Blood–brain barrier

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Key points
The blood–brain barrier (BBB) is a dynamic and functional neurovascular unit comprised of the capillary endothelium, astrocytes, pericytes, and extracellular matrix.

The cerebral capillary endothelium has a junction complex which restricts paracellular permeability. Controlled reversible opening of the BBB is essential for normal physiological function, and pathological disruption is detrimental to the underlying brain.

P-glycoprotein is a membrane transporter actively involved in the extrusion of drugs and toxic metabolites from the brain.

There are several methods that affect drug delivery across the BBB, including hyperosmotic or chemical disruption of endothelial tight junctions.

The blood–brain barrier (BBB) is a highly regulated interface that separates the peripheral circulation and central nervous system (CNS). It functions primarily as a selective diffusion barrier at the level of the cerebral microvascular endothelium. The BBB is often defined structurally as specialized endothelial cells lining the intraluminal portion of brain capillaries, but a more dynamic and functional definition recognizes the periendothelial accessory structures as integral components of the BBB. In addition to endothelial cells, the BBB is composed of the astrocytes, pericytes, neurons, and the extracellular matrix. This established neurovascular unit is essential for providing protection to the underlying brain cells and preserving the CNS homeostasis necessary for stable and coordinated neuronal activity.1

In certain strategic positions, the BBB is functionally deficient. The unprotected regions include the pineal gland, neurohypophysis, area postrema, subfornical organ, median eminence, and the vascular organ of the lamina terminalis. These regions are collectively referred to as circumventricular organs and permit diffusion of blood-borne molecules, allowing regulation of endocrine and autonomic nervous system functions.

Structure of the BBB
The structure of the BBB is complex and the component parts together form a functional neurovascular unit (Fig. 1).

Cerebral endothelium
Anatomically, the endothelial cells of the BBB can be differentiated from the peripheral tissue endothelia as they possess uniquely distinguishing characteristics (Table 1). The interendothelial spaces of the cerebral microvasculature are characterized by the presence of junctional complexes, which comprises tight junctions (TJs) and adherens junctions (AJs) that restrict the paracellular permeability across the endothelium.1,2

Adherens junctions
AJs mediate the adhesion of endothelial cells to each other and regulate paracellular permeability. They are composed of the membrane calcium-dependent protein cadherin that joins the actin cytoskeleton via intermediary proteins (catenins) to form adhesive contacts between cells. TJ and AJ components, particularly ZOs and catenins, are known to interact and further restrict permeability across the endothelium.

Astrocytes
Astrocytes are the star-shaped cellular elements of the neuroglia (literally nerve glue or nerve cement) which is the non-neuronal supporting tissue of the CNS. Glial cells have endfeet that form a lattice of fine lamellae closely apposed to the outer surface of the endothelium, thereby separating the capillaries from the neurons. They serve as scaffolds, guiding neurons to...
Sucrose and K

Mitochondrial content Greater number and volume Pinocytic vesicular transport Minimal Carrier-mediated transport Glucose, amino acids Established polarity–polarized transporters Present (e.g. amino acids) Exposure to flow membrane Luminal Carrier-mediated transport Glucose, amino acids

Enzymatic barrier (BBB-specific markers) Present (metabolizing neuroactive blood-borne solutes) Inductive influence from astrocytes Mandatory Fenestrations Absent Tight junctions Present Transendothelial electrical resistance $>1500 \ \Omega \ \text{cm}^2$ (low paracellular permeability) Pinocytic vesicular transport Minimal Mitochondrial content Greater number and volume (increased energy demands of active transport) Sucrose and K$^+$ permeability Low

Table 1 Description of the main cellular and functional properties of BBB endothelia

Table

their proper location during development and directing vessels of the BBB. Astrocytic glia plays a major role in the induction and maintenance of the BBB phenotype. Astrocyte–endothelial interaction and intercellular signalling is essential for optimal BBB function, and there is growing evidence that endothelial cells have a reciprocal inductive influence on astrocytes.3

Astrocytes may also have a direct influence on the dynamic control of the brain microcirculation. Dilatation of arterioles triggered by neuronal activity is dependant on intercellular calcium responses within the astrocyte network. Neuron–astrocyte signalling is necessary for regulating the energy supply to support neuronal function.

