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Review

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Aberrant blood-brain barrier dynamics in cerebral small vessel disease - a review of associations, pathomechanisms and therapeutic potentials

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Abstract

This review explores the pivotal role of the blood-brain barrier (BBB) in maintaining central nervous system (CNS) homeostasis and its dynamic involvement in the pathogenesis of cerebral small vessel disease (CSVD), i.e., the major precursor of age-related neurodegenerative diseases such as vascular dementia and Alzheimer's disease. It underscores the BBB as a critical physiological boundary that regulates the exchange between the bloodstream and the cerebral milieu through a complex and dynamic interface composed of endothelial cells, astrocyte endfeet, and pericytes. The integrity of this barrier is paramount for neural function, shielding the brain from toxicants and pathogens while facilitating the transport of essential nutrients. Nevertheless, BBB dysfunction is recognized as a lead in the pathogenesis of neurodegeneration including CSVD, emphasizing the need for focused research on maintaining or restoring BBB function. This review highlights recent advancements in our understanding of BBB dynamics in both health and disease states, its involvement in CSVD pathomechanisms, and the challenges and

future directions of translational research and emerging technologies. The review advocates for a multidisciplinary approach to uncover the complexity of BBB dysfunction in CSVD, as well as insights into potential therapeutic targets aimed at preserving BBB integrity, thereby minimizing its impact given the notable world's aging demographics.

Keywords: Blood-brain barrier, cerebral small vessel disease, BBB dysfunction, translational research

INTRODUCTION

The blood-brain barrier (BBB) represents a vital physiological frontier in the central nervous system (CNS) that meticulously orchestrates the selective exchange between the circulation and the cerebral microenvironment. This complex and dynamic interface is composed of endothelial cells (ECs) sealed by tight junctions (TJs) and adherens junctions (AJs), and supported by other cell types, such as astrocytes and pericytes. Together, they form a highly selective barrier that not only shields the brain from potentially neurotoxic compounds, but also facilitates the transportation of essential nutrients and molecules necessary for an optimal neural function. The regulatory mechanisms of BBB ensure cerebral homeostasis by safeguarding the delicate microenvironment essential for neural signaling and brain health[1,2]. Disruption to the integrity and/or dynamics of BBB is increasingly implicated in the pathogenesis of various CNS disorders[3]. Such disruptions can lead to the uncontrolled entry of harmful substances, inflammation, and subsequent detrimentally compromise neural function, thus emphasizing the critical need for research focused on maintaining or restoring BBB functionality[2,4].

In addition to the BBB, the glymphatic pathway plays a pivotal role in the maintenance of cerebral homeostasis in facilitating the clearance of interstitial solutes, such as metabolic by-products, from the brain. This complex pathway is distinguished by the movement of cerebrospinal fluid (CSF) into the brain parenchyma through perivascular space (PVS) that accompanies the brain's vascular network. Astrocytes, a specific type of glial cells, play a crucial role in this process. Their endfeet envelop the brain's blood vessels, housing aquaporin-4 (AQP4) water channels that help in the facilitation of CSF influx into the interstitial space[5-7]. The glymphatic pathway functions in a series of steps: CSF enters the brain along para-arterial spaces fueled by arterial pulsation, integrates with interstitial fluid within the brain tissue that assists in clearing the metabolic by-products, and afterward through the para-venous passages to be cleared into the lymphatic systems[5].

Cerebral small vessel disease (CSVD) is a prevalent neurological condition of significant concern with the growing global aging population that contributes to full-blown stroke and vascular dementia[8,9]. In clinical practice, the neuroimaging features of CSVD as seen on magnetic resonance imaging (MRI) include recent small subcortical infarct, lacune, white matter hyperintensity (WMHs), enlarged perivascular space (ePVS), cerebral microbleed, cortical superficial siderosis, cortical cerebral microinfarcts, and brain atrophy[10-12]. Every case of CSVD has an underlying microvascular pathology, which adds to the complexity of its pathophysiological mechanism that is yet unknown[13]. The breakdown of the BBB has been specifically linked with the development and progression of CSVD, impacting cerebral blood flow (CBF) dynamics, and thus increasing the risk for cognitive impairment and dementia[14,15]. In fact, with increasing age and the presence of chronic hypertension, damage to the cerebral endothelium of the BBB is inevitable owing to the stress caused by hemodynamic changes[16].

Therefore, in this narrative review, we aimed to explore the relationship between BBB integrity and CSVD, with a focus on preclinical studies that shed light on how aberrant BBB dynamics affects the progression

and development of CSVD. By delving into the complexities of BBB dynamics in the context of CSVD, this review underscores the importance of the BBB, the neuro-glio-vascular unit (NGVU), and the glymphatic system in both the maintenance of brain health and the development of neurodegenerative diseases. Finally, we discussed the current research status and potential therapeutic options to overcome aberrant BBB dynamics in CSVD.

