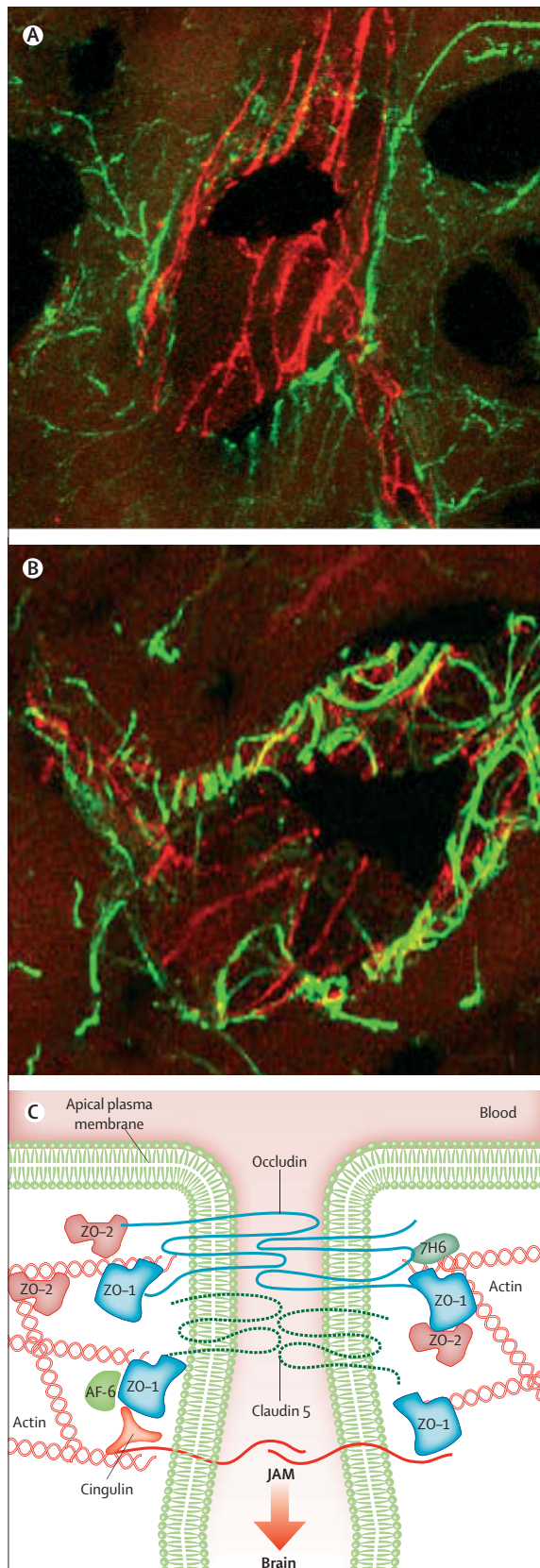


Figure 2: In vivo studies of the BBB in the rat after transient ischaemia and reperfusion with the suture method

(A) Brain section of a spontaneously hypertensive rat after 90 min of ischaemic middle cerebral artery occlusion with a suture and 3 h of reperfusion. A confocal image of a blood vessel in the non-ischaemic piriform cortex is shown. The astrocytes are immunolabelled with GFAP (green) and the tight junction protein claudin-5 is immunostained red. Note the linear bands of claudin-5 surrounded by the GFAP-positive astrocytic foot processes. (B) A similar image from the ischaemic piriform cortex showing disruption of the claudin-5-positive bands. (C) The molecular components of the tight junction.^{1,22}

of adequate in vitro and translational models and clinical studies of the BBB.

Recommendations

We recommend that resources are set aside to fund research into the BBB and a BBB resource centre that includes a database of BBB-specific genes and BBB-specific reagents. Courses on the BBB in higher education, particularly in postgraduate programmes, are important for the advancement of this area and should be encouraged. Also, the recognition that BBB research is an interdisciplinary subject that requires the knowledge and collaboration of cellular and molecular biologists, neurobiologists, immunologists, physiologists, pharmacologists, and behavioural scientists is crucial.

Injury and the brain barriers

Neurotrauma and related neuropathological events (stroke, head injury, ischaemia and reperfusion, haemorrhage, and infarction) share a common feature: the alteration of the BBB at and around the site of injury.¹⁷ The brain barriers have important roles in the overall homeostasis of the CNS, which include regulation of the exchange of nutrients, ions, water, metabolites, and xenobiotics between the blood and the CNS^{1,18} and the prevention of entry of potentially neurotoxic plasma constituents into the CNS.^{19,20} Thus, changes in the BBB can profoundly affect the progression of injury, including the loss of neurons and specific CNS functions, the response to therapy, and the time course and extent of recovery. Clearly, it is important to understand the underlying function and molecular biology of the barrier tissues and how these parameters change with injury.^{1,18,21} This requirement is complex because the barriers constitute the combined function of many proteins, cellular structures, and cell types that sense and respond to their immediate microenvironment with a range of responses, from the subtle to the dramatic.