**Pericytes and extracellular matrix**

The basal lamina, in which pericytes are embedded, is interposed between the endothelium and astrocytes and consists of collagens, proteoglycans, laminin, fibronectin, and other extracellular matrix proteins. It provides mechanical support for cell attachment via integrins (transmembrane receptor proteins) and acts as a barrier to the passage of macromolecules. Pericytes send out cellular projections, which penetrate the basal lamina and attach at irregular intervals to the abluminal membrane of the endothelium, covering ~30% of the microvascular circumference. There is some evidence that pericytes have the ability to induce BBB ‘tightness’ by regulating endothelial cell proliferation, differentiation, and formation of endothelial TJs. Pericytes of the BBB demonstrate a phagocytic capacity which may be involved in neuroimmune functions. Additionally, pericytes express a number of receptors for vasoactive agents, including catecholamines, angiotensin II, endothelin I, and vasopressin, indicating that they may also be involved in cerebral autoregulation.2 3

**Transport at the BBB**

The brain capillaries are 50–100 times ‘tighter’ than peripheral microvessels as a result of the complex tight junctions severely restricting the paracellular flux of hydrophilic solutes. Penetration across the brain endothelium is therefore effectively confined to selective bidirectional transcellular mechanisms.

In the case of transcellular simple diffusion, the general rule is that the higher the lipophilicity of a substance, the greater the diffusion across the BBB into the brain. For example, if two substances, identical on all other fronts, vary in molecular weight, the smaller substance will penetrate the BBB more rapidly. Consequently, small inorganic molecules (e.g. oxygen, carbon dioxide, nitric oxide, and water) diffuse freely across the lipid membranes of the endothelium along their concentration gradient. Additionally, hydrogen bond reduction of a compound will enhance its membrane permeability. On the other hand, many essential metabolic substances are highly polar solutes and have poor endothelium membrane permeability. Such substances are conveyed across the BBB by facilitated diffusion, a form of carrier-mediated transport in which solute molecules bind to specific membrane protein carriers and then moved along concentration gradients. Facilitated diffusion is energy independent and contributes to transport at the BBB of substances such as glucose, amines, amino acids, nucleoside, monocarboxylates, and small peptides. The BBB has several specific transport systems to meet the metabolic demand of the brain, including glucose-transporter 1 (GLUT-1), the L-system, and A-system amino acid carriers.

Another important mechanism of transport across the BBB is endocytosis. Non-specific bulk-phase endocytosis occurs to a very limited degree in the endothelial cells of the brain vasculature, which therefore has lower levels of endocytosis than peripheral capillaries. However, specific mediated endocytosis, of which there are two types, receptor-mediated endocytosis (RME) and absorptive-mediated endocytosis (AME), provide a means for selective uptake of macromolecules into the CNS. There are receptors for the uptake of many different types of ligands, including hormones, growth factors, enzymes, and certain plasma proteins. RME occurs for substances such as transferrin, insulin, and insulin-like growth factor, and is a highly specific type of energy-dependent transport at the BBB.3
Increased trans-membrane water transport: aquaporin-4 up-regulation

TBI; tumours: astrocytomas; hypoxia/ischaemia; chemical mediators of inflammation: (TNF-α, IL-1β, IL-8, VEGF, NO, histamine, serotonin, bradykinin, adenosine nucleotides, arachidonic acid, thrombin and reactive oxygen species); autoimmune encephalitis; tumours: glioma and metastatic adenocarcinoma

Enhanced transcytosis: ↑ pinocytosis, ↑ vesicular endothelial transport

TBI; septic encephalopathy; hepatic encephalopathy; hypoxia/ischaemia; seizures; tumours; multiple sclerosis; chemical mediators of inflammation (as above); lead and mercury poisoning; tricyclic antidepressants

Extracellular matrix destruction; matrix metalloproteinases activation

TBI; inflammation: multiple sclerosis; HIV-encephalitis; tumours; cytokines: IL-6

