

# Blood-brain barrier dysfunction in epilepsy: Mechanisms, therapeutic strategies and future orientation (Review)

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**Abstract.** The blood-brain barrier (BBB) is a crucial structure for maintaining homeostasis within the central nervous system, and its integrity plays a pivotal role in the onset and progression of epilepsy. Epileptic seizures can disrupt the molecular architecture of the BBB, including the loss of tight junction proteins, activation of matrix metalloproteinases and dysfunction of supporting cells. Various pathological changes, such as transmembrane transport disorders, upregulation of platelet-derived growth factor receptor  $\beta$  and vascular endothelial growth factor signalling pathways, and activation of astrocytes and microglia, accompany these alterations. These modifications exacerbate the entry of toxic molecules (such as albumin) into the brain parenchyma, triggering neuroinflammation and neuronal damage, thereby establishing a vicious cycle of epilepsy, BBB disruption and recurrent epilepsy. Consequently, repairing or protecting the BBB is a novel strategy for controlling epileptic seizures and

treating drug-resistant epilepsy. Consequently, compared with current treatment approaches that primarily focus on suppressing neuronal excitability, repairing or protecting the BBB is a novel strategy for controlling epileptic seizures and treating drug-resistant epilepsy. Drugs such as botulinum, levetiracetam and angiotensin receptor blockers show the potential for BBB protection. The development of nanomaterials can enhance drug concentrations in affected areas, thereby offering new avenues for refractory epilepsy. The present study systematically reviews the critical role of the BBB in the pathogenesis of epilepsy, untangles the complex association between BBB dysfunction and the course of the disease, aims to deepen our understanding of the molecular mechanisms underlying BBB damage, and explores new approaches for epilepsy prevention and treatment from a BBB perspective. This review provides a theoretical foundation and research direction for the development of diagnostic and

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**Abbreviations:** ABCC, ATP-binding-cassette transporters sub-family C; ANGPT-1, angiotensin-1; AQP4, aquaporin 4; ARB,

angiotensin receptor blocker; ASD, anti-seizure drugs; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; BMEC, brain microvessel endothelial cell; CNS, central nervous system; DBS, deep brain stimulation; DRE, drug-resistant epilepsy; EEG, electroencephalographic; FUS, focused ultrasound; GABA,  $\gamma$ -aminobutyric acid; GLUT1, glucose transporter 1; KA, kainic acid; Kir4.1, inwardly rectifying potassium channel 4.1; LEV, levetiracetam; MB, microbubble; MCT, monocarboxylate transporter; MLCK, myosin light chain kinase; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; MRP, multidrug resistance protein; NIR, near-infrared; PDGFR $\beta$ , platelet-derived growth factor receptor  $\beta$ ; P-gp, P-glycoprotein; PKC, protein kinase C; rhEPO, recombinant human erythropoietin; SE, status epilepticus; SHH, sonic hedgehog; Tf, transferrin; TGF- $\beta$ R, transforming growth factor  $\beta$  receptor; TJ, tight junction; VEGFR, vascular endothelial growth factor

**Key words:** blood-brain barrier, epilepsy, tight junction proteins, neuroinflammation, nano-drug delivery system, antiepileptic therapy

treatment strategies that are safer and more effective than current standard therapies.

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## 1. Introduction

Epilepsy is one of the most prevalent and severe neurological disorders, and its global disease burden continues to pose substantial challenges. Epidemiological studies estimate that ~70 million individuals worldwide are affected by epilepsy (1). The mortality rate among individuals with epilepsy is three-fold higher than that of the general population, with a number also suffering from comorbidities such as depression and bone fractures, imposing significant burdens on families and society (2). The annual global burden of epilepsy is >13 million disability-adjusted life years, with combined direct medical expenditures and indirect productivity losses amounting to US\$28 billion, underscoring its status as a critical public health priority (2). The primary mechanism underlying epileptic seizures is an imbalance between the excitatory and inhibitory processes within the central nervous system (CNS) (3). Clinically, it manifests as transient recurrent neurological dysfunction caused by abnormal hyper-synchronous neuronal discharges. Symptom heterogeneity is strongly correlated with epileptogenic focus localisation: Focal seizures may involve motor manifestations (e.g. limb automatisms), sensory disturbances (e.g. olfactory/visual hallucinations) or autonomic dysfunction (e.g., tachycardia, facial flushing); and generalised seizures are characterised by impaired consciousness, generalised tonic-clonic movements or respiratory arrest (4). Approximately 30% of patients with epilepsy demonstrate resistance to existing anti-seizure drugs (ASDs) (5), and patients with drug-resistant epilepsy (DRE) experience significantly elevated risks of progressive brain damage as the frequency of seizures increases. Chronic recurrent seizure activity accelerates the progression of hippocampal sclerosis, leading to irreversible neuronal loss and abnormal neural network reorganisation (6). This results in a self-perpetuating 'seizure-damage-increased seizure susceptibility' cycle that ultimately drives disease progression toward the refractory stages (7). DRE is characterised by the failure to achieve seizure control with at least two established ASDs (administered alone or in combination) (8). Globally, DRE has an estimated prevalence of 13.7%, with a cumulative incidence of 25% in paediatric populations and 14.6% in adult and mixed cohorts (9). Compared with treatment-responsive patients, individuals with DRE demonstrate elevated risks of depression, epilepsy-related injuries and neurological deficits (10,11),

coupled with increased mortality rates (12,13). Current neuromodulation approaches for DRE include deep brain stimulation (DBS), vagus nerve stimulation and responsive neurostimulation. A longitudinal follow-up study revealed that these interventions achieve >50% seizure frequency reduction in 50-60% of recipients (14). Nevertheless, most drug-resistant patients fail to derive substantial benefits, and a few are ineligible for neurostimulation due to incomplete evaluations or contraindications. This clinical reality underscores the critical need to explore novel therapeutic targets and intervention strategies to deliver safer and more effective options to patients with refractory epilepsy populations.

The blood-brain barrier (BBB) plays a vital role in regulating epileptogenesis by maintaining CNS homeostasis (15). Brain stability, particularly neurotransmitter balance, depends on the controlled exchange of substances between blood and brain tissue. As a dynamic interface, the BBB regulates the passage of ions, proteins and other molecules, and maintaining this balance is preserved. The structure of the BBB comprises capillary endothelial cells, tight junctions (TJs) connecting these cells, a basement membrane and surrounding glial cells. Under normal physiological conditions, the BBB tightly controls the entry of substances into the brain parenchyma through paracellular and transcellular pathways, thereby preventing the infiltration of harmful elements into the CNS and ensuring a stable internal environment (16,17). When the BBB is compromised, toxic substances and immune cells can infiltrate brain tissue, contributing to the pathogenesis of neurological disorders, such as traumatic brain injury, multiple sclerosis, epilepsy and brain tumours (18). BBB integrity is pivotal in disease progression, influencing both the onset of neurological conditions and the efficacy of therapeutic interventions. Research suggests that epilepsy can disrupt BBB function, with seizures often triggering an excessive release of the excitatory neurotransmitter glutamate (Glu), leading to increased BBB permeability (19). Additionally, alterations in angiogenesis, and changes in the expression of receptors, transporters and ion channels, further increase the risk of recurring seizures (19-21).

The present review is focused on the alterations in the structure and function of the BBB and presents recent advances in understanding its effect on epilepsy. This review examines the mechanisms by which BBB dysfunction contributes to epilepsy progression and discusses emerging therapeutic strategies aimed at targeting the BBB to enhance treatment outcomes for epilepsy.

## 2. Structure and function of the BBB

Approximately 140 years ago, the German scientist Paul Ehrlich firstly observed that a dye injected into the blood vessels of mice stained peripheral organs but not the brain or spinal cord, suggesting the existence of a barrier (22,23). This barrier, now known as the BBB, was microscopically confirmed in the 1960s (24).

The BBB consists of three primary layers, with the first being formed by brain microvascular endothelial cells (BMECs) connected by TJs. BMECs are characterised by fewer pore structures and endocytic vesicles than peripheral endothelial cells, along with a high mitochondrial density, which is indicative of reduced permeability and increased metabolic activity.