The effects of stroke (figure 2)^{1,22} and head injury are two main areas of clinical research into the BBB. The insights from in vitro studies of the molecular features of the BBB have been translated into in vivo studies, where disruption of the tight junction proteins is seen in experimental models of injury, including reperfusion after stroke. Neutrophils are reported to have great potential as neuroprotective agents in brain ischaemia or injury, but the results of clinical trials have been disappointing.²³ Although studies in animals have found that brain-derived neurotrophic factor has a pronounced protective effect after ischaemic lesions,²⁴ clinical trials with brain-derived neurotrophic factor for the treatment of acute ischaemic stroke or neurodegenerative diseases have, thus far, failed owing to poor blood–brain permeability and the short plasma half-life (<10 min) of brain-derived neurotrophic factor.²⁵ Fibroblast growth factor is a cationic peptide that is transported across the BBB by absorptive-mediated transcytosis after intravenous

administration.²⁶ Studies in rats have shown that fibroblast growth factor protects hippocampal neurons against ischaemia–reperfusion injury.²⁷ However, clinical trials of fibroblast growth factor in patients with acute ischaemic stroke were stopped owing to hypotension and higher mortality rates in the treated groups compared with the control groups.²⁸ Perhaps the greater understanding of the extracellular matrix that has come from recent studies on the proteolytic disruption of basal lamina proteins (eg, laminin, fibronectin, and type IV collagen) and tight junctions might explain these clinical results.²⁹ The fact that the BBB response to injury is biphasic is often overlooked and warrants further study.

Important issues for injury and the brain barriers

Future research into trauma and stroke will involve studies of the natural progression of the disruption of brain barriers in human beings and parallel studies that analyse the molecular events *in vivo* and *in vitro* in animals. The delineation of timing of the history of the disruption of the brain barriers in human beings is crucial to improve our experimental models; however, modern genomic and proteomic methods are also important for the discovery of new mechanisms that could lead to successful therapies. Analysis of the signalling pathways should identify upstream genes as potential therapeutic targets for amelioration of the deleterious effects of BBB disruption in trauma and other pathologies. To limit brain injury and speed recovery, there is an urgent need for high-resolution, non-invasive, *in vivo* tools (molecular probes and detection technology) that will help us monitor brain barrier function (tight junctions, transporters, and channels) within the components of the NVU. These tools should include CNS-deliverable, molecular-level reporters of expression, (eg, small molecules with specific affinity for the protein of interest) activity (substrates), and inhibitors that can be used in therapy. In addition, appropriate imaging technology (PET, multiphoton confocal microscopy, and MRI) should enable us to monitor in time and space over a range of resolutions. The development of imaging technology is the single most crucial topic for the advancement of research into brain injury.

Research priorities

The quantification of the time course of barrier breakdown in response to molecules of different sizes after different types of neurotrauma is a research priority. Researchers should also investigate the molecular targets and changes in the BBB signalling pathways after brain injury, the role of tight junction proteins in BBB homeostasis, and the changes to the BBB that are associated with brain injury or diseases of the CNS. The determination of how patterns of barrier disruption depend on the nature and location of the injuries and the contribution of brain barrier breakdown to secondary injury after neurotrauma are also research priorities.

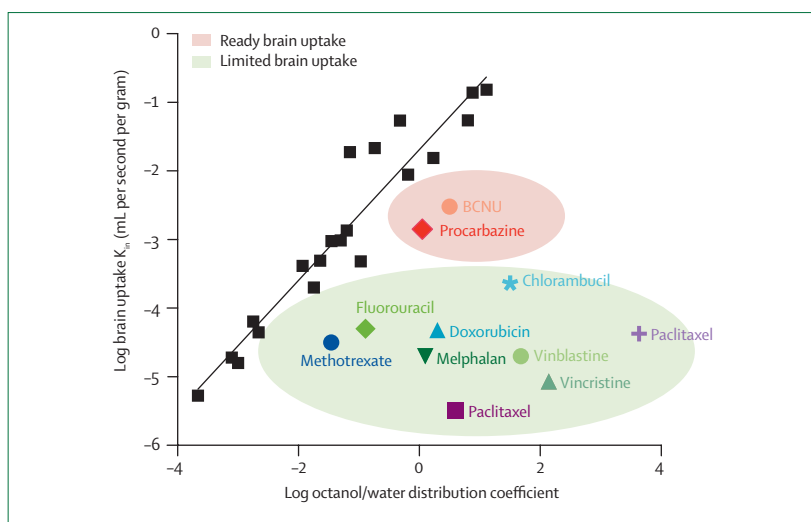


Figure 3: Relationship between BBB permeability and the octanol/water partition coefficient for chemotherapeutic drugs

The solid line is the least-squares fit to the data for compounds that are not actively taken up by the brain or pumped out by the BBB. The permeability to specific chemotherapeutic drugs is shown. Many chemotherapeutics have limited uptake into the brain because they are either polar (low octanol/water distribution coefficient), have high molecular weight (>500), are bound to plasma proteins, or are transported out of brain by active efflux pumps.³³

Barriers to progress

A main barrier to progress is the spatial resolution of current imaging methods, which cannot resolve the localisation of increased BBB permeability in the original trauma and the tissue that surrounds it. Additionally, barrier tissues have not been regarded as therapeutic targets, and because there is a lack of defined models for specific CNS injuries that translate to human beings, the progress of translational science in injury and the brain barriers is slowed. Progress is also hindered by the cost of imaging equipment, the need for teams of scientists and engineers to carry out imaging studies of brain injury, and the limited cross-disciplinary research into the BBB.