THE BLOOD-BRAIN BARRIER: FUNDAMENTAL CONCEPTS

The human brain is home to over 80 billion neurons, a myriad of glial cells, and more than 600 km of blood vessels[3,17]. The BBB serves a crucial role in maintaining the homeostasis of the brain's microenvironment by acting as a dynamic interface between the CNS interstitial fluid and the circulating blood. This separation regulates solutes and toxicants, maintaining a stable environment conducive to neuronal function[18]. In essence, as illustrated in Figure 1, this homeostasis is delicately regulated by the NGVU and glymphatic system, which control the flow of CSF, interstitial fluids (ISF), metabolic waste, and their clearance through the venous circulation. NGVU stands for the integration of neuronal structures, glial cells (including the microglial), and vasculature (specifically capillaries, arteries, and/or arterioles) that is controlled by the astrocytes. As the brain's metabolic rate increases, the NGVU continuously releases neurotoxic soluble waste products into the ISF area. The glymphatic system is recognized as a principal route of draining these metabolites. This pathway involves the perivascular spaces around capillaries, arteries, venules, and veins, facilitating the bi-directional flow of CSF and ISF within the brain[5-7]. The driving force behind the movement of interstitial fluid reverting to the glymphatic system is an intricate process. Arterial pulsation assists in the convective influx of CSF into the interstitial space while the perivenular spaces act as the conduit for the CSF to exit, thus securing efficient removal and clearance across the glymphatic system[5,6]. These mechanisms accentuate the importance of both arterioles and venules in sustaining the homeostasis of ISF. The hypothesis states that the perivascular space acts as a conduit for the CSF to flow into the brain parenchyma perivascular space[19].

Endothelial cells

The BBB contains specialized cells called endothelial cells (ECs), which are crucial for preserving the microenvironment of the brain and controlling the flow of substances between the bloodstream and the brain[20]. The brain is protected from neurotoxins, drugs, and other potentially harmful substances by the ECs within the BBB[21]. This function is fully supported by TJ proteins and the high densities of transporters and receptors to ensure that essential molecules can readily enter the brain[22]. Moreover, the interaction between ECs and the adjacent astrocytes emphasizes the structural integrity of the BBB. ECs within the BBB physiologically balance CNS protection and immune cell infiltration by regulating neurovascular coupling, CBF, and immune surveillance[23]. Additionally, to maintain CNS homeostasis, TJs comprised of several proteins including claudins, occludin, and junctional adhesion molecules [JAMs], are interconnected through cytoplasmic accessory proteins such as zonula occludins [ZOs], which help maintain the integrity of the BBB, resulting in high trans-endothelial electrical resistance and limited paracellular permeability[24]. Therefore, BBB responses are dynamically regulated by crosstalk between ECs and surrounding cells in response to both physiological and pathological stimuli[25].

Astrocytes

Astrocytes are essential parts of the NGVU, which maintains the homeostasis of the CNS. Their wide branching processes, which encircle the capillaries in the brain, allow them to come into close contact with ECs, which is essential for the structural and functional integrity of the BBB[21]. Astrocyte endfeet, which make up more than 99% of the brain's capillary surfaces, support the glia limitans and maintain the integrity of the BBB[26]. By releasing substances like glial-derived neurotrophic factor (GDNF) and angiopoietin-1 (Ang1), which improve TJs proteins expression and structure in ECs, astrocytes influence the BBB integrity