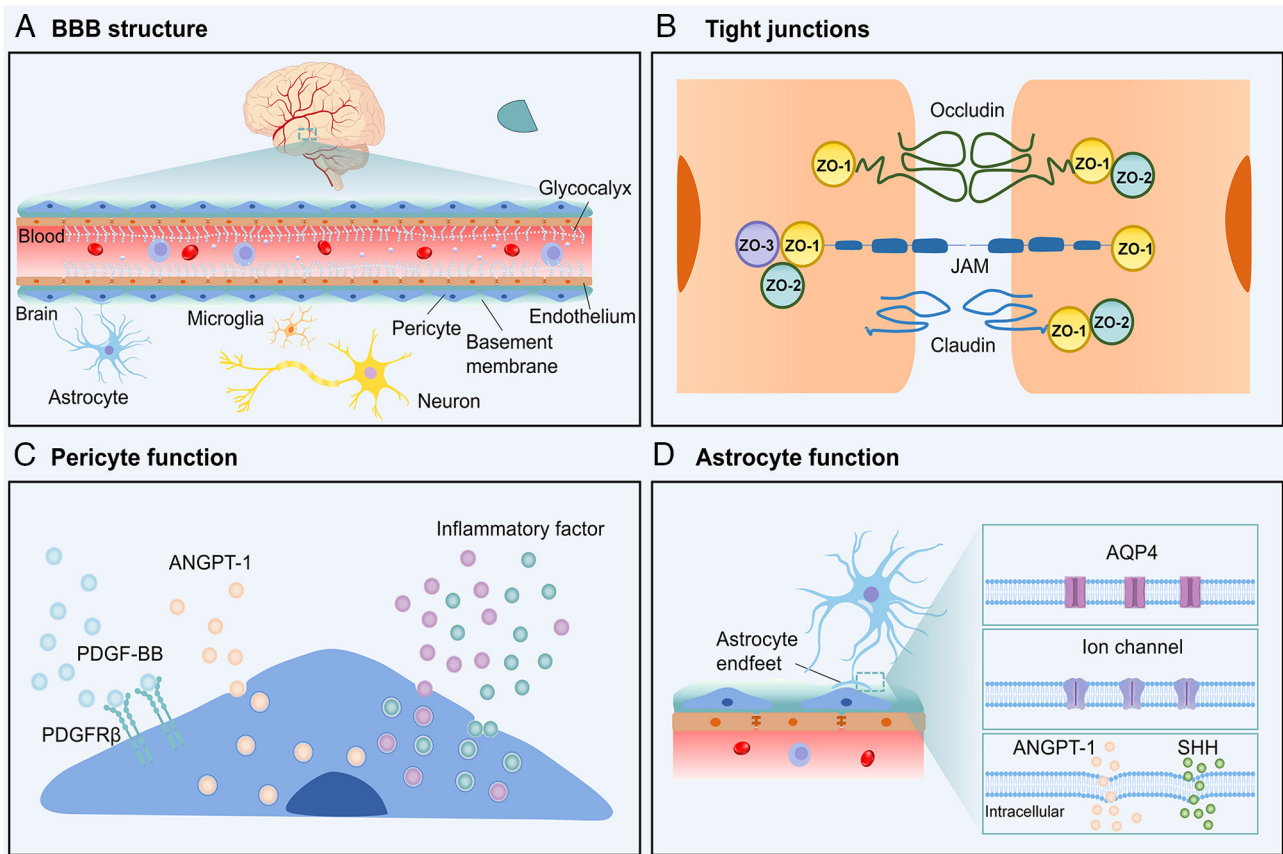


Figure 1. Physiological structure and molecular characteristics of the BBB. (A) The BBB is composed primarily of BMECs connected by TJs and is encased by a basal lamina, pericytes, astrocytic end-feet and narrow extracellular spaces. (B) BMECs are connected by TJs complexes formed by proteins such as claudins, occludins, ZO-1, ZO-2, ZO-3 and junctional adhesion molecules. These complexes tightly regulate non-specific substance transport, ensuring the low permeability of the BBB. (C) Pericytes are essential for BBB functionality. PDGFR $\beta$  receptors on pericytes interact with PDGF-BB secreted by BMECs, facilitating vascularisation. Pericytes release ANGPT-1, which supports BMEC development and maintains BBB stability. Pericytes modulate inflammatory responses by releasing both pro-inflammatory chemokines and anti-inflammatory cytokines. (D) Astrocytes form expanded end-feet surrounding the outer basal surface of BMECs and establish close connections with them. These end-feet are rich in AQP4 and ion channels, which contribute to blood flow regulation, ion homeostasis and nutritional support. Astrocytes also produce cytokines, such as ANGPT-1 and SHH, to enhance BBB integrity by upregulating TJ proteins and modulating BMEC transport properties. BBB, blood-brain barrier; TJ, tight junction; ZO-1, zona occludens protein 1; ANGPT-1, angiotensin-1; PDGFR $\beta$ , platelet-derived growth factor receptor  $\beta$ ; PDGF-BB, platelet-derived growth factor-BB; SHH, sonic hedgehog; AQP4, aquaporin 4; JAM, junctional adhesion molecule.

These cells feature transcellular transport pathways involving solute carriers, receptors and ion transporters (25,26). Additionally, BMECs produce a glycocalyx layer that prevents direct contact between BMECs and leukocytes, and plays a critical role in reducing inflammation and maintaining BBB integrity (27,28) (Fig. 1A). TJ complexes composed of proteins, such as claudins, occludin, zona occludens protein 1 (ZO-1), ZO-2 and ZO-3, further restrict paracellular diffusion, ensuring low BBB permeability (29) (Fig. 1B). The second layer of the BBB comprises a basement membrane and pericytes around the vascular endothelium. The basement membrane is composed of collagen IV, laminin, nidogen and heparan sulphate proteoglycans. This layer anchors cells and provides structural support, and its disruption leads to increased BBB permeability through altered expression of TJ proteins (30). Pericytes, a critical component of the BBB (31), maintain CNS homeostasis by promoting vascularisation via platelet-derived growth factor (PDGF) signalling and secreting factors such as angiotensin-1 (ANGPT-1), which facilitates the development of BMECs and enhances BBB stability (32). Pericytes modulate inflammatory responses by releasing pro-inflammatory

chemokines, such as CCL2 and CXCL1 (33) (Fig. 1C). The third layer consists of astrocyte end-feet that enwrap capillaries, forming a sheath-like structure. Astrocytes, which are the most abundant glial cells in the brain (34), regulate ion transport, blood flow and nutrient supply through ion channels and aquaporins (35,36); they secrete cytokines, including ANGPT-1 and sonic hedgehog (SHH), strengthening BBB integrity by upregulating TJ proteins and modulating BMEC transport properties (37,38). Furthermore, astrocytes contribute to neurotransmission by participating in 'tripartite synapses' with neurons, forming a link between nerve cells and blood vessels (39) (Fig. 1D). These three interconnected layers work synergistically to maintain brain homeostasis by tightly regulating the entry of substances into the brain and facilitating the clearance of harmful compounds.

### 3. BBB dysfunction in epilepsy

Maintaining the structural and functional integrity of the BBB is crucial to ensure a stable internal environment within the CNS. In 1986, Cornford and Oldendorf introduced the

pivotal concept that seizures can affect BBB function (40). Subsequent studies have confirmed BBB disruption in seizure models induced by agents such as pilocarpine, fluoroethyl, kainic acid (KA) and bicuculline (41-44). Under normal physiological conditions, the BBB plays an essential role in stabilising the brain microenvironment. However, seizures compromise its integrity, accelerate epilepsy progression and potentially lead to drug-resistant forms of the disorder. This creates a detrimental feedback loop wherein further damage to the structural, molecular and immune regulatory mechanisms of the BBB exacerbates its dysfunction (45).

*Epilepsy-induced BBB structural disruption.* Epileptic activity significantly compromises BBB molecular integrity, primarily manifested by the downregulation of TJ proteins (occludin, ZO-1 and claudin-8), which culminates in elevated paracellular permeability (46,47). The activation of matrix metalloproteinase (MMP)-2/9 plays a pivotal role in mediating these alterations by degrading TJ components and remodelling the perivascular extracellular matrix architecture, further compromising barrier functionality (48). Clinical investigations have revealed persistent BBB compromise in epilepsy, as evidenced by elevated serum levels of MMP-2 and related biomarkers even during interictal periods (49). Tuberous sclerosis complex 1 emerges as a critical regulator of TJ assembly, with its deficiency being mechanistically linked to epileptogenesis via aberrant mechanistic target of rapamycin pathway activation that disrupts TJ protein expression and localization (50,51). This cascade of molecular events orchestrates structural and functional BBB destabilisation, highlighting the core vulnerability of the neurovascular interface (Fig. 2).

*Epilepsy-induced neurovascular unit dysfunction.* Epileptogenesis induces pathological alterations extending beyond cerebrovascular endothelial cells to encompass the entire neurovascular unit (NVU), which comprises BMECs, pericytes, astrocytes and microglia. In KA-induced epileptogenesis models, BMECs demonstrate significant Kv7.5 channel downregulation, potentially serving as early biomarkers for endothelial dysfunction (21). Concurrent seizure activity drives the marked activation of vascular endothelial growth factor (VEGF)/VEGF receptor signalling cascades, where sustained VEGF upregulation exerts dual inhibitory effects on ZO-1 expression while potentiating MMP activity, collectively diminishing BBB structural integrity (46,47,52,53). Pericytes undergo phenotypic reorganisation with concomitant PDGF receptor  $\beta$  (PDGFR $\beta$ ) upregulation during epileptogenesis, synergising with activated microglia to form pro-fibrotic niches that exacerbate barrier impairment (54). Electrophysiological analyses have revealed seizure-induced inward currents in pericytes, indicating a functional compromise (55). Astrocyte activation enhances gap junction coupling and electrical synapse formation, amplifying neuronal hypersynchrony (56), whereas microglial activation propagates neuroinflammation through pro-inflammatory cytokine release, establishing a chronic inflammatory milieu that destabilises BBB permeability (57). This multifaceted cellular interplay ultimately drives NVU functional decoupling, thereby perpetuating epileptogenic cascades (Fig. 2).

*Epilepsy-mediated BBB transport dysregulation.* The selective barrier function of the BBB is maintained through the coordinated regulation of paracellular and transcellular transport. Epileptic seizures activate multiple signalling pathways that impair both routes, increasing the risk of entry of harmful substances into the brain. In the paracellular pathway, seizure-induced activation of the mitogen-activated protein kinase (MAPK) pathway leads to the degradation and redistribution of TJ proteins. This disrupts intercellular junctions and amplifies local inflammatory responses (58,59). Abnormal activation of the phosphoinositide 3-kinase/Akt pathway further reduces claudin-5 expression. This triggers cytoskeletal remodelling and compromises the barrier integrity (60,61). Oxidative stress enhances these effects by activating the nuclear factor- $\kappa$ B (NF- $\kappa$ B) signalling pathway. This promotes the expression of pro-inflammatory cytokines and MMPs, thereby accelerating TJ protein degradation (62). These alterations are particularly evident in the early stages of epilepsy, where changes in cerebral blood flow and increased mechanical stress further exacerbate BBB dysfunction (63,64).

In the transcellular pathway, efflux transporters, such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance-associated proteins (MRPs) are upregulated. This limits the penetration of ASDs across the BBB (65-69). BCRP is upregulated in vascular endothelial cells and astrocytes, further restricting drug distribution within the brain (70,71). Members of the ATP-binding-cassette transporters sub-family C (ABCC) transporter family, including ABCC2, are overexpressed in endothelial and astroglial cells in the brains of patients with epilepsy. This expression profile contributes to the reduced efficacy of ASDs (72). Inhibition of these transporters enhances drug accumulation in the brain tissue and improves therapeutic outcomes (73,74). Monocarboxylate transporters (MCTs), including MCT1 and MCT2, regulate lactate and pH homeostasis in the brain. Decreased expression of these transporters in the hippocampus of patients with epilepsy leads to lactate accumulation and the formation of an acidic microenvironment. This contributes to neuronal damage, calcium dysregulation and oxidative stress, ultimately compromising BBB integrity (75,76).

Epilepsy disrupts the BBB via multiple mechanisms. These include degradation of TJ proteins, dysfunction of the NVU, impaired transporter systems and homeostatic imbalances within the brain microenvironment. Such changes increase brain vulnerability to harmful substances and may drive the progression of epilepsy and the development of pharmacoresistance. These alterations are considered to be key contributors to the pathophysiology of DRE. Furthermore, evidence from multimodal imaging techniques, including magnetic resonance imaging (MRI) and positron emission tomography, has revealed a significant spatial and temporal overlap between BBB dysfunction and epileptic activity. This provides imaging-based support for the causal relationship between BBB impairment and seizure generation (77-79).

#### **4. Possible mechanisms underlying BBB dysfunction in epilepsy**

The structure and function of the BBB are dynamic and influenced by various internal and external factors. In