Recommendations

We recommend the designation of specialised centres for human and molecular studies of brain injury. Improved collaboration between academia and pharmaceutical companies will speed the discovery of therapies for head trauma and stroke. We also recommend studies that investigate the ability of several drugs to prevent damage to the BBB. Improvements in the training of the next generation of experts on the BBB is crucial to continue advancement within the field of injury and the brain barriers.

Tumours and the brain barriers

The study of the BBB is crucial to understand and manage primary and metastatic brain tumours. Many factors that modify vascular endothelial function and the tightness of the barrier in brain tumours have been identified,³⁰ physiological factors,³¹ including tumour interstitial fluid pressure, markedly influence drug

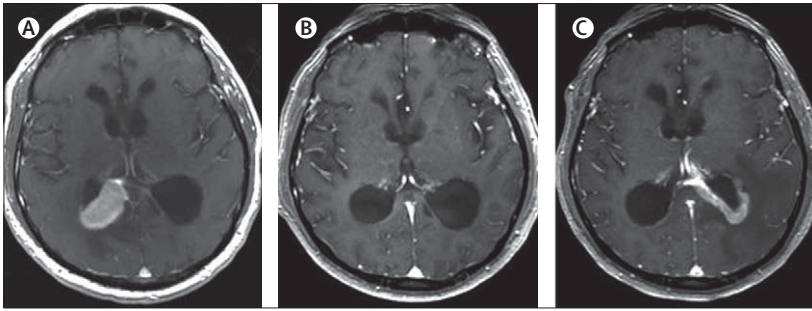


Figure 4: Delivery and efficacy of zevalin for primary CNS lymphoma
T1-weighted MRI with gadolinium contrast axial scans. (A) Scan before giving ^{90}Y -zevalin shows enhancing tumour (high signal) around the occipital horn of the ventricle. (B) Complete response 2 months after giving ^{90}Y -zevalin. (C) Relapse around the opposite occipital horn 3 months after treatment with ^{90}Y -zevalin, with continuing complete response at the site of the original tumour. Figure reproduced with permission from the American Society of Clinical Oncology.³⁵

penetration into tumours.³² The complex pathophysiology of the blood–tumour barrier can be manipulated to improve our knowledge and management of brain tumours. Neuroimaging shows clear disruption of the BBB in many tumours; however, the drug-based therapies available are often variably excluded from the tumour, in part, by the activity of efflux transporters (figure 3).³³

Tumour and brain pharmacokinetics

The adequate delivery of chemotherapeutic drugs to brain tumours is crucial; drug delivery can be impeded by the BBB and tumour interstitial fluid pressure. An important question for any drug delivery approach is how much drug is delivered and to which area of the tumour. The determination of drug concentrations in the tumour and the local effects of the drugs under investigation are important in early-phase clinical trials. There are multiple techniques to develop and assess drug delivery to brain tumours, including microdialysis in animals and humans.³⁴ Furthermore, the delivery of large biomolecule therapeutics to the brain and tumours might be particularly low (figure 4).^{35,36}

Delivery strategies

Multiple strategies to circumvent, disrupt, or manipulate the BBB have been used to improve the delivery of drugs to brain tumours. Reduction of the interstitial fluid pressure can enhance the time–concentration exposure of a drug to tumour tissue.³⁷ Convection-enhanced delivery, where chemotherapy is infused directly into the tumour through strategically placed catheters, has progressed from animals to clinical trials in the past 10 years. Polymer-based, local drug delivery to tumours has been part of the standard care for selected patients with malignant gliomas since 1996, when the FDA approved the use of carmustine-impregnated wafers for recurrent glioblastoma multiforme. The limitations of polymers and convection-enhanced delivery include uneven drug distribution in heterogeneous infiltrating tumours and variable release of the chemotherapeutic drug.

BBB disruption, with or without intra-arterial chemotherapy, has been extensively investigated as a technique to enable the delivery of drugs, which can be as large as the herpes virus,³⁸ across the BBB. Most commonly, this approach uses osmotic BBB disruption with hyperosmotic mannitol followed by intra-arterial chemotherapy, although BBB disruption guided by MRI and induced by ultrasound might also be effective.^{39,40} Although relatively small numbers of patients have been treated, there have been promising results with osmotic BBB disruption and intra-arterial chemotherapy. There is evidence for long-term complete responses without the cognitive loss associated with whole brain radiation therapy when this technique is used with methotrexate-based therapy for primary CNS lymphoma⁴¹ and isolated brain parenchyma relapse of systemic lymphoma.⁴² The delivery of antibodies, such as rituximab, can now be studied in animals with primary CNS lymphoma⁴³ and in the clinic.⁴⁴ Similar long-lasting responses after enhanced delivery have been seen in previously difficult-to-treat embryonic tumours, such as pineoblastoma.⁴⁵

Gene therapy and brain tumours

The gene therapies developed over the past 20 years have the potential to successfully treat malignant brain tumours. Although clinical studies have found that there is no danger for patients with brain tumours, the clinical benefits have been limited, possibly because of hindrance by the BBB. The published results of clinical trials have been summarised by Fulci and Chicocca.³⁶

Imaging

Nanoparticle MRI contrast agents have been developed to measure tumour blood volume and flow.⁴⁶ When combined with traditional gadolinium imaging, accurate measures of tumour permeability can be made, which help to assess the responses to therapeutics, such as antiangiogenic drugs. Brain imaging technologies have been successfully applied to measure the delivery and local effects of anticancer drugs in brain tumours (figure 4).³⁵

Delivery of drugs to tumours is often associated with pathological changes that can confound assessments of tumour progression. New applications of PET, single photon emission computed tomography, and new MRI techniques might be useful to distinguish between tumour growth and injury caused by treatment. The changes seen with imaging (eg, pseudo progression at the end of radiation)⁴⁷ complicate the clinical management of patients with brain tumours after the delivery of drugs across the BBB.