Increased trans-membrane water transport: aquaporin-4 up-regulation

TBI; tumours: astrocytomas; hypoxia/ischaemia; chemical mediators of inflammation: VEGF, TNF-α, IL-β, hepatocyte growth factor

Fenestration formation

VEGF, alpha-1 adrenergic agonist (methoxamine); tricyclic antidepressants (chlorpromazine)

Disease induced nutrient transport changes

Diabetes (GLUT-1); Alzheimer’s disease (β-amyloid); Wernicke-Korsakoff syndrome (thiamine); ischaemic stroke (GLUT-1); multiple sclerosis (ICAM-1)

### Opening of the BBB in physiology

The BBB affords the CNS a ‘privileged’ microenvironment which is not exposed to abrupt fluctuations in plasma composition or circulating neuroactive agents that may disturb neural function. However, modest and reversible BBB opening has physiological advantages. The plasma is a rich source of factors required for the normal repair processes of the brain. Barrier opening also allows immunological surveillance of the CNS as well as allowing neurons to sample plasma composition as part of the brain’s key function in normal regulatory mechanisms.

BBB opening is a well-regulated process under normal physiological conditions and occurs in response to agents released locally. The chemical molecules capable of modulating BBB permeability arise from three major sources; the endothelium, the astrocytes, and nerve terminals of neurons close to capillaries. Several of the mediators of BBB opening have been identified and include glutamate, aspartate, ATP, endothelin-1, nitric oxide, tumour necrosis factor-alpha (TNF-α), and interleukin 1-beta (IL-1β). Other humoral agents that influence BBB permeability are bradykinin, serotonin, histamine, and substance P. This increase in BBB permeability occurs due to a transient opening of the TJ paracellular pathway.3 4

### Opening of the BBB in pathophysiology

The opening of the BBB is a critical event in the development and progression of several diseases that affect the CNS. Enhanced BBB permeability is an invariable response to a primary brain insult and is commonly associated with a variety of brain injury paradigms, including traumatic brain injury (TBI), ischaemic stroke, intracerebral haemorrhage, primary and metastatic neoplasms, inflammatory diseases (meningitis, ventriculitis, and cerebral abscess), and severe toxic–metabolic derangements (encephalopathy). Specific pathological changes at the BBB, such as opening of the endothelial tight junctions, enhanced transcytosis, damage to the extracellular matrix, upregulated transmembrane water transport, changes in nutrient transport, and pore formation lead to barrier failure (Table 2). Although the initial

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**Table 2** Several potential conditions and factors shown to induce changes at the BBB (↓ or ↑ represents up- or down-regulated expression)

<table>
<thead>
<tr>
<th>Condition/Event</th>
<th>Factors/Challenges</th>
</tr>
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<tbody>
<tr>
<td>Opening of TJs: ↓ occludin, ↓ ZO-1, ↓ claudin-1, disorganization of AJ proteins</td>
<td>Hypoxia/ischaemia; TBI; septic encephalopathy; burn encephalopathy; multiple sclerosis; HIV-induced dementia; Alzheimer’s disease; chemical mediators of inflammation (TNF-α, IL-1β, IL-8, VEGF, NO, histamine, serotonin, bradykinin, adenosine nucleotides, arachidonic acid, thrombin and reactive oxygen species); autoimmune encephalitis; tumours: glioma and metastatic adenocarcinoma</td>
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**Fig 2** The consequences of BBB breakdown.

Insults to the brain in various pathologies are quite different, there is a common final pathway of loss of BBB integrity (Fig. 2).

**Traumatic brain injury**

The damage to the brain tissue after TBI is determined by two substantially different pathological processes. At the moment of impact (primary insult), the CNS is exposed to shear forces with resultant mechanical deformation of the BBB neurovascular unit. The increased cerebrovascular permeability and subsequent vasogenic oedema is a major cause of the increased brain tissue water after TBI. Protein extravasation secondary to BBB compromise and damage to extracellular proteins increases the osmotic gradient and abnormal fluid accumulation. Under normal conditions, a ‘sink effect’ is provided by the ventricles and subarachnoid CSF to allow circulation and replenishment of the extracellular space. This is overwhelmed in TBI, and the extravasated proteins retard the clearance of fluid from the brain.