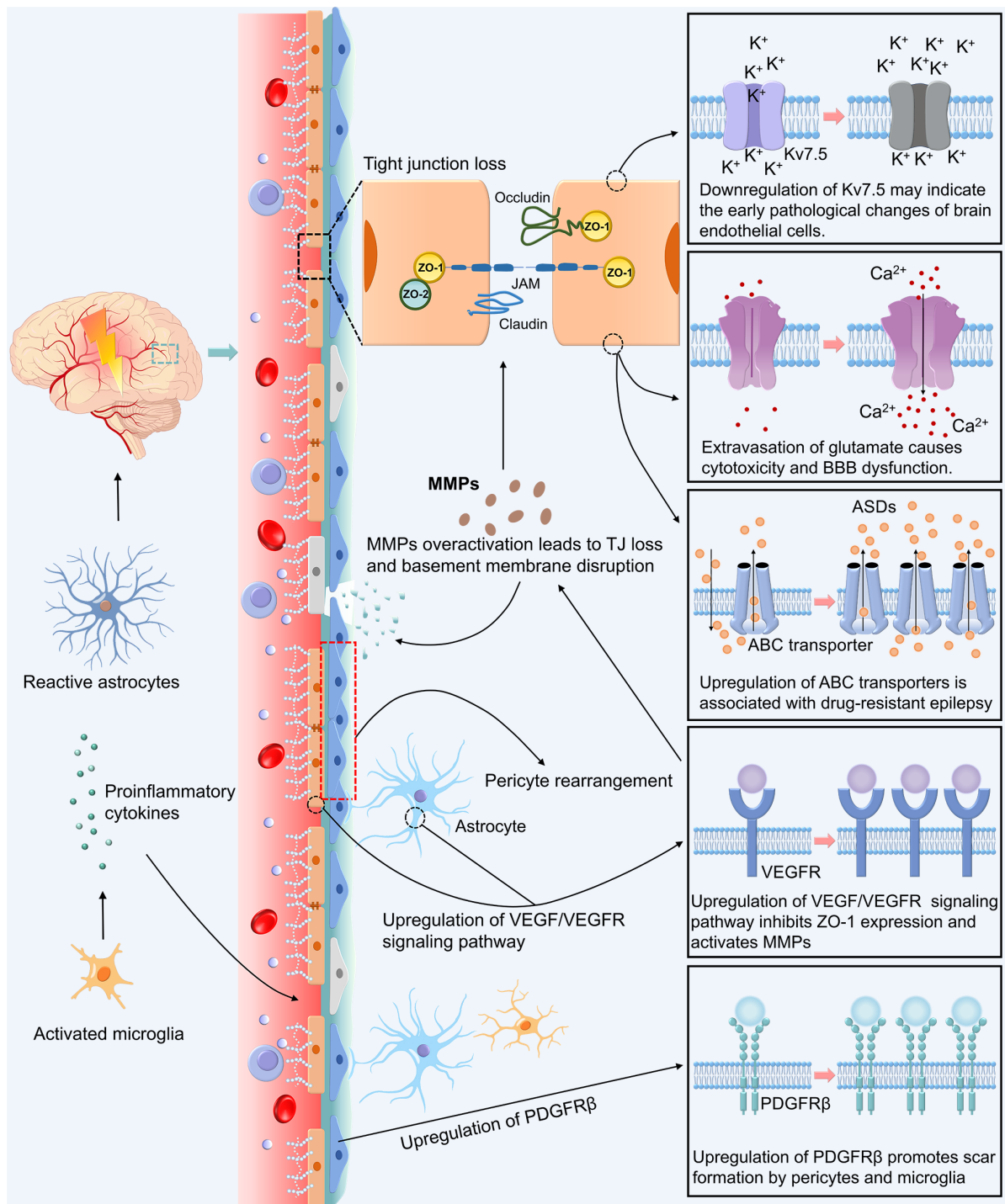


Figure 2. Functional changes of the BBB in the epileptic brain. Following epilepsy, the expression of TJ proteins, including occludin, ZO-1 and claudin-8, in BBB endothelial cells, as well as the KV7.5 ion channel in endothelial cells, is reduced significantly. In status epilepticus, enrichment of a large amount of glutamate in the brain leads to N-methyl-D-aspartate activation in endothelial cells and further increases the intracellular  $Ca^{2+}$  concentration, leading to excitotoxicity, abnormal expression and function of TJs, and increased permeability of the BBB. The upregulation of ABC transporters, such as P-glycoprotein and breast cancer resistance protein, in vascular endothelial cells limits the brain penetration of anti-seizure drugs and is closely related to drug-resistant epilepsy. Following seizures, the expression levels of the VEGF/VEGFR signalling pathway in glial and endothelial cells are upregulated, the expression of the TJ protein ZO-1 is inhibited and MMPs are activated, which destroys the vascular endothelial extracellular matrix and degrades TJ proteins. Seizures cause pericyte rearrangement and PDGFR $\beta$  overexpression, further compromising BBB integrity. The activation of astrocytes and microglia enhances electrical synaptic coupling and promotes the release of pro-inflammatory mediators, thereby increasing BBB permeability. BBB, blood-brain barrier; TJ, tight junction; ZO-1, zona occludens protein 1; ABC, ATP-binding cassette; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; MMP, matrix metalloproteinase; PDGFR $\beta$ , platelet-derived growth factor receptor  $\beta$ ; JAM, junctional adhesion molecule.

the pathogenesis of epilepsy, several mechanisms, either directly or indirectly, disrupt the BBB, contributing to the aggravation and persistence of epileptic conditions. The

primary factors associated with epilepsy and the main pathways through which they affect the BBB are outlined below.

*Glu and neurotransmitter imbalance in BBB dysfunction.* The BBB physiologically maintains cerebral Glu homeostasis through selective transport systems to prevent neurotoxic leakage (80). However, in epilepsy, excessive extracellular Glu accumulation triggers excitotoxicity, inducing neuronal damage and BBB structural-functional compromise (81). Glu exerts dual effects through ionotropic receptor [N-methyl-D-aspartate (NMDAR)/ $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)] and metabotropic Glu receptors (mGluRs) expressed in neurons, BMECs and astrocytes (82-84). In BMECs, activation of GluN1/GluN2B-containing NMDAR elevates intracellular  $Ca^{2+}$  levels, activates  $Ca^{2+}$ /calmodulin (CaM)-dependent kinase II/protein kinase C (PKC) pathways to downregulate occludin/claudin-5, and induces cytoskeletal remodelling, ultimately impairing barrier integrity (19,85). Concurrent RhoA/Rho-associated protein kinase (ROCK) and MAPK kinase 1/2-Extracellular signal-regulated kinase (ERK)1/2 signalling exacerbates vascular permeability (19,85). Astrocytic AMPAR (GluA1/A2 subtypes) activation promotes  $Na^{+}/Ca^{2+}$  influx, stimulating pro-inflammatory cytokines [interleukin (IL)-1 $\beta$  and tumor necrosis factor (TNF)- $\alpha$ ] and MMP-2/9 secretion via paracrine mechanisms to amplify BBB disruption (19). Metabotropic mGluR5 demonstrates paradoxical BBB modulation: Genetic ablation reduces BBB leakage and neutrophil infiltration (86), whereas receptor activation enhances ZO-1 expression via phospholipase C/PKC $\mu$ /c-Jun signalling through  $\alpha$ -actinin-1 protein complexes (87). In addition to Glu, epilepsy-associated  $\gamma$ -aminobutyric acid (GABA) depletion impairs astrocytic support to endothelia (88), whereas 5-hydroxytryptamine hyperactivation increases BBB permeability (89). Emerging evidence has implicated ATP/adenosine disequilibrium in BBB pathology. Seizure-elevated extracellular ATP activates microglial P2X7 receptors, triggering NLRP3 inflammasome-mediated IL-1 $\beta$  release (90), which induces MMP-9 production and ZO-1 downregulation. Purinergic P2X receptor 7 inhibition attenuates BBB damage in models of traumatic epilepsy models (91,92). These neurotransmitter disturbances collectively orchestrate BBB breakdown through receptor-specific signalling cascades, establishing a bidirectional interplay between epileptic hyperexcitability and barrier dysfunction (Fig. 3).

*Abnormal cerebrovascular remodelling.* During epileptic seizures, abnormal electrical activity in the brain affects neurons and induces pathological remodelling of blood vessels, thereby weakening the BBB. Studies using rat status epilepticus (SE) models have identified complex vascular networks in lesion areas, which are indicative of vascular remodelling (93,94). Similarly, increased vascular density observed in the hippocampus of patients with temporal lobe epilepsy is correlated with seizure frequency, suggesting that abnormal angiogenesis may contribute to epilepsy progression (95).

VEGF is a key pro-angiogenic factor that is significantly upregulated during seizures and promotes endothelial cell proliferation, migration and neovascularisation (96). Although neovascularisation enhances local blood supply, the newly formed vessels are often structurally unstable and exhibit increased permeability, leading to a compromised BBB (52). Seizure-induced local hypoxia activates hypoxia-inducible

factor-1 $\alpha$  (97), further accelerating angiogenesis by upregulating VEGF transcription (98). However, VEGF also downregulates TJ proteins, such as occludin and ZO-1, weakening BBB integrity and increasing vascular permeability (Fig. 2). This allows the infiltration of pro-inflammatory factors and immune cells across the BBB (47,99). Additionally, these infiltrating immune cells and inflammatory mediators exacerbate these effects by releasing proteases and pro-inflammatory factors that degrade the vascular structure, further aggravating BBB damage (99).

Taken together, the above findings indicate that abnormal vascular remodelling significantly contributes to BBB disruption through mechanisms involving angiogenesis, VEGF-mediated downregulation of TJ proteins and MMP-induced degradation, thereby playing a pivotal role in the pathogenesis of epilepsy (Fig. 3).

*Dysregulation of ion channels.* During seizures, ion channel dysfunction, particularly in the sodium, potassium and calcium channels, leads to excessive neuronal discharge and abnormal electrical activity. Dysregulated sodium and potassium channels cause prolonged depolarisation, maintaining neurons in a hyperexcited state and destabilising surrounding structures such as astrocytes, which impair BBB stability (100,101). Calcium ions ( $Ca^{2+}$ ) are pivotal in neuronal excitability, and intracellular  $Ca^{2+}$  imbalances frequently triggers seizures (102). Seizure-induced intracellular  $Ca^{2+}$  overload activates  $Ca^{2+}$ /CaM complexes, triggering sequential activation of myosin light-chain kinase (MLCK), which phosphorylates MLC. This  $Ca^{2+}$ -dependent cascade induces actomyosin contraction, drives cytoskeletal reorganisation and destabilises the intercellular TJ integrity (103,104). The pharmacological inhibition of MLCK using ML-7 has demonstrated therapeutic efficacy in ameliorating BBB dysfunction (104,105). Excessive  $Ca^{2+}$  influx activates PKC isoforms, with the PKC $\zeta$  subtype directly phosphorylating occludin to disrupt its binding with ZO-1 scaffolding proteins, thereby destabilising intercellular TJs and compromising BBB integrity (103,104).

Under physiological conditions, coordinated aquaporin 4 (AQP4)-inwardly rectifying potassium channel 4.1 (Kir4.1) expression in astrocytic end-foot domains maintains cerebral water-potassium homeostasis through extracellular ion clearance and osmotic balance. Epileptic activity significantly downregulates AQP4 expression (106-108), induces mislocalisation with reduced polarised distribution (109,110) and impairs vascular structural support, thereby collectively destabilising TJ integrity. These pathological alterations increase the BBB permeability (111,112) and the risk of neurotoxic infiltration. Experimental validation using AQP4-knockout models demonstrates exacerbated BBB damage post-epileptogenesis (113). Additionally, AQP4 downregulation additionally hinders Kir4.1-mediated potassium reuptake, resulting in elevated extracellular  $K^{+}$  levels and increased intracellular osmotic pressure (114) (Fig. 3).