Important issues for tumours and brain barriers

Imaging is the single most important topic and a research priority. Current methods restrict the accurate assessment of tumour size, location, type, and response to therapy.

The information obtained in most studies is limited to a small subset of properties, such as BBB passive permeability, blood volume, blood flow, and glucose metabolism. Improved imaging techniques have the potential to monitor and quantitate accurately tumour invasion, growth, angiogenesis, cellular apoptosis, necrosis, inflammation, oedema, gene expression, and response to therapeutic intervention in human beings and small animals. Imaging techniques can be combined with continuous assays of drug delivery to the tumour (eg, microdialysis) to assess more appropriately the in vivo response to therapy.

Research priorities

The priorities should be the study of changes in the proteome of the BBB–tumour interface during tumour progression and treatment. Researchers should also investigate the role of the BBB in tumour invasion and micrometastases, particularly tumours that develop when the BBB is intact. Additionally, factors that determine drug transport across the BBB should be studied so that brain-specific or brain-tumour-specific drugs can be developed. Approaches such as nanotechnology-based, receptor-mediated, and carrier-mediated targeted drug delivery to the CNS tumour should be investigated. Other major research priorities include imaging of tumour invasion, growth, angiogenesis, cellular apoptosis, necrosis, inflammation, oedema, and gene expression.

Barriers to progress

The current in vitro and animal models are inadequate for studying the BBB–brain tumour interface, which is a main barrier to progress in the field. Although brain metastases are, by far, the most common brain tumour, they are one of the least studied. The lack of information about pharmacokinetic and pharmacodynamic endpoints in the brain also hinders advancement in the study of tumours and the brain barriers. Lastly, the expense and regulatory requirements related to the conduct of clinical trials creates a challenge to further this area of science.

Recommendations

We recommend the development of representative tumour model systems that can be used to monitor tumour growth and drug action in a non-invasive way. The development of surrogate measures of the BBB–brain tumour interface (eg, permeability and drug delivery) is also important. The development of new imaging modalities that are validated with actual tissue assays will be crucial to advance this science. We also recommend phase zero clinical trials to optimise drug and tissue assays and validate the delivery of new drugs or delivery strategies before proceeding to larger trials. Funding initiatives for BBB and blood–tumour barrier research are vital for progress in this field, in addition to

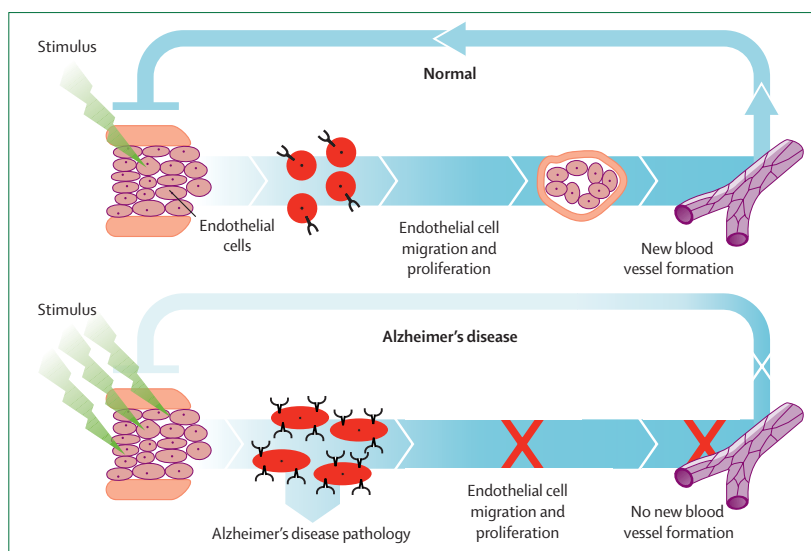


Figure 5: Proposed scheme for endothelial activation in healthy patients and patients with Alzheimer's disease

Top. Under normal conditions, endothelial cells respond to stimuli such as hypoxia or IL-1 β by upregulating several gene products with biological activity, including inflammatory cytokines and proteases. Endothelial cells then migrate, proliferate, and form new blood vessels, which, through a negative feedback loop, turn off the process. Bottom. In Alzheimer's disease, endothelial cells are activated in response to stimuli, but there is no migration, proliferation, or angiogenesis. Thus, in patients with Alzheimer's disease, endothelial cells continue to release inflammatory cytokines and proteases, with deleterious consequences for neurons.

improvements to the grant review process for interdisciplinary projects.

Neurodegenerative diseases and brain barriers

Research to understand the pathogenesis of neurodegenerative diseases and to design effective therapies has focused mainly on neurons. This neuron-centric view has contributed to our knowledge of neuronal dysfunction, neuronal death pathways, and the accumulation of proteinaceous aggregates during chronic neurodegenerative processes; however, this approach has not resulted in therapeutics that modify the disease. The pathogenesis of neurodegenerative disorders (figure 5) is more complex than previously thought, and the lack of success of neurocentric-based therapies might be due to the participation of non-neuronal cells in the disease process.