Secondary brain injury is essentially ischaemic in nature and induces a cellular component to the oedema formation—cytotoxic oedema. This occurs because of disruption of the BBB secondary to disorganization of TJ proteins, release of inflammatory
mediators, such as inter-cellular adhesion molecule (ICAM-1) and vascular endothelial growth factor (VEGF), and mitochondrial dysfunction. Activation of matrix metalloproteinases (MMP) causes proteolysis of the basal lamina and contributes to the progression of tissue damage.5

In addition to these mechanisms, trans-membrane water transport also plays a role in the brain-swelling process. In normal brain, the water channel protein, aquaporin-4 (AQP4), is expressed in perimicrovessel astrocyte foot processes. TBI results in up-regulation of AQP4 in reactive astrocytes after TBI and there is significant correlation between AQP4 expression and the degree of contrast-enhanced CT scan-determined cerebral oedema. Up-regulation of AQP4 is not restricted to TBI, but is also present in several other intracranial disorders.

The development of therapeutic strategies to abate the different stages of oedema formation is focused on the manipulation of barrier dynamics after injury. Recent studies have demonstrated that early neurosurgical intervention, such as decompressive craniectomy and contusiectomy, correlates with a decrease in MMP levels and limitation of the pathological processes of BBB breakdown.6

Ischaemic stroke

Ischaemic stroke-related cerebral ischaemia sets in motion a series of events that leads to increased BBB permeability. The main factors include cytokines, VEGF, and nitric oxide. The release of VEGF induces fenestration formation in endothelial cells and enhances BBB capillary permeability. This leads to cerebral oedema formation with the risk of ongoing ischaemia and the potential for further infarction of the penumbra. VEGF antagonism limits TJ protein disruption and paracellular permeability, and reduces post-ischaemic oedema thereby halting the progression of ischaemic brain injury.1 5

Septic encephalopathy

Septic encephalopathy is the commonest form of encephalopathy in general intensive care units and is associated with increased morbidity and mortality. It results in a symmetrical, diffuse brain dysfunction associated with breakdown of the BBB and cerebral oedema. The cellular pathology underlying septic encephalopathy-related BBB disruption includes increased pinocytosis in cerebral microvessel endothelium and disorganized interendothelial junctional proteins leading to a ‘leaky’ TJ. These are the consequences of the effects of circulating inflammatory mediators on the cerebral endothelium, the neurotransmitter composition of the reticular activating system, astrocyte dysfunction, and direct neuronal injury.2 7

Tumour

The microvasculature of brain tumours is poorly developed, lacks a BBB, and leaks fluid that leads to cerebral oedema and additional mass effect that often exceeds that related to the tumour itself. Investigations of the ultrastructure of human gliomas and metastatic adenocarcinoma have confirmed opening of interendothelial TJs, which is associated with an increase in paracellular permeability and peri-microvessel oedema. Neoplastic astrocytes fail to produce the necessary factors to maintain BBB integrity in peritumoral areas and may also secrete permeability factors that counteract normal barrier function. The mainstay of therapy of brain tumour-related oedema is corticosteroids and, although the exact mechanism of action is unclear, they appear to reduce endothelial permeability.7

Transport of drugs across the BBB

The BBB is a major impediment to the entry of many therapeutic substances acting on the CNS and the delivery of drugs to the brain after systemic administration requires transport through the BBB. In determining whether a given drug may undergo significant transport across the BBB, the two most important factors are its lipophilicity and molecular weight. Lipophilic drugs enter the brain compartment by either passive diffusion or by becoming solubilized into the lipid bilayer of the endothelial cell membrane. The lipophilicity of a drug as an index of BBB transport is only valid when the molecular weight is less than or approximately equal to 400–600 Da. Even if a drug is highly lipid soluble, it will not be transported across the BBB in pharmacologically significant amounts if its molecular weight exceeds 600 Da.