Thus, ion channel abnormalities during seizures initiate cellular damage and TJ disruption, thereby impairing BBB permeability. Cytoskeletal changes and PKC activation driven by  $Ca^{2+}$  influx weaken TJs, while AQP4 and Kir4.1 dysfunctions further disturb the water and potassium balance. These

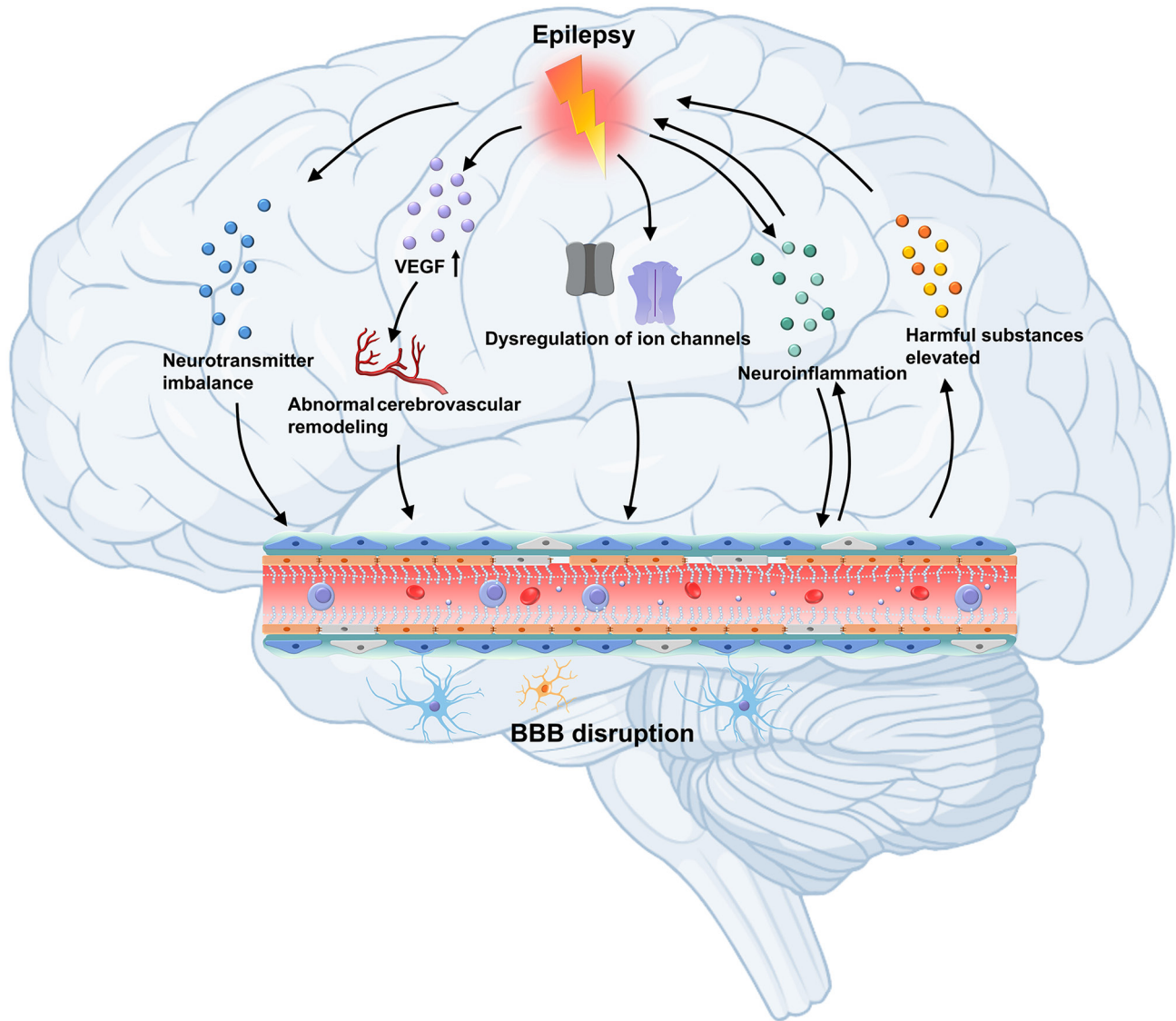


Figure 3. Interplay between BBB damage and epileptogenesis. Epilepsy leads to neurotransmitter imbalances, abnormal remodelling of cerebral blood vessels and abnormal changes in ion channels, thereby destroying the BBB. Epilepsy can cause microglial activation, which releases a number of pro-inflammatory mediators, and the long-term inflammatory response leads to increased BBB permeability. BBB damage exacerbates the inflammatory response through various pathways and accumulates harmful substances in the brain, thereby promoting the occurrence and development of epilepsy. BBB, blood-brain barrier; VEGF, vascular endothelial growth factor.

interconnected mechanisms contribute to BBB dysfunction and exacerbate the brain tissue damage during seizures.

### 5. BBB injury promotes epileptogenesis

An impaired BBB is increasingly recognized not only as a consequence of CNS disorders, such as epilepsy, but also as a pathogenic factor capable of promoting seizures. Early investigations demonstrated that BBB dysfunction could induce focal epilepsy, as shown in rat models, where bile salt-induced BBB disruption led to epileptiform activity within 4-49 days post-disruption (115). Notably, experimental disruption of the BBB in SE models using mannitol to induce osmotic opening has been shown to exacerbate seizure frequency in rats (116). Recent findings have highlighted the role of BBB disruption in seizure pathogenesis. *In vitro* introduction of thrombin has been shown to rapidly induce epileptiform discharge

activity in hippocampal neurons, effectively replicating the consequences of BBB impairment (117). Additionally, the deletion of claudin-5, a crucial TJ protein, results in spontaneous seizures, severe neuroinflammation and mortality in mice. These effects can be mitigated by RepSox, which modulates claudin-5 expression, highlighting its critical role in maintaining BBB integrity and preventing seizures (118). Furthermore, in BMECs, selective knockout of cyclin-dependent kinase 5, an essential regulator of neuronal excitability, induces spontaneous epilepsy. This process involves astrocyte dysfunction mediated by the CXCL1/CXCR2 signalling pathway, linking BBB dysfunction to broader neuronal and astrocytic abnormalities (119).

*Impaired BBB allows albumin to penetrate brain parenchyma.* BBB dysfunction results in serum albumin leakage and significantly contributes to epileptogenesis (115,116). The leakage of

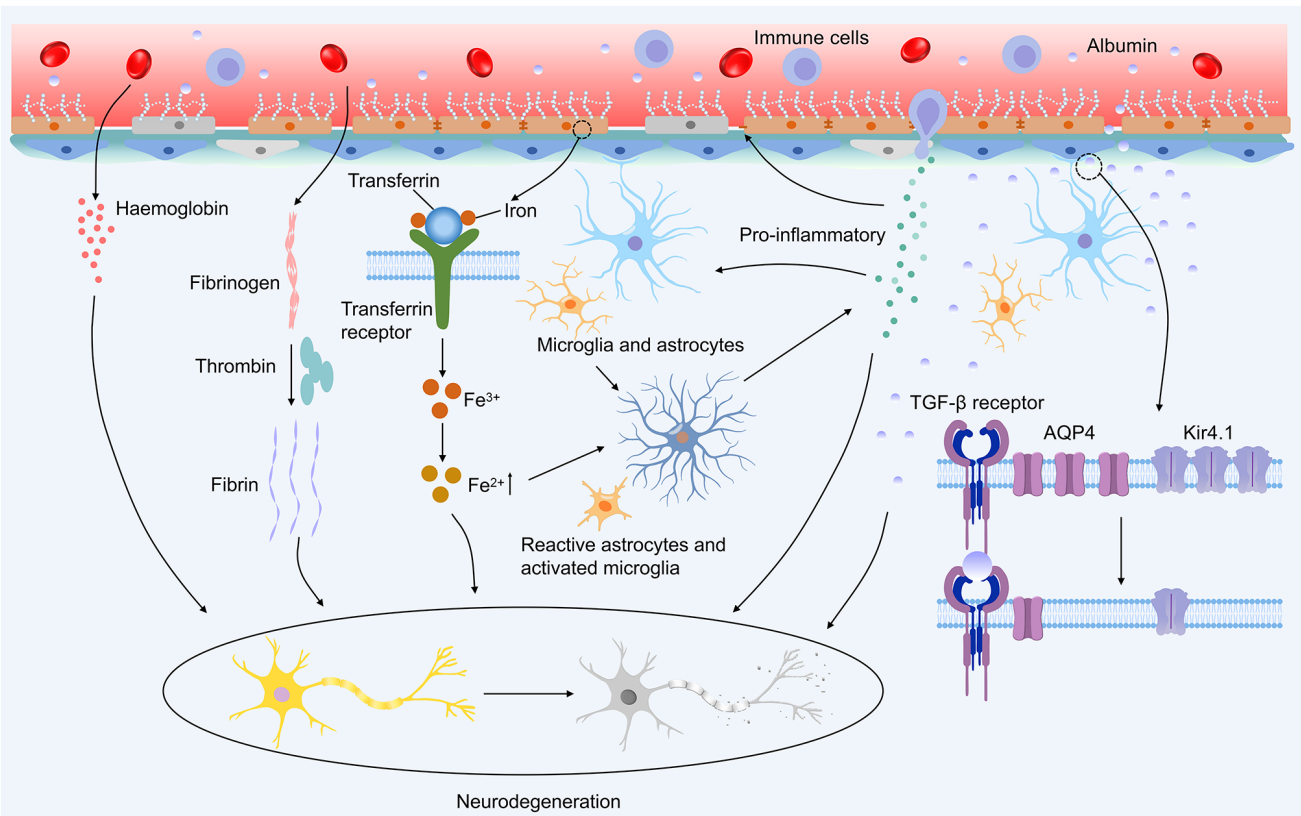


Figure 4. Mechanisms of epilepsy progression caused by BBB damage. Serum albumin leakage caused by BBB damage is a key factor in epileptogenesis. After serum albumin enters the brain parenchyma, it binds to TGF- $\beta$  receptors on astrocytes, resulting in a decrease in the expression of Kir4.1 and AQP4, disrupting potassium and water balance, lowering the excitability threshold and activating the associated signalling cascade. This may eventually lead to seizures, and the exuded serum albumin can be absorbed by neurons, causing neurons to die and dissolve. Furthermore, when the BBB is compromised, immune cells from the periphery infiltrate brain tissue to release pro-inflammatory factors, such as tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , high mobility group box-1 and toll-like receptor 4. These mediators can activate glial cells in the brain, such as microglia and astrocytes, and promote them to release more pro-inflammatory factors, aggravate neuronal damage, increase neuronal excitability and reduce the threshold of seizures. These pro-inflammatory factors can further aggravate the destruction of the BBB structure. BBB dysfunction increases the permeability of blood vessels, resulting in a severe local inflammatory response, which jointly promotes the transport of a large amount of iron from the blood to the brain parenchyma, causing iron overload. Oxidative stress caused by iron overload causes further neuronal damage. The accumulation of iron activates microglia and astrocytes, releases a large number of pro-inflammatory factors, aggravates neuronal damage, reduces the threshold of seizures and promotes the development of epilepsy. Additionally, BBB disruption allows plasma components such as fibrinogen, thrombin and haemoglobin to enter the brain, further promoting neuronal death and aggravating seizure progression. BBB, blood-brain barrier; TGF- $\beta$ , transforming growth factor- $\beta$ ; AQP4, aquaporin 4; Kir4.1, inwardly rectifying potassium channel 4.1.

albumin into the brain parenchyma, often in combination with antiepileptic drug exposure, may play a role in the development of drug resistance in patients (120). Albumin infiltration facilitates epilepsy by lowering the threshold for spreading depolarisations, enhancing neuronal hyperexcitability and impairing normal neuronal function (121). In normal rats, electroencephalographic (EEG) monitoring following intraventricular administration of albumin revealed rapid spiking activity in the hippocampus within 15-60 min, and a substantial reduction in seizure threshold was observed 3 months later (122).

Additionally, albumin alters synaptic plasticity in the hippocampal CA1 region, potentially influencing progression of epilepsy (123). In guinea pig brain slices with KA-induced epileptiform activity, albumin application intensifies epileptiform discharges and triggers seizure recurrence upon subsequent KA exposure, highlighting its role in promoting acute seizures (124). When albumin infiltrates the brain parenchyma, it interacts with transforming growth factor  $\beta$  receptors (TGF- $\beta$ R) on astrocytes, resulting in reduced expression of Kir4.1 and AQP4 in these cells (Fig. 4).