In the clinical arena of neurodegenerative disorders, the BBB is traditionally seen as irrelevant or simply as a barrier to treatment. In reality, the brain barriers have important roles in the pathology and progression of a broad spectrum of CNS disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis. From a clinical and basic science perspective, the brain barriers are vital for CNS homeostasis and the preservation of neuronal integrity. The alterations to the cerebral microcirculation and BBB in neurodegenerative diseases need to be investigated, and the CNS microvasculature might be a new target for therapeutic development.

BBB and amyloid beta transport in and out of the brain

The receptor for advanced glycation end products (RAGE) is thought to be a primary transporter of amyloid beta ($A\beta$) from the systemic circulation across the BBB and into the brain, whereas low-density lipoprotein receptor-related protein-1 mediates transport of $A\beta$ out of the brain.⁴⁸ Alzheimer's disease is associated with changes in the distribution of RAGE and low-density lipoprotein receptor-related protein-1 in the hippocampus and cortex of human beings, which suggests that Alzheimer's disease might develop as a clearance storage disorder, in which the accumulation of $A\beta$ leads to neurodegeneration.⁴⁹ On the basis of the BBB transport-clearance hypothesis, various immune and non-immune $A\beta$ clearance treatments for Alzheimer's disease are currently under development. These include inhibitors of the RAGE- $A\beta$ interaction at the BBB, which reduces $A\beta$ transport into the brain, reduces neuroinflammation, and increases the responses of cerebral blood flow to neuronal activity.⁵⁰

Circumvention of the BBB

One of the great successes in the treatment of neurodegenerative disorders was the discovery of the depletion of dopamine in Parkinson's disease and the recognition that the precursor of dopamine, levodopa, was transported across the BBB and provided symptomatic benefit to patients. Other therapies for Parkinson's disease do not cross the BBB to any notable extent, which leads to the need for improved delivery strategies. A good example of this is glial-derived neurotrophic factor, a potent neurotrophin that can revive damaged dopaminergic nerve terminals in animal models of Parkinson's disease.⁵¹ Glial-derived neurotrophic factor will not cross the BBB, and alternative methods of administration have been attempted.⁵² In patients with Parkinson's disease, intracerebroventricular administration of glial-derived neurotrophic factor with implanted reservoirs and ventricular catheters proved that glial-derived neurotrophic factor was biologically active but lacked clinical efficacy. Despite direct intraparenchymal infusion to ensure sufficient delivery to the CNS and accurate targeting, the distribution of glial-derived neurotrophic factor to the required tissue is still a major problem.⁵³ The development of less invasive methods to deliver therapeutic molecules, such as proteins, small RNAs, and genes, to specific regions of the CNS is clearly important.⁵⁴

Important issues for neurodegenerative diseases and brain barriers

Ageing of the vascular system may be the most important risk factor for neurodegeneration. We hypothesise that the alterations in the normal neuronal milieu that occur with age, due to the alterations to the cerebral microvasculature, CSF circulation, and disruption to the

NVU, predispose the CNS to neurodegeneration. We propose the investigation of the changes in the NVU and CSF turnover that occur with ageing: the secretion of endothelial toxins and inflammatory factors; alterations to other structural components of the NVU (tight junctions, basal lamina, pericytes, and astrocytic foot processes); CSF production, clearance, and composition; clearance of amyloid and other proteinaceous aggregates that accumulate in the brain interstitial fluid or cells; the role of non-neuronal cells in the pathogenesis of neurodegenerative disorders; and aberrant brain angiogenesis.

Research priorities

To increase our knowledge of neurodegenerative diseases and brain barriers, the roles of RAGE, low-density lipoprotein receptor-related protein-1, P-glycoprotein, and $A\beta$ carriers on the influx, efflux, and neurological effects of $A\beta$ protein in patients with Alzheimer's disease must be determined. Other crucial areas of research include the roles of the genes that are expressed in the cerebral vasculature (eg, *MEOX2*, *SRF*, and *MYOCD*) in Alzheimer's disease. Investigation of the changes in barrier structure, receptor complement, and transport that occur in normal ageing, dementia, and ischaemia should not be overlooked. Oxidative stress and the BBB, such as reactive oxygen species and nitric oxide, is a research priority; aberrant angiogenesis in neurodegenerative diseases is also important. Research should also examine the communication within the NVU to identify how these cells respond to, process, and synthesise inflammatory mediators. A final research priority is the circumvention of the BBB.

Barriers to progress

An impediment to progress in this field is the lack of understanding of the continuum between normal ageing and age-related dementias. The lack of standardised in vitro and in vivo models that incorporate comorbidity of the BBB in neurodegeneration is a barrier to progress. Current research is focused on the neurobiology of disease rather than the integrative biology of the entire brain. Improved neuroimaging techniques are needed to visualise subtle or transient changes in the permeability of brain barriers. Additionally, there are few clinical trials of anti-inflammatory drugs to prevent or delay the progression of neurodegenerative diseases.

Recommendations

We recommend that interinstitutional, multidisciplinary teams of scientists and clinicians who are interested in the BBB and the cerebrovasculature are encouraged and funded. The genomes of the NVU and choroid plexus must be investigated and we must develop strategies to control aberrant brain angiogenesis and image the brain microcirculation in vivo.