An additional stumbling block for drug delivery to the CNS is the presence of an active drug efflux system within the BBB. P-glycoprotein (P-gp) is an ATP-dependent 170 kDa phosphorylated glycoprotein membrane transporter that is involved in the active cellular extrusion of drugs and potentially toxic metabolites from the brain. P-gp is located on the apical surface of the endothelial cells of the brain capillaries and contributes to the poor BBB penetration of many highly lipophilic drugs by blocking their entry at the level of the capillary endothelium. The presence of P-gp in the BBB is a potential target for drug design. P-gp inhibitors with low toxicity are in trial for the treatment of multi-drug resistance in tumours.8

Drug delivery and the BBB

In order to overcome the difficulties in CNS penetration, various drug delivery strategies have been developed. These generally fall into one or more of the following three categories: drug manipulation, disruption of the BBB, and alternative routes of drug delivery.

Drug manipulation

Lipophilic analogues

CNS penetration is favoured by low molecular weight, lack of ionization at physiological pH, and lipophilicity. For example, diamorphine, a diacyl derivative of morphine, crosses the BBB 100 times
more easily than its parent drug just because it is more lipophilic. Hence, it is possible to ‘smuggle’ compounds across the BBB as their lipophilic precursors. A drug’s lipophilicity correlates strongly with cerebrovascular permeability so, in theory, lipophilic analogues of small hydrophilic drugs should penetrate the BBB more readily. This strategy has been frequently used, but the results have been disappointing.

Prodrugs
Drugs can be administered as prodrugs which, by virtue of their improved characteristics, are brought closer to the receptor site and maintained there for longer periods of time. At the receptor site, the prodrug gets converted to the active form. Unfortunately, simple prodrugs suffer from several important limitations. Whilst increased lipophilicity may improve movement across the BBB, it also tends to increase uptake into other tissues causing an increased systemic tissue burden. This is especially detrimental in the case of potent drugs such as steroids or cytotoxic agents when toxicity is exacerbated at non-target sites.

Carrier- and receptor-mediated transport
Carrier- and receptor-mediated transport pathways are available for certain circulating nutrients and peptides and can be utilized as portals of entry to the brain for many drugs. Utilization of differences in the affinity and maximal transport activity among these transport systems is an attractive strategy for controlling the delivery and retention of drugs in the brain. Receptor-mediated drug delivery uses chimeric peptide technology wherein a non-transportable drug is conjugated to a transport vector, usually a modified protein- or receptor-specific monoclonal antibody, which undergoes receptor-mediated transcytosis through the BBB. Various therapeutic agents have been delivered to the brain using this technology, including peptide-based pharmaceuticals such as a vasoactive peptide analogue, neurotrophins, and small molecules incorporated within liposomes. However, the effective utilization of this technique is still under investigation. 9

Disruption of the BBB
This is an invasive strategy for enhanced CNS drug delivery which involves transient BBB disruption in association with systemic drug administration. This results in enhanced extravasation rates in the cerebral endothelium leading to increased brain parenchymal drug concentrations.

Chemical disruption
Various techniques have been used to disrupt the BBB, but many are unacceptably toxic and hence not clinically applicable. These include the infusion of solvents such as dimethyl sulphoxide or ethanol and metals such as aluminium. Irradiation and the induction of pathological conditions, including hypertension, hypercapnia, hypoxia, or ischaemia, have similar effects.

Hyperosmotic BBB disruption
Osmotic opening of the BBB involves intracarotid injection of an inert hypertonic solution such as mannitol or arabinose to initiate endothelial cell shrinkage and opening of TJ for a period of a few hours. This has been used to permit delivery of anti-neoplastic agents to the brain. Although this treatment is still investigational, there are anecdotal data to suggest that it may be beneficial in some patients who fail to respond to systemic chemotherapy.10

Alternative routes to CNS drug delivery
A third class of strategies aimed at enhancing CNS penetration of drug molecules is composed of delivery methodologies that do not rely on the cardiovascular system. The important methods in this group are administration via intraventricular/intrathecal and olfactory routes.

Conflict of interest
None declared.

References

Please see multiple choice questions 13–15.