Decreased Kir4.1 disrupts intracellular potassium clearance, potentially contributing to neuronal hyperexcitability and synchronised neural activity (125). The downregulation of AQP4 further exacerbates this imbalance by affecting Kir4.1, altering cellular potassium and water homeostasis, and activating signaling pathways that may ultimately lead to seizures (106,126). Additionally, BBB dysfunction has been linked to reduced levels of the gap junction proteins connexin 30 and 43 in the cortical astrocytes. This reduction results in gap junction uncoupling, which impairs extracellular potassium clearance and creates favourable conditions for seizure generation (127,128).

These observations underscore the critical role of BBB dysfunction in allowing serum albumin to penetrate the brain parenchyma, triggering a cascade of molecular and cellular changes that contribute to the onset and progression of epilepsy (Fig. 4).

*BBB damage facilitates inflammation to accelerate epilepsy progression.* In physiological states, the BBB maintains its impermeability to peripheral immune cells and macromolecules

(e.g. albumin) via a tripartite mechanism involving endothelial TJs (claudin-5 and occludin), astrocytic end-feet ensheathment and the supportive basement membrane. This structural and functional synergy safeguards the chemical and immunological homeostasis of the CNS parenchyma. A pathological breach of BBB integrity allows serum proteins (e.g., albumin) to penetrate the brain parenchyma, directly triggering TGF $\beta$ RII-mediated SMAD phosphorylation in astrocytes (129). This signalling event drives the transcriptional upregulation of proinflammatory cytokines (IL-1 $\beta$  and TNF- $\alpha$ ), thereby promoting neuroinflammation and lowering the seizure threshold (129). BBB dysfunction enables the translocation of serum proteins into the brain parenchyma while simultaneously creating a permissive pathway for peripheral immune cells (e.g. T cells and macrophages) to infiltrate through disrupted endothelial junctions. This dual invasion drives neuroinflammation and contributes to seizure susceptibility by destabilising the neurovascular niche (130-133). These infiltrating immune cells release large quantities of proinflammatory mediators, such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6, which further activate microglia and astrocytes, initiating a neuroinflammatory cascade (Fig. 4). Progressive neuroinflammation drives marked upregulation of proinflammatory cytokines (e.g. IL-1 $\beta$  and TNF- $\alpha$ ) and alarmins [e.g. high mobility group box 1 (HMGB1)], coupled with the activation of pattern recognition receptors, such as Toll-like receptor 4 (TLR4). Preclinical and clinical evidence has demonstrated that the magnitude of this inflammatory cascade directly correlates with seizure severity, likely through the amplification of excitotoxicity and synaptic dysfunction via glial-derived cytokine signalling (134). These factors exacerbate BBB permeability and act directly on neurons and synapses, promoting neuronal hyperexcitability and facilitating epileptogenesis.

TNF- $\alpha$  drives BBB disruption through NF- $\kappa$ B-dependent inhibition of claudin-5 transcription, ultimately decreasing claudin-5 expression (131,135). Moreover, TNF- $\alpha$  activates the RhoA/ROCK signalling pathway, inducing cytoskeletal remodelling and spatial redistribution of claudin-5 and occludin, ultimately increasing BBB permeability (136,137). IL-1 $\beta$  contributes to BBB dysfunction by downregulating ZO-1 expression via the MAPK signalling pathway (138), and promoting ZO-1 ubiquitination and degradation through the ERK pathway in cooperation with TNF- $\alpha$  (139). As BBB integrity continues to deteriorate, inflammatory factors, such as HMGB1, accumulate in the brain parenchyma, where they, together with IL-1 $\beta$ , activate neuronal NMDAR (GluN2B subunits), triggering an influx of Ca<sup>2+</sup>, leading to increased neuronal excitability and synaptic plasticity abnormalities (140,141). Moreover, IL-1 $\beta$  disrupts the excitatory-inhibitory balance by inhibiting astrocytic GABA reuptake while simultaneously increasing Glu release, further lowering the seizure threshold (141). Additionally, the inflammatory response resulting from BBB disruption exacerbates BBB degradation via MMPs, thereby contributing to epileptogenesis. IL-1 $\beta$  enhances MMP-9 activity via the ATP/P2X7R pathway (142), while HMGB1 upregulates MMP-9 through a TLR4-mediated, but TNF- $\alpha$ -independent, mechanism (143). This process further stimulates IL-1 $\beta$  release via the NLRP3 inflammasome, accelerating basement membrane degradation (144). Excessive MMP

activation compromises BBB integrity and alters the neural network architecture by remodelling the perisynaptic extracellular matrix. This contributes to the formation of chronic epileptogenic foci (145).

Simultaneously, TNF- $\alpha$  upregulates AMPA receptor expression at neuronal synapses, enhances excitatory synaptic transmission and reduces astrocytic Glu uptake. These changes increase synaptic Glu concentrations, ultimately leading to excitotoxicity (146-148). Furthermore, elevated IL-1 $\beta$  levels downregulate GABA receptor expression, further weakening inhibitory neurotransmission. This disruption of excitation-inhibition homeostasis significantly increases the likelihood of epileptiform discharges (146,149). The breakdown of the BBB initiates a self-reinforcing cycle comprising inflammatory responses, neuronal hyperexcitability and seizure-promoting activities. As BBB permeability increases, more inflammatory mediators infiltrate the brain parenchyma, further activating the neurons and glial cells. This sustained activation leads to the excessive release of inflammatory factors, exacerbating neuronal damage and increasing the incidence of epileptiform discharges (150). Chronic neuroinflammation dysregulates synaptic homeostasis and glial-neuronal crosstalk, culminating in the destabilisation of neural circuits and the emergence of epileptogenic zones (151). Consequently, this pathological cycle significantly complicates epilepsy treatment, highlighting the critical need for therapeutic strategies targeting BBB protection and the attenuation of neuroinflammation (Fig. 4).

*BBB damage-induced iron overload exacerbates seizure severity.* The BBB plays a critical role in maintaining CNS iron homeostasis by regulating iron transport via transferrin (Tf) and its receptor, transferrin receptor protein 1, which are essential for myelin synthesis and catecholamine neurotransmitter metabolism, and contribute to neurodevelopment and protection under normal conditions (152). However, BBB damage disrupts this regulation, allowing excessive iron to enter the brain tissue from the bloodstream, leading to its accumulation in neurons and glial cells (153,154).

The studies by Willmore *et al* (155,156) were the first to demonstrate that cortical injections of FeCl<sub>2</sub> or FeCl<sub>3</sub> in rats and cats induced chronic seizures, establishing a connection between iron overload and epilepsy. In patients with focal epilepsy and in mouse hippocampal slices, significant iron deposition has been observed in seizure-prone regions, particularly in the hippocampus (157). A clinical study revealed elevated Tf saturation in individuals with epilepsy compared with controls, supporting the role of iron dysregulation in the pathogenesis of epilepsy (158).

Iron overload contributes to the epilepsy pathophysiology by triggering oxidative stress, which lowers the seizure threshold. The accumulated iron catalyses the Fenton reaction, generating free radicals that accelerate lipid peroxidation, disrupt neuronal membrane integrity and increase neurotoxicity (159,160). Elevated levels of reactive oxygen species and malondialdehyde, along with decreased GPX4 expression observed in mouse models of epilepsy, underscore the role of oxidative stress in epilepsy progression (161,162). Oxidative stress damages mitochondria, leading to neuronal apoptosis and necrosis, weakening neural networks, fostering

epileptic foci and increasing the likelihood of spontaneous seizures (154,159).

Iron accumulation also activates microglia and astrocytes, driving the release of inflammatory mediators, such as TNF- $\alpha$  and IL-1 $\beta$  (163). These inflammatory factors increase neuronal excitability by reducing GABAergic inhibition and enhancing excitatory neurotransmitter release, thereby increasing seizure frequency. Oxidative stress and inflammation caused by iron overload exacerbate BBB dysfunction, facilitating further infiltration of iron and inflammatory mediators into the brain. This self-reinforcing cycle exacerbates neuronal damage, increases seizure frequency and complicates epilepsy management (153,164) (Fig. 4).

In conclusion, BBB-related iron overload plays a significant role in epilepsy pathophysiology. Oxidative stress and inflammation lower the seizure threshold, exacerbate neuronal damage and establish a cycle that drives epilepsy progression.

*Harmful substances are elevated in the brain after BBB damage.* The BBB plays a crucial role in regulating the movement of molecules in and out of the CNS and ensuring a stable chemical composition in the neuronal environment, which is essential for normal neural function. Under physiological conditions, the BBB acts as a protective barrier, preventing the entry of neurotoxic plasma components, blood cells and pathogens into the brain (165). However, in pathological states, BBB dysfunction manifests as cerebrovascular hyperpermeability, neurovascular decoupling or blood flow dysregulation, and is associated with disorders such as stroke, glioma, epilepsy, traumatic brain injury and neurodegenerative diseases (166,167). Disruption of the BBB integrity allows neurotoxic plasma components to leak into the brain, facilitates immune cell infiltration and disturbs the CNS environment, leading to neuronal death and worsening disease progression (53,166). Research using transgenic mouse models with chronic BBB disruption has revealed that the accumulation of neurotoxic proteins, including fibrinogen, thrombin, hemoglobin, iron-rich hemosiderin and plasmin, within neurons can initiate or aggravate neurodegeneration (31,168,169) (Fig. 4). A strong link exists between BBB dysfunction and epilepsy, suggesting that a compromised BBB may contribute to epileptogenesis or exacerbate seizure propagation. An experimental study demonstrated that artificial disruption of the BBB, such as through osmotic shock, can induce seizures (170). Furthermore, conditions that impair BBB integrity, including infection, inflammation, stroke and traumatic brain injury, are well-documented triggers of seizures and epilepsy development (116,171) (Fig. 4).

Disruption of the BBB plays a central role in both the initiation of epileptogenesis and the chronic progression of epilepsy. Emerging evidence suggests that BBB disruption facilitates albumin infiltration into the brain parenchyma, exacerbates neuroinflammation, induces iron overload and allows the entry of harmful substances into the CNS (172-174). These pathological changes collectively drive the chronic progression of epilepsy and seizure initiation. Given the integral role of BBB integrity in disease modulation, therapeutic strategies aimed at restoring BBB function are gaining increasing attention (175). Potential interventions include pharmacological agents that reinforce BBB integrity, anti-inflammatory therapies, iron

chelators, advanced nanodelivery systems and cell-based therapies. The following section discusses these innovative approaches, highlighting their potential to optimise antiepileptic interventions, disrupt the pathological cycle and pave the way for precision medicine in epilepsy treatment.

## 6. Current novel therapeutic strategies targeting the BBB

As outlined, BBB dysfunction plays a critical role in epileptogenesis, and the interplay between BBB disruption and epileptic activity forms a self-reinforcing cycle that exacerbates both conditions and accelerates disease progression (43,44). Given the pivotal role of the BBB in epilepsy, the development of therapeutic interventions targeting BBB dysfunction is increasingly prioritised. Strategies that focus on restoring BBB integrity, decreasing BBB permeability and blocking the infiltration of neurotoxic substances may help interrupt this detrimental cycle, slow disease progression and enhance patient outcomes.