Specialised neural barriers

Specialised neural barriers comprise the only interface between the blood and neural tissue, apart from the endothelium of the brain parenchymal capillaries. These specialised neural barriers include the blood–CSF barrier (choroid plexus epithelium), the meninges and the blood–subarachnoid CSF barrier, the blood–retinal barrier (retinal capillary endothelium and retinal pigment epithelium), the blood–nerve barrier (endoneural capillary endothelium and perineurium), the blood–labyrinth barriers (cochlea and vestibular system), and the blood–spinal cord barrier (figure 6).^{55,56} These barriers are less well studied than the BBB but need to be regarded as sites that influence the normal physiology of the neural microenvironment and undergo specific patterns of developmental regulation, deteriorate with age, and express specific pathologies in several neurological disorders. The recognition that disease can affect these barriers presents challenges to understand the disorder and develop new therapies. Moreover, several of the neural barrier sites and mechanisms work in parallel; therefore, any comprehensive overview needs to acknowledge their individual functions and their interactions. Specialised neural barrier sites contain capillaries with tight junctions that can be disrupted or made leaky by processes such as inflammation.

Choroid plexus

The choroid plexus is a highly vascular tissue, with leaky, fenestrated capillaries in series with the barrier layer formed by the epithelial cells.⁵⁷ The choroid plexus is the main site of CSF production and it has specific transport and polypeptide secretory functions that contribute to the growth, development, and maintenance of neural tissue.⁵⁸ The choroid plexus and the CSF are involved in several disorders, and analysis of the CSF can give insights into conditions that affect the brain parenchyma and the BBB.

Meninges

The arachnoid layer(s), which border the dura, are coupled by tight junctions and form the meningeal barrier layer. The classic view of CSF drainage is that it flows out through valve-like outpouchings of the arachnoid layer (granulations) into the dural venous sinuses; however, alternative routes for CSF drainage are recognised, for example, through the olfactory nerves and carotid sheath.⁵⁹

Blood–retina barrier

The inner blood–retina barrier is formed by the endothelial cells of the retina capillaries and has properties similar to the BBB. The outer blood–retina barrier is formed by the retinal pigment epithelium. Overall the blood–retina barrier is leakier than the BBB, which enables greater penetration of hydrophilic compounds.

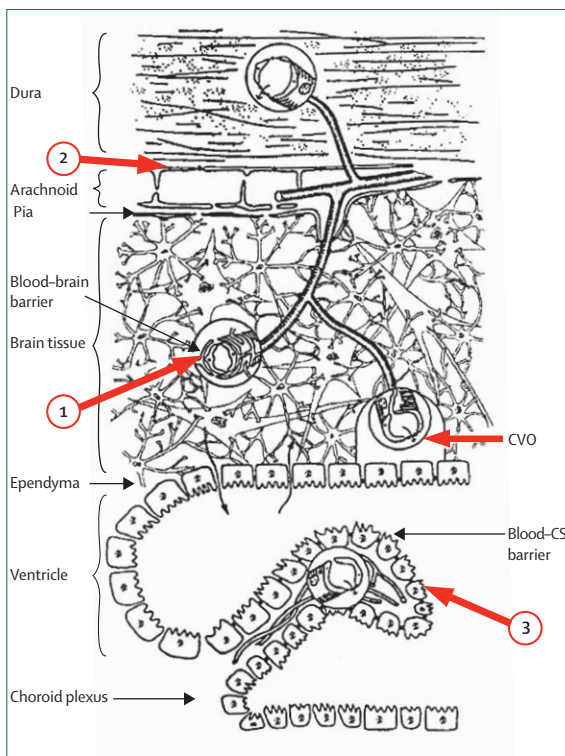


Figure 6: Barrier sites in the CNS

The three main sites of barriers in the CNS. (1) The brain endothelium, which forms the BBB. (2) The arachnoid epithelium, which forms the middle layer of the meninges. (3) The choroid plexus epithelium, which secretes CSF. At each site, the physical barrier formed by the tight junctions reduces the permeability of the paracellular (intercellular cleft) pathway. In the circumventricular organs (CVO), which contain neurons that are specialised for neurosecretion or chemosensitivity, the endothelium is leaky, which enables tissue–blood exchange; however, because these sites are separated from the rest of the brain by an external glial barrier and separated from the CSF by a barrier at the ependyma, CVOs do not form a path across the BBB. Reproduced with permission from Elsevier.⁵⁶ The other specialised endothelial barriers covered in this report are similar to the brain endothelial barrier, whereas the other epithelial barriers (eg, eye, nerve, or labyrinth) have regional specialisations.

Blood–nerve barrier

The blood–nerve barrier comprises two barrier sites—the endothelial layer of the endoneural capillaries and the perineurium (sheath) that surrounds the bundles (fascicles) of axons. Both barriers can be disrupted, particularly after surgery or trauma, but also in autoimmune disorders (eg, Guillain Barre syndrome), inflammation (diabetic neuropathy), and chronic demyelinating polyradiculoneuropathy. Disruption of the barriers is accompanied by intraneural oedema.