*BBB-targeted therapeutic strategies for epilepsy management.* BBB dysfunction is a critical contributor to epilepsy initiation and progression. The pathological translocation of serum albumin and other blood-derived molecules into the CNS parenchyma disrupts the neurovascular niche, amplifying neuronal injury and seizure activity (176,177). This microenvironmental disruption amplifies oxidative stress and synaptic hyperexcitability, progressively lowering the seizure threshold and accelerating epileptogenesis. Given its pivotal role in epileptogenesis, restoration of BBB integrity has emerged as a novel and promising therapeutic strategy. Various approaches are being actively investigated to restore BBB integrity and function, with the goal of accelerating their translation into clinical use as effective therapies for epilepsy.

Among these strategies, natural compounds, such as the neuroprotective flavonoid vitexin, have demonstrated efficacy in promoting BBB integrity through the upregulation of TJ proteins, thus decreasing susceptibility to epilepsy (178). A derivative of vitexin, VB-001, has completed a phase I safety trial (CTR20190758) and may soon enter phase II clinical trials. Certain ASDs have been shown to exert protective effects on the BBB. For example, potassium channel modulators, such as retigabine, suppress neuronal hyperexcitability and upregulate TJs proteins, such as occludin and claudin-5, thereby decreasing BBB permeability (21). However, despite its initial approval by the U.S. Food and Drug Administration in 2011, retigabine was withdrawn due to adverse effects, such as skin and retinal pigmentation. The optimised variant, XEN1101, exhibited a  $\geq 50\%$  reduction in seizure frequency in 47% of participants with drug-resistant focal epilepsy during a phase II clinical trial (NCT03796962), with no reported retinal adverse effects. Another ASD, levitracetam (LEV), has demonstrated the potential to maintain BBB integrity following febrile seizures by upregulating occludin and claudin-5 expression, and inhibiting BMEC pinocytosis (179). Angiotensin receptor blockers (ARBs) are attracting interest as adjunctive treatments for epilepsy, as they improve cerebral blood flow, prevent cerebral haemorrhage, preserve BBB function, reduce neuroinflammation and provide neuroprotection (180). Preclinical studies have

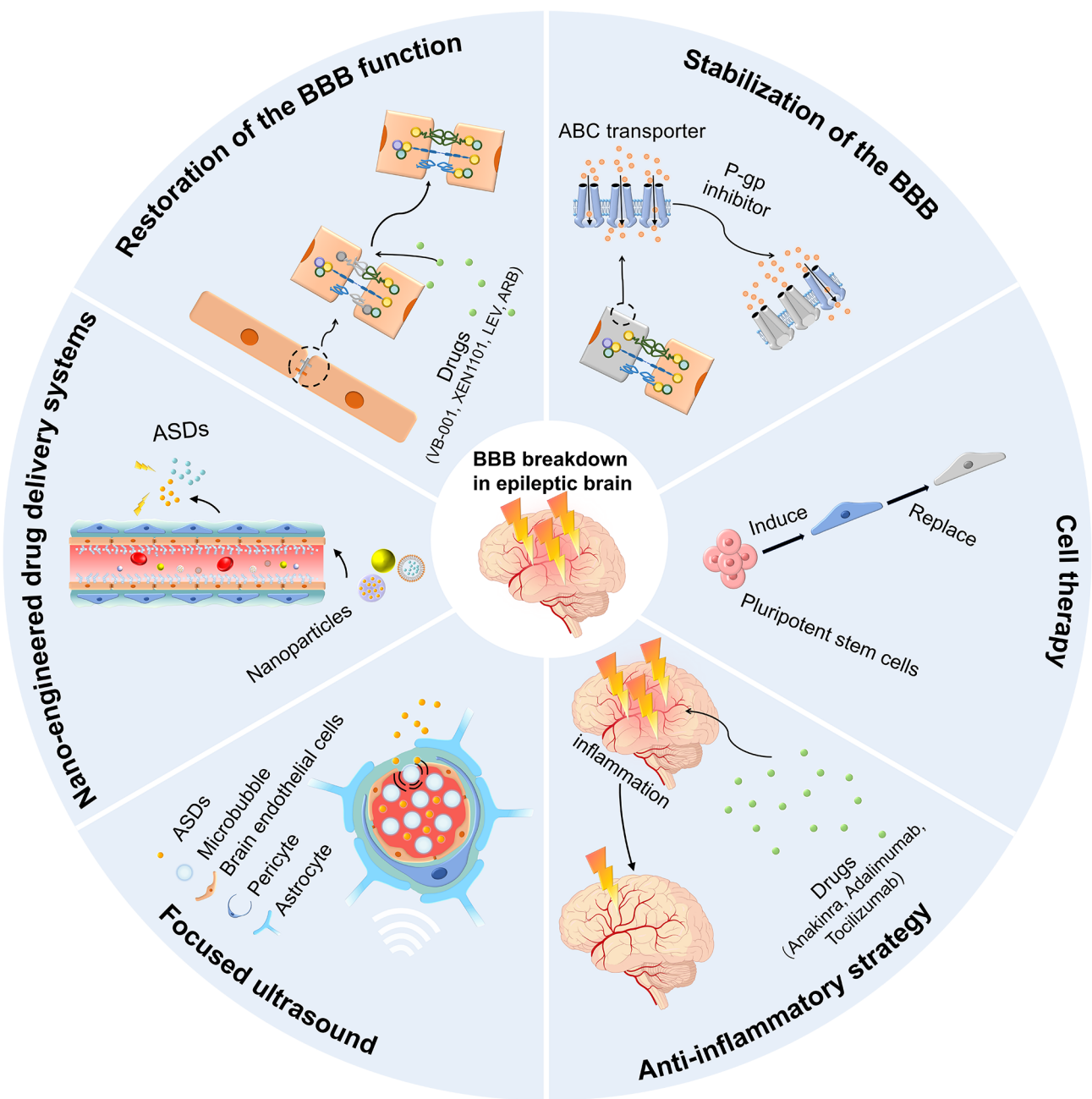


Figure 5. Novel treatment strategies for epilepsy based on BBB regulation. Emerging strategies for treating epilepsy have focused on restoring BBB integrity and improving drug delivery. Restoring the damaged BBB and eliminating the negative effects caused by BBB dysfunction (such as anti-inflammatory therapy) may be key strategies for the treatment of epilepsy, such as the vitexin derivative VB-001, potassium channel modulators, levetiracetam, flavonoids and high mobility group box-1 antagonists. Cell therapies using induced pluripotent stem cell-derived pericytes to replace dysfunctional pericyte function may potentially restore the BBB. Focused ultrasound combined with microbubbles, which instantaneously opens the BBB, is a promising non-invasive technique that plays an important role in targeted delivery therapy. Nanomaterial drug delivery systems can pass through the BBB and play an important role in treating epilepsy. BBB, blood-brain barrier; ASD, anti-seizure drug.

shown that ARBs upregulate claudin-5 and ZO-1 expression, thereby facilitating structural restoration of the BBB in rodent models of senescence (181). Additionally, ARBs have been shown to significantly reduce seizure severity in experimental epilepsy models (182) (Fig. 5). An alternative therapeutic approach focuses on mitigating the detrimental consequences of BBB dysfunction. Iron chelation and antioxidant therapies have been proposed to counteract neurotoxic effects associated with BBB damage, such as intracerebral iron accumulation and oxidative stress (183).

*Novel therapeutic strategies targeting BBB stabilisation for epilepsy.* BBB-stabilising drugs are a promising strategy for epilepsy treatment. RepSox, a claudin-5 modulator, has been shown to inhibit seizure activity in a KA-induced mouse model of epilepsy (118). A promising therapeutic approach involves the administration of P-gp inhibitors to enhance the efficacy via BBB drug retention. P-gp is an efflux transporter that actively pumps drugs out of the brain, contributing to drug resistance in epilepsy. Inhibition of P-gp can increase intracranial ASD concentrations and improve therapeutic

outcomes (184) (Fig. 5). Ferreira *et al.* (73) reported that P-gp-inhibiting flavonoids significantly increased ASD brain penetration, underscoring the value of targeting P-gp to improve BBB drug delivery. Deng *et al.* (185) further showed that miR-146a-5p, an anti-inflammatory microRNA, reduced P-gp expression in SE-model-derived cerebrovascular endothelial cells through NF- $\kappa$ B pathway inhibition, highlighting its potential as a novel therapy for drug resistance caused by SE. Recombinant human erythropoietin (rhEPO) inhibits P-gp activity and reverses P-gp-mediated drug resistance in refractory epilepsy (186). Although the inhibition of transporters can enhance drug accumulation in the brain, significant challenges remain. P-gp inhibition may cause systemic toxicity (e.g. liver damage) due to elevated drug levels (187), but trigger off-target effects that alter drug distribution in normal tissues. Therefore, transporter inhibition strategies require caution and precise control for clinical use. Critical factors include dosing timing and regimen selection, with a recommended low-dose initiation strategy combined with real-time blood drug monitoring and clinical effect evaluation to adjust inhibitor/anti-seizure medication doses and schedules (188). Close monitoring of liver/kidney function, infection markers and systemic toxicity risks is essential for minimising intervention-related hazards during the initial treatment.

*Novel cell-based therapies for epilepsy.* Cell-based therapies are promising strategies for BBB repair. Induced pluripotent stem cell (iPSC)-derived pericytes have been proposed to replace dysfunctional pericytes and restore BBB integrity, thereby increasing vascular stability, reducing neuroinflammation and improving selective BBB permeability (189). Chen (190) demonstrated that transplantation of iPSC-derived pericytes enhances BMEC interactions, upregulates the expression of TJ proteins, and suppresses the expression of inflammatory markers (TNF- $\alpha$  and IL-1 $\beta$ ), ultimately decreasing BBB permeability and conferring neuroprotection. These findings suggest that iPSC-derived pericyte transplantation is a promising therapeutic approach for BBB restoration in patients with epilepsy. However, further studies are needed to refine transplantation protocols, increase cell survival rates, and evaluate the long-term safety and efficacy of this method (Fig. 5).

*Novel anti-inflammatory strategies for epilepsy.* A bidirectional pathological interaction exists between epileptic seizures and BBB disruption, both of which are accompanied by a pronounced neuroinflammatory response. Neuroinflammation increases BBB permeability and promotes seizure generation and chronicity. Therefore, targeting the inflammatory cascades has emerged as a promising strategy for epilepsy therapy. Recent studies have identified several critical cytokines and receptors as potential therapeutic targets, including IL-1R1, HMGB1 and TNF- $\alpha$  (191-193). In a KA-induced SE mouse model, conditional deletion of IL-1R1 in endothelial cells significantly suppressed hippocampal inflammation during KA-induced SE, reduced seizure susceptibility and improved behavioural outcomes (194), indicating the IL-1 $\beta$ /IL-1R1 axis as a viable target. HMGB1, a prototypical damage-associated molecular pattern, activates inflammatory signalling via TLR4 and the receptor for advanced glycation

end products. Clinically, HMGB1 has been identified as a potential biomarker of refractory epilepsy (195). Experimental studies have suggested that HMGB1 antagonists or neutralising monoclonal antibodies can decrease seizure frequency and severity (196). TNF- $\alpha$ , a classical pro-inflammatory cytokine, regulates neuronal excitability and apoptosis; its inhibition in preclinical models has demonstrated neuroprotective effects and reduced seizure activity (197). However, systemic TNF- $\alpha$  suppression may increase the risk of infections and malignancies, thereby limiting its clinical application (198). Regarding efficacy, interventions targeting inflammatory mediators have shown the potential to reduce seizures and delay epileptogenesis in preclinical settings (199). Under certain conditions, such as SE or DRE, these strategies may offer advantages over conventional ASDs, which primarily control symptoms (200). Nevertheless, the risks associated with immune suppression necessitate precise regulation of the treatment dose, timing and duration to achieve therapeutic benefits while ensuring safety (201).

Several clinical trials have investigated the application of BBB-stabilising and anti-inflammatory agents in the treatment of epilepsy. For example, anakinra, an IL-1 receptor antagonist, has demonstrated efficacy in reducing seizure burden and recurrence in paediatric patients with febrile infection-related epilepsy syndrome (FIRES) (202). Humanised monoclonal antibodies targeting neuroinflammatory pathways have been used. Adalimumab, an anti-TNF- $\alpha$  agent, has shown seizure-reducing effects in clinical contexts (203). Tocilizumab, which blocks IL-6R, is currently under phase IV clinical trials for the treatment of paediatric FIRES (Chinese Clinical Trial Registry no. ChiCTR2400085185). Moreover, specific HMGB1 antagonists are under preclinical development and can potentially advance into clinical testing. In summary, IL-1R1, HMGB1 and TNF- $\alpha$  represent the most promising molecular targets in current anti-inflammatory approaches for epilepsy treatment. This strategy improves seizure control and delays disease progression, particularly in refractory cases. However, rigorous multicentre trials are essential to validate its clinical utility and define the optimal therapeutic windows and indications (Fig. 5).

*Novel strategies for epilepsy treatment based on enhancing drug delivery to target brain regions.* In addition to anti-inflammatory approaches, improving drug delivery to epileptogenic brain regions is another critical therapeutic strategy for epilepsy. Although epilepsy can alter the permeability of the BBB, elevated expression of efflux transporters such as BCRP, MRP1 and MRP2 continues to restrict the effective entry of drugs into the brain. Major challenges include the reduced bioavailability, solubility, stability and efficacy of therapeutic compounds before they reach their intended targets within the brain. To address these limitations, researchers have proposed various strategies to improve the efficiency of brain-targeted drug delivery. With the rapid growth of nanomedicine, nanoparticle-based drug delivery systems have become prominent solutions for increasing drug accumulation in the brain. Drug-loaded nanoparticles can cross the BBB and reach specific lesion sites in the brain without losing their activity, thereby improving drug utilization (204). Glucose transporter 1 (GLUT1) is abundantly expressed in the BBB, and several

GLUT1-targeting nanocarriers have been developed to facilitate BBB penetration (205-207). Anraku *et al* (208) designed glucose-modified nanomaterials that could recognise and bind to GLUT1. This strategy takes advantage of the increased GLUT1 expression following fasting-induced hyperglycaemia, which promotes BBB penetration and brain accumulation of nanocarriers. Although this system has not yet been applied in epilepsy models, it may overcome the challenge of reduced GLUT1 expression in the epileptic brain and achieve effective drug delivery. Serum albumin, which leaks into the brain parenchyma following BBB disruption, has been considered a viable carrier for constructing brain-targeted nanocarriers. Although most albumin-based systems are currently used for anticancer drug delivery (209-211), their potential application in epilepsy treatment merits further investigation (Fig. 5).

Beyond optimising nanomaterials, the transient disruption of BBB integrity has been explored to enhance drug transport from the circulatory system into the CNS. Common BBB-opening approaches include focused ultrasound (FUS), static magnetic fields and near-infrared (NIR) laser stimulation, all of which are non-invasive. FUS, often used in combination with intravenously injected microbubbles (MBs), temporarily disrupts the BBB through acoustic cavitation, thereby improving drug penetration into brain tissues (212,213). A clinical study by Gasca-Salas *et al* (214) demonstrated that MRI-guided FUS combined with MBs successfully and reversibly opened the BBB at the temporoparietal junction in patients with Parkinson's disease and dementia, achieving success in 8 out of 10 treatments in 5 patients. In addition, Ozdas *et al.* developed FUS-responsive nanoassemblies that enabled non-invasive, targeted drug release in the brain with drug accumulation levels approximately 1300-fold higher than free drug, demonstrating significant potential for treating epilepsy and other neurological diseases (215).

Static magnetic fields can enhance the BBB penetration of magnetic nanoparticles, increasing the permeability rate from 3.36 to 8.47% (216). Gupta *et al* (217) reported that magnetic field-induced heating can temporarily loosen TJs in endothelial cells, facilitating drug release and delivery. Zhang *et al* (218) constructed a magnetoelectric core-shell nanostructure ( $\text{Fe}_2\text{O}_4@ \text{BaTiO}_3$ ) using a static magnetic field-assisted interface co-assembly strategy. These nanostructures can be externally activated to produce electrical stimulation and modulate neuronal currents, with initial validation of their feasibility for the wireless modulation of epilepsy symptoms (219). Given the characteristic hypersynchronous discharges observed during epileptic seizures, electroresponsive nanomaterials containing Fc groups have been developed to enable on-demand drug release, increase local drug concentration and improve treatment outcomes (218).

NIR-activated photothermal nanomaterials can generate local heating, which facilitates BBB opening by enhancing interstitial fluid flow and increasing endothelial membrane permeability (220). Wu *et al* designed a dopamine-pyrrole hybrid system that combined receptor-mediated endocytosis with NIR-triggered photothermal conversion, thereby enhancing the brain delivery of ASDs (221). Yang *et al* (222) further demonstrated that under NIR stimulation, black phosphorous nanosheets modulate membrane currents in hippocampal neurons and suppress epileptiform discharges

in mouse models, offering a new therapeutic direction for DRE. In addition, macrophage membrane-coated biomimetic nanoparticles (MA@RT-HMSNs) constructed by Geng *et al* showed excellent targeting ability. These nanoparticles successfully delivered the death-associated protein kinase (DAPK)1 inhibitor TC-DAPK6 across the BBB and selectively accumulated in inflammatory regions within epileptogenic zones, showing significant therapeutic benefits in both acute and chronic epilepsy models. Hou *et al* (223) developed a dual-target nanoparticle system using microfluidic techniques to encapsulate lamotrigine in polyethylene glycol for targeted delivery to epileptic neurons. This system significantly alleviated seizure symptoms. DBS, an established method for managing DRE, was recently integrated with FUS to achieve minimally invasive neuromodulation. Chen *et al* reported that low-intensity FUS pulses effectively suppress seizure-related EEG discharges in pentylentetrazole-induced epileptic rats, optimising exposure parameters and improving the antiepileptic effect (224). Clinical research has confirmed the safety and feasibility of FUS in targeting epileptogenic brain areas without significant adverse effects, laying the groundwork for its clinical translation (225). A summary of studies investigating BBB-penetrating antiepileptic therapies is provided in Table I.

Despite encouraging progress in nanotechnology-based drug delivery systems for epilepsy, several significant challenges hinder their successful translation from laboratory studies to clinical applications. First, there are unresolved safety concerns *in vivo*. While some nanomaterials demonstrate satisfactory biocompatibility *in vitro*, their behavior in living organisms may differ considerably. The mechanisms of the metabolism, degradation and excretion of nanodrugs remain poorly understood, leading to concerns about their potential long-term toxicity. Nanoparticles may accumulate in vital organs, impairing physiological functions, and such accumulation over time could result in organ dysfunction or failure. Additionally, the degradation products of nanomaterials may be toxic, posing continuous risks to human health even after therapeutic effects have ended. To address these safety issues, comprehensive studies on the biocompatibility, immunogenicity and chronic toxicity of nanomaterials are essential. Second, the potential immunogenicity of different nanoparticle formulations remains a concern. Although various nanomaterials have been extensively studied for the diagnosis of CNS disorders, relatively few studies have focused on their cytotoxicity, genotoxicity and immunotoxicity (226-228). Factors such as surface properties, particle size, morphology and chemical composition can all influence immune responses. Immunomodulatory therapies are currently under investigation for autoimmune-related epilepsy, in which abnormal immune activity contributes to seizure generation. Third, translating pharmacokinetic data from rodent models to humans presents additional complexity. The pathogenesis of a number of CNS disorders remains unclear, and significant physiological differences exist between humans and animal models. As a result, most rodent models may fail to accurately replicate the pathophysiological features of human epilepsy. Furthermore, controlling drug release *in vivo* with high precision is still challenging. Variations in the rate, timing and quantity of drug release can significantly impact therapeutic

Table I. Summary of other strategies for crossing blood-brain barrier antiepileptic therapy.

A, Nanomaterial drug delivery systems				
Mediated mode	Epilepsy type	Advantages	Disadvantages	(Refs.)
Fe <sub>3</sub> O <sub>4</sub> @BaTiO <sub>3</sub> nanochains	PTZ-induced seizure model	Epilepsy is treated with a wireless deep magnetic computer therapy platform that facilitates on-demand long-term radio stimulation	Not translated in clinical applications	(219)
TPGS-Fc	PTZ-induced seizure/KA-induced epilepsy/kindling model	Provides on-demand drug release at lower doses, improving efficacy and reducing side effects	Not translated in clinical applications	(218)
PPY-PDA-PHT-ANG together with NIR irradiation	PTZ-induced seizure/KA-induced epilepsy/pilocarpine-induced SE model	To enhance brain targeting and sustained electro-responsive drug release for on-demand therapy of epilepsy	Suitable only for the encapsulation and delivery of PHT	(221)
Black phosphorus together with NIR irradiation	4-Aminopyridine-induced epilepsy model	A potential wireless, non-genetic, non-implantable promising treatment for epilepsy	Not translated in clinical applications	(222)
ANG-PHT-ERHNP	Kindling seizure model	Reduced ignition stimulus-induced seizures	Suitable only for the encapsulation and delivery of PHT	(239)
MA@RT-HMSNs	KA-induced epilepsy model	Promotes the recovery of cognitive function, thereby exerting neuroprotective and therapeutic effects on epilepsy	Not translated in clinical applications	(240)
D-T7/Tet 1-lipids@PL	KA-induced epilepsy/Pilocarpine-induced seizure model	It has a good therapeutic effect on seizures in both acute and chronic epilepsy models, and has high biosafety	Suitable only for the encapsulation and delivery of LTG	(223)
P85-PHT-PBCA-NPs	Pilocarpine-induced seizure model	Novel and effective ways to overcome drug-resistant epilepsy	Suitable only for the encapsulation and delivery of PHT	(241)
Poloxamer-188 CBZ-NP	Isoniazid-induced seizure model	It opens up new prospects for the treatment of drug-resistant epilepsy caused by overexpression of ABC transporters	Suitable only for the encapsulation and delivery of CBZ	(242)
PHT@TGN-HBc	Pilocarpine-induced seizure model	Improves the therapeutic effect of ASDs and reduces side effects	Suitable only for the encapsulation and delivery of PHT	(243)

## B, Focused ultrasound

Mediated mode	Epilepsy type	Advantages	Disadvantages	(Refs.)
Transcranial FUS pulsation	PTZ-induced seizure model	Inhibits acute epilepsy neuronal activity in an animal model of PTZ-induced acute epilepsy	Not translated in clinical applications	(224)

Table I. Continued.