Blood–labyrinth barrier

The concept of the blood–labyrinth barrier comes from the observation of the markedly different chemical compositions of blood and the inner ear fluids in the cochlea, utricle, saccule, and semicircular canals.⁶⁰ The determination of the anatomy of the blood–labyrinth barrier requires reference to several barriers, which

include the blood–endolymph barrier and the endolymph–perilymph barrier.⁶¹

Blood–spinal cord barrier

The blood–spinal cord barrier is an endothelial cell barrier that is reinforced by astrocytes, pericytes, and the extracellular matrix and has several distinct features. The spinal cord has no choroid plexus: the transition to the peripheral system is related to nerve root entry zones, rather than circumventricular organs. The general permeability of the blood–spinal cord barrier is higher than the permeability of the BBB. The regional blood supply has a different array of segmental distribution and anastomoses than that in the brain, which leads to different distribution of watershed zones and white matter vulnerability to ischaemia. The CSF contacts the spinal cord at the spinal subarachnoid space and the central canal; the interface layers are formed by the leptomeninges and the ependyma, respectively, but these do not have appreciable barrier function under normal conditions.

Changes in spinal cord blood flow and tissue perfusion, mechanisms of secondary injury, and the biphasic opening of the blood–spinal cord barrier show how a better understanding of the blood–spinal cord barrier will help to optimise drug delivery.⁶² Experimental autoimmune encephalomyelitis is a good model to investigate the ascending progression of demyelination, inflammation, and the intricate relationship between tissue damage and increased permeability of the blood–spinal cord barrier.⁶³

Pharmacokinetic studies done with small tracers (sucrose, insulin, mannitol, or aminoisobutyric acid) and larger tracers (albumin, horseradish peroxidase, immunoglobulin, or luciferase) show dynamic changes in the blood–spinal cord barrier during disease processes.^{64,65} Histological examination shows distinctive differences in the blood–spinal cord barrier between white matter and grey matter, and among the epicentre, caudal, and rostral regions of the spinal cord. In vivo and post mortem MRI provides high-resolution information on haemorrhage, oedema, and breakdown of the blood–spinal cord barrier. Comparison of primary microvessel endothelial cells from the brain and the spinal cord complement in vivo studies and show lower expression of some tight junction proteins and higher concentrations of transferrin receptor in the blood–spinal cord barrier.⁶⁶ Despite many groups rushing to find cures for spinal cord injury, studies on the blood–spinal cord barrier require specialised techniques and a thorough understanding of spinal cord anatomy.

Most important issues for specialised barriers

Many themes are common to all the specialised neural barriers: the nature of the cell layers that form the barrier, their permeability and transport function, the consequences of pathology, and the promising therapies. However, each barrier site has unique features that not

only arise through a tissue-specific developmentally regulated process but also function differently in normal physiology and have particular vulnerabilities in the pathological setting. The most important problem is to understand the similarities and differences of the various barriers. Although examination of each barrier site can give valuable insights into the development, physiology, and pathology of the barrier, many barrier sites work together in such a coordinated way that systems and models are needed to explore their complexity.

Research priorities

Knowledge of the function of neural barriers in health, pathology, and ageing is a priority to understand specialised neural barriers. A second priority is to determine the roles of reactive oxygen and nitrogen species, cytokines, and second messenger pathways in inflammation at the neural barriers. Research should focus on structure–function and cross-species studies, to delineate the endothelial and epithelial components of specialised neural barriers. The important research priorities also include the imaging of leukocyte trafficking, the modulation of permeability, and the investigation of the changes with age and pathology in the blood–retinal barrier and other barriers.

Barriers to progress

The current lack of good experimental models of specialised neural barriers, including the choroid plexus, the arachnoid and pial barrier layers, the blood–retinal barriers, blood–cochlear barriers, and blood–nerve barriers, are a hindrance to progress. A second bottleneck to progress in this field is that CNS pathologies commonly affect several barrier sites (BBB, choroid plexus, or meninges) but are rarely regarded together. Finally, there are few scientists who research the specialised neural barriers, and the availability of human tissue samples is limited.

Recommendations

We recommend that clinicians and basic scientists in this field are brought together; so too are funding initiatives and focused research programmes. Also, the development of nanolitre scale analytic resources and facilities and techniques for high-resolution in vivo imaging is crucial.

Delivery of drugs to the brain

Delivery of therapeutic compounds to the brain is complicated.^{35,67} Despite great strides in the basic science of brain physiology and disease in the past decade, problems of delivery have received negligible attention. Current estimates are that 98% of all small molecule drugs cross the BBB only negligibly, and miniscule amounts of large molecule drugs cross the BBB, except when dysfunction of the BBB causes leakage or at those sites with transport systems. This has slowed the application of pharmacotherapy and immunotherapy in brain diseases.

Major recent advances include approaches to circumvent the BBB for brain delivery through the endogenous transport mechanisms or bypassing the BBB altogether, as discussed in the section on tumour barriers.

Receptor-mediated transcytosis

The BBB has systems for the transport of endogenous peptides, such as insulin or transferrin. The systems for receptor-mediated transcytosis operate in parallel with the classic carrier-mediated transporter systems, which transport small molecule nutrients, vitamins, and hormones. The carrier-mediated transporter systems are portals of entry for small molecule drugs that mimic the molecular structure of their endogenous ligands. A related receptor-mediated transcytosis system to deliver biopharmaceuticals is the membrane-bound precursor of heparin-binding epidermal growth factor,⁶⁸ and a siRNA molecule was delivered by this approach.