B, Focused ultrasound				
Mediated mode	Epilepsy type	Advantages	Disadvantages	(Refs.)
Low-intensity FUS	KA-induced epilepsy model	Pulse FUS can suppress epileptic-like discharges in EEG	Not translated in clinical applications	(244)
Thalamus FUS ablation	Adult epilepsy patients	Reduction of epileptic seizures	Patient's language and working memory decline	(245)
Repetitive transcranial FUS stimulations	PTZ-induced seizure model	Bidirectional control of epileptic-like activity using repetitive transcranial ultrasound stimulation	Not translated in clinical applications	(246)
Low-intensity pulsed FUS	KA-induced epilepsy model	Low intensity pulsed ultrasound has neuro-protective and beneficial behavioral effects in KA epilepsy animal models	Not translated in clinical applications	(247)
Low-intensity FUS	Patients with DRE	Effectively regulates brain circuits or neuronal activity	No sham treatment was used, and the number of patients was limited	(225)
MRgFUS	Pilocarpine-induced seizure model	To support non-invasive surgical modalities for the treatment of drug-resistant focal epilepsy	The size of the animal sample is small	(248)
Low-intensity transcranial pulsed ultrasound	KA-induced epilepsy model	It provides a new idea for ultrasound neuromodulation in the treatment of epilepsy.	Not translated in clinical applications	(249)

ANG, angiopep-2; CBZ, carbamazepine; D-T7, D-form T7; ERHNP, electro-responsive hydrogel nanoparticle; FUS, focused ultrasound; HBc, hepatitis B core protein; LTG, lamotrigine; MA@RT-HMSNs, macrophage membrane-biomimetic-hollow mesoporous silica nanocarriers; MRgFUS, low-intensity focused ultrasound; NP, nanoparticle; PHT, phenytoin sodium; PTZ, pentylenetetrazole; P85-PHT-PBCA-NPs, pluronic P85-coated phenytoin poly(butylcyanoacrylate) nanoparticles; PPY, polypyrrole; PDA, polydopamine; PLGA, poly(lactic-co-glycolic acid); KA, kainic acid; SE, status epilepticus; TPGS-Fc, ferrocene (Fc)-conjugated D- $\alpha$ -tocopherol polyethylene glycol succinate (TPGS); TGN, protein sequence TGNKALHPHNG; Tet, tetracycline; NIR, near-infrared.

efficacy. Rapid release may cause excessive local concentrations, while slow or incomplete release may prevent the drug from reaching therapeutic levels at the target site. For instance, Zhang *et al* (229) observed that 2 h after treatment with FUS combined with MBs, Evans blue dye was unable to penetrate the brain parenchyma, indicating that the effect of FUS is reversible. MBs have shown good biocompatibility and safety in multiple studies (230-232), and initial clinical trials applying this method to temporarily open the BBB have reported no adverse patient outcomes (231). It has also been reported that FUS combined with MBs is safe and effective in patients with Parkinson's disease, with the BBB returning to normal within 24 h post-treatment (214). Nevertheless, before this technique can be widely adopted in clinical settings, its complete safety must be validated. In particular, the optimal frequency range for ultrasound application requires further clarification, as frequencies that are too high may pose serious safety risks, whereas lower frequencies may not sufficiently increase BBB

permeability. Magnetically guided brain-targeted delivery using ionic nanoparticles has also been validated by several studies (217,233). However, recent findings indicate that nanomaterials, especially metal-based ones, may induce oxidative stress, inflammatory responses and alterations in neurotransmitter expression (234). Xiong *et al* (235) found that increased BBB permeability induced by photothermal effects is reversible, with barrier function returning to baseline within 48 h after treatment, providing further evidence for the safety of this strategy. Despite these findings, the long-term use of nanodrugs in epilepsy treatment may pose health risks and requires thorough evaluation through extensive preclinical and clinical studies.

In conclusion, nanodrug delivery systems can improve the solubility, stability and BBB permeability of antiepileptic compounds, offering a promising approach for overcoming drug resistance and reducing adverse effects. Although their unique capacity to cross the BBB has shown considerable

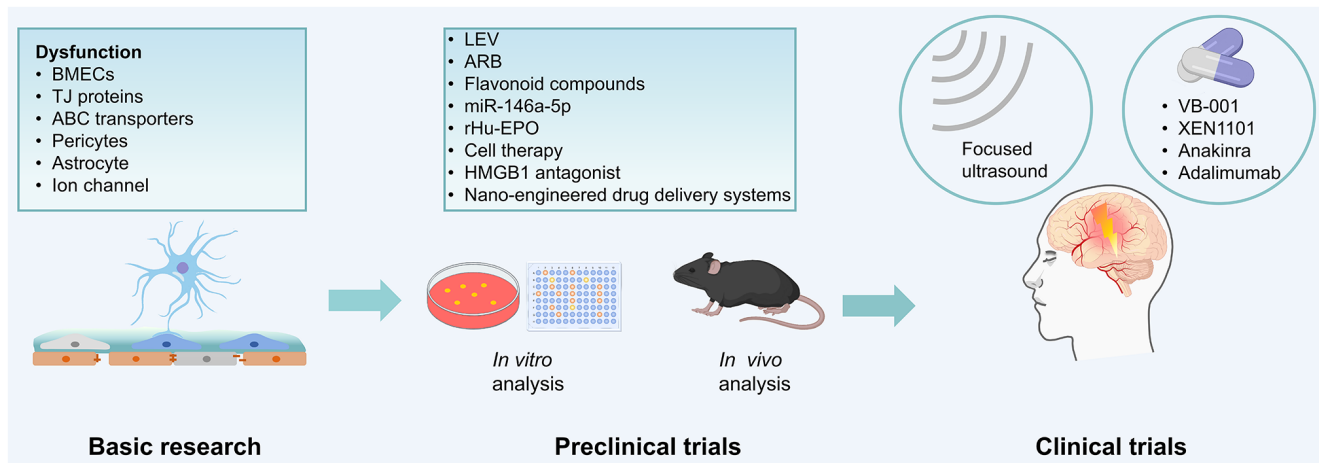


Figure 6. BBB-based basic research to clinical translation. Basic research on BBB damage in preclinical and clinical trials to explore BBB-based treatments for epilepsy. BBB, blood-brain barrier; TJ, tight junction; ABC, ATP-binding cassette; LEV, levetiracetam; ARB, angiotensin receptor blocker; miR, microRNA; rHu-EPO, recombinant human erythropoietin; HMGB1, high mobility group box-1.

potential in neurological therapies, their safety and efficacy remain major barriers to clinical translation. Furthermore, the development of wireless and minimally invasive DBS platforms is urgently required. Early diagnosis of CNS diseases can improve patient outcomes (236), and understanding the prognostic factors of epilepsy can help guide personalised treatment strategies. Accurate and reliable survival predictions can support clinical decision-making for epilepsy and related neurological disorders (237,238).

To illustrate the translational pathway of BBB-targeted therapies in epilepsy, a schematic overview of the developmental pipeline from basic research to clinical applications is presented in Fig. 6. Foundational studies have focused on BBB components such as endothelial cells, TJ proteins, astrocytes and transporters, which inform therapeutic target identification. Preclinical studies have used *in vitro* and *in vivo* models to assess candidate therapies, including LEV, ARBs, miR-146a-5p, rhEPO, flavonoids and nanocarrier systems, to control seizures and preserve BBB integrity. At the clinical stage, techniques such as FUS are being explored for BBB modulation, and agents such as VB-001, XEN1101, anakinra and adalimumab are being evaluated for their efficacy and safety. This framework highlights the stepwise process required to translate BBB-focused strategies from the laboratory to the clinic (Fig. 6).

## 7. Conclusion

The BBB is a complex and highly selective structure essential for maintaining a stable, extracellular environment in the CNS and ensuring optimal brain function. BBB integrity is closely associated with the progression of various neurological disorders, with BBB dysfunction serving as a significant pathological factor in CNS diseases. Evidence highlights a bidirectional relationship between epilepsy and BBB disruption. Epilepsy alters the molecular structure and functionality of the BBB through multiple mechanisms, whereas BBB damage can accelerate the onset and progression of epilepsy.

Seizure activity can compromise the BBB through mechanisms such as the release of excitatory neurotransmitters, abnormal vascular remodelling and altered ion channel

function, resulting in increased permeability. These changes may increase brain excitability, facilitate seizure progression and elicit inflammatory responses. Simultaneously, BBB disruption allows plasma proteins such as albumin to enter the brain parenchyma, leading to inflammatory reactions, iron accumulation and infiltration of neurotoxic substances, which further aggravate seizure activity. This reciprocal relationship underscores the intricate connection between epilepsy and BBB integrity, with each condition exacerbating the other. Understanding these dynamics is vital for the development of novel therapeutic strategies.

Advancements in research have significantly enhanced the understanding of BBB molecular and cellular functions, as well as the pathological consequences of its dysfunction in CNS diseases. Disruptions in the brain microenvironment can impair neuronal function, promote cellular damage and trigger apoptosis, accelerating the pathological progression of epilepsy. Restoring BBB integrity is increasingly recognised as a critical strategy in treating epilepsy.

Given the unpredictability of epileptic seizures and the necessity for long-term drug therapy, the development of innovative treatment strategies to effectively manage seizures remains a priority. Future research should focus on several key areas: i) BBB repair and protection. Advancing therapeutic interventions to restore BBB integrity to mitigate the heightened neuronal excitability and inflammatory responses resulting from BBB injury. ii) Application of nanotechnology. Nanotechnology can be utilised to enhance drug penetration across the BBB and develop more efficient brain-targeted drug delivery systems, thereby achieving significant reductions in seizure frequency. iii) Ultrasound-mediated BBB opening. Exploring ultrasound technology to temporarily open the BBB, facilitating improved drug delivery through a non-invasive approach that offers potential for epilepsy treatment. iv) Comprehensive treatment strategies. Integrating BBB repair, cutting-edge drug delivery techniques and antiepileptic therapies to create a holistic treatment model for superior therapeutic outcomes.

Nanomaterial-based drug delivery systems have demonstrated great potential due to their favourable bioavailability and low toxicity, demonstrating efficacy in inhibiting or

reducing seizure activity. However, additional clinical trials are required to validate their safety and efficacy. BBB-centred interventions represent a promising breakthrough in epilepsy treatment, particularly for mitigating disease progression and enabling secondary prevention. When integrated with other therapeutic approaches, these interventions could pave the way for novel strategies to prevent, control and reverse epilepsy progression. Comprehensive research on the chemical mediators and receptors involved in neuroinflammation may provide critical insights into the neurobiological mechanisms of epileptogenesis. This understanding could serve as a basis for identifying new biomarkers and therapeutic targets, facilitating the screening of individuals at high risk for epilepsy, and advancing both preventative and therapeutic strategies. A deeper understanding of the pathophysiological mechanisms underlying epilepsy is necessary to facilitate the development of novel drugs and therapeutic approaches. Currently, several new diagnostic and treatment strategies are being progressively implemented in clinical practice, including wearable devices for automatic seizure detection, minimally invasive surgical techniques for selective ablation of epileptogenic zones and precision medicine based on the genetic causes of epilepsy. Gene therapy for epilepsy has advanced significantly over the past decade, with some approaches entering clinical trials. In the future, clinical research should adopt more precise methods for regulating neural networks, such as optogenetics, to specifically target hyperactive neurons with abnormal gene expression. These efforts are expected to provide safer and more effective treatment options for epilepsy patients, improve their quality of life and contribute to major advances in epilepsy therapy.

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#### Authors' contributions

NH, YWH and ZYD reviewed the literature and drafted the manuscript. SYQ and WZ were involved in reviewing and editing the manuscript. GHT and YYL revised and approved the final manuscript. All authors have read and approved the manuscript. Data authentication is not applicable.

#### Ethics approval and consent to participate

Not applicable.

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#### Competing interests

The authors declare that they have no competing interests.

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