Monoclonal antibody molecular trojan horses, which can be either chimeric or humanised, can be produced with genetic engineering.^{69,70} The most potent antibody-based molecular trojan horse is a monoclonal antibody against the human insulin receptor.⁷¹ The peptidomimetic monoclonal antibodies are molecular trojan horses that ferry an attached drug, protein, antisense nucleotide, or non-viral plasmid DNA across the BBB.⁷²⁻⁷⁵

Non-antibody delivery systems have been developed, including histones,⁷⁶ p97,⁷⁷ receptor-associated protein,⁷⁸ the Tat transduction domain peptide,⁷⁹ and other cationic peptides or polymers.⁸⁰ Although the transport of ligands is hypothesised to be receptor-mediated,⁷⁸ the transport of cationic peptides might be mediated on the basis of charge interactions by absorptive-mediated endocytosis systems.⁷⁶ The conjugation of low-density lipoprotein apoproteins to the surface of nanoparticles triggers receptor-mediated transcytosis across the BBB by the low-density lipoprotein receptor in the BBB.⁸¹

Techniques to image drug targets to the brain

Imaging has made important contributions to drug discovery and brain drug targeting, particularly pharmacokinetics and quantification of the therapeutic response. Breakthroughs have been achieved with MRI, PET, optical imaging, radioisotope imaging, and X-rays. The various imaging techniques are complementary, and multi-modal imaging strategies have the potential to greatly accelerate drug discovery.

Important issues for drug delivery to the brain

The overall goal of future research to target drugs to the brain is to expand the CNS drug formulary from small, lipid-soluble molecules to large pharmaceuticals and drugs that do not normally cross the BBB.

The application of advanced *in vivo* imaging techniques helps preclinical and clinical investigations of the many steps required for drug discovery. Measurements of neuropharmacokinetics and neuropharmacodynamics

with good spatial and temporal resolution are important to evaluate many of the drug delivery systems. The function of brain barrier systems is known to change under pathological conditions, and these changes need to be characterised. For example, the investigation of the *in vivo* spatial distribution of receptor-mediated transcytosis systems in health and disease is important if drug delivery is to be optimised. Multiple imaging modalities will probably be needed and should provide important information at different phases of the discovery process. Therefore, an important goal of targeting drugs to the brain is increased emphasis on the development of novel imaging agents and techniques for optical imaging, nuclear imaging, X-ray, and MRI.

Research priorities

The priority is for research to target therapeutics to specific regions or cell types of the brain, in addition to pharmacokinetic modelling of drug delivery. The toxicity associated with technologies to deliver drugs to the BBB and neural tissue should be investigated. Researchers should also investigate the molecular interactions among BBB transport systems and determine the roles of the extracellular matrix on the microvascular permeability barrier and component cell function. Another priority is the molecular imaging of the vasculature and brain with targeted contrast agents and the regulation of BBB transport by astrocyte foot processes and other components of the NVU.

Barriers to progress

To research further the delivery of drugs across the brain barriers, opportunities to train and fund scientists that specialise in brain drug delivery must be established.

Greater emphasis should be placed on the importance of drug targeting to the BBB and CNS in the pharmaceutical industry. The current lack of *in vivo* validation of *in vitro* studies of the BBB and the lack of controlled clinical trials that address CNS drug delivery hinder the advancement of brain drug delivery.

Recommendations

Systems that target drugs to the brain and enable the delivery of recombinant protein neurotherapeutics should be developed. *In vivo* models should be used to validate new systems that target drugs. Genomic and proteomic discovery platforms that enable the identification of new BBB transporters should be developed. We also advocate the development of cross-disciplinary, integrated centres that bring together membrane transport biologists, pharmaceutical scientists, bioengineers, and imaging scientists.

Conclusions

Several common themes and topic-specific needs are priorities to advance the field of brain barrier research. Cross-topical themes include advances in imaging

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Search strategy and selection criteria

The International Brain Barriers Society (IBBS) is a new organisation that addresses the scientific and clinical needs for research into brain barriers. The IBBS panel initially identified 12 topics as priorities to advance the field of brain barrier research and treat CNS disease. These topics were then narrowed down to the six topics deemed to be the most clinically relevant to the field: inflammation, injury, tumours, neurodegeneration, specialised neural barriers, and delivery. A committee of international specialists in brain barrier research, co-chaired by a basic scientist and a clinician, was assigned to each of the topics (webappendix 1). Each committee produced a report on their topic, which summarised the state of the science, including the key advances since 2000, barriers to progress in the field, recommendations to advance the science in each area, and the resources needed to accomplish these goals. The IBBS initial meeting—Strategies for Advancing Brain-Barriers Translational Research—was held on March 22–23, 2007, in Stevenson, WA, USA. The draft reports were discussed by approximately 120 scientists and clinicians from around the world (webappendix 2). This Review is a synopsis of the six reports written by the committees, which are available at the IBBS website.

techniques, characterisation of the mechanisms, problems of the delivery of therapeutics, training of new investigators, and building of consortiums among institutions and laboratories. The cited reports that accompany this synopsis (see panel) should be consulted for more details on each topic. Clearly, brain barriers are more than just tight junctions, as emphasised by the many features of the NVU. Translational scientists who design drug and delivery strategies to the brain in the 21st century will be challenged by this complexity in order to achieve the highest quality CNS health demanded by our societies and agencies.

Contributors

All authors contributed equally to the writing and approval of this Review.

Conflicts of interest

We have no conflicts of interest.

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See Online for webappendices 1 and 2

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