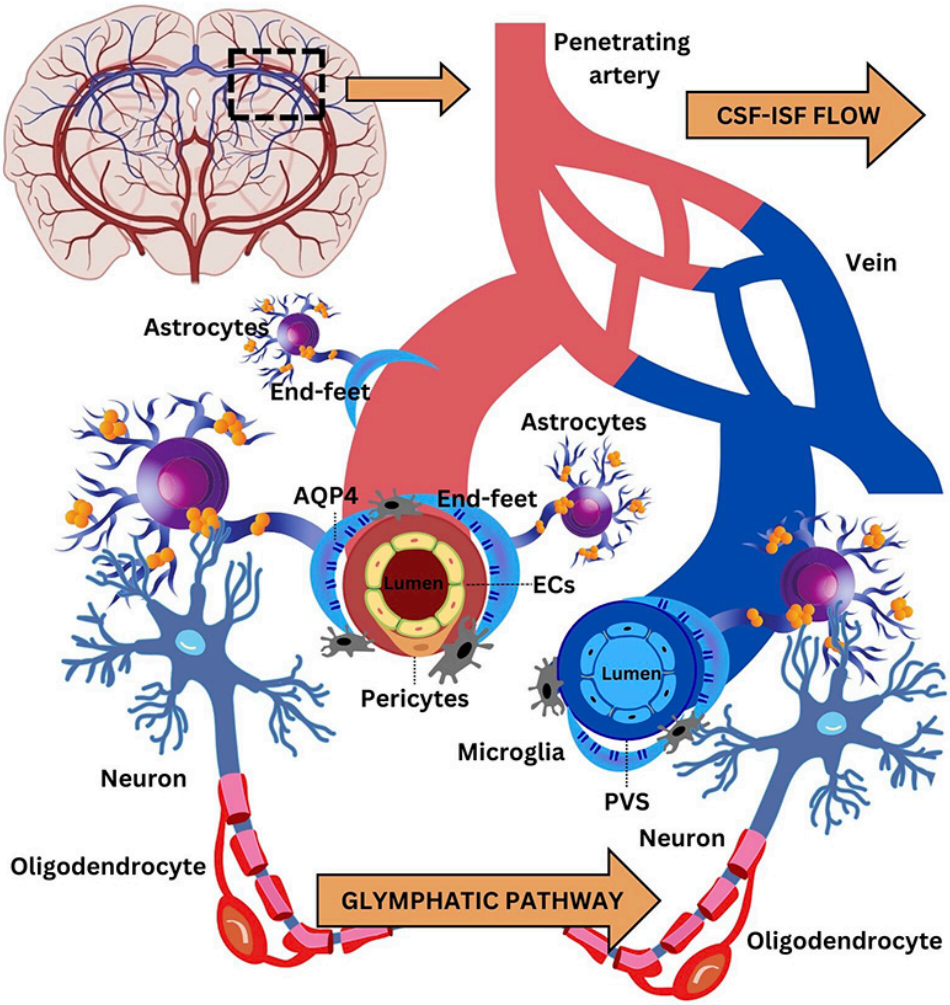

Figure 1. The components of BBB, NGVU, and the glymphatic system. The NGVU is composed of neuronal cells, glial cells, and the vascular system that includes arteries, veins, smooth muscle cells (not depicted in the figure), and pericytes that encircle the vascular endothelial cells. The glymphatic system is primarily made up of astrocytes and the endfeet of astrocytes that envelop the vasculature. It drains waste products through the CSF from the artery, flowing into the ISF through astrocytic AQP4 channels, thereby mixing the CSF with ISF. Waste solutes and/or by-products subsequently traverse the glymphatic pathway for absorption and processing by the waste clearance system.

and dynamics[27]. Moreover, through neurovascular coupling, they control CBF, aid in the movement of nutrients and waste products, and modify local metabolic demands[28,29]. Furthermore, astrocytes play a crucial role in CNS immunological processes such as inflammatory response orchestration, modulation of BBB permeability, pathogen and injury detection and response, and immune cell recruitment[30]. Thus, these diverse functions highlight the importance of astrocytes in CNS defense and BBB physiology.

Pericytes

Essential to the structural and functional dynamics of the BBB, pericytes are a fundamental component of the NGVU. Pericytes are distributed in capillary basement membranes and interact intimately with extracellular matrix constituents, astrocytes, and ECs. Pericytes, with a spindle-shaped morphology and distinct markers such as platelet-derived growth factor receptor-beta (PDGFR-β) and neuron-glial antigen 2 (NG2), govern the function of the endothelium barrier and capillary blood flow via direct contact and paracrine signaling[31,32]. Moreover, pericytes play roles in angiogenesis and the integrity of the BBB

dynamics, whereby they stabilize ECs and respond to endothelium-derived PDGF-β by multiplying and differentiating[32]. Furthermore, by tightening endothelial connections and controlling capillary blood flow through alpha-smooth muscle actin (α-SMA) expression, pericytes impact waste disposal, nutrient delivery and reduce paracellular leakage[33]. Finally, pericytes modulate BBB permeability to immune cells and control leukocyte trafficking as part of CNS immune surveillance[34]. Thus, in the setting of neurodegenerative disease, where the buildup of toxic chemicals contributes to pathology, this element of pericyte activity is aptly pertinent[35].

Microglia

Microglia cells, the resident immune cells of the CNS, play an essential role in supporting BBB integrity. In 1919, Pío del Río-Hortega depicted microglia as 'vascular satellites" because of their inseparable relationship with cerebral vessels[36]. This historical outlook emphasizes the well-known acclaim of the close association between microglia and the brain's vascular system. Newer research has clarified the importance of this perivascular positioning in brain angiogenesis[37,38]. Microglia, by their interactions with ECs and other elements of the NGVU, play an essential role in sustaining vascular integrity and enhancing the configuration of new blood vessels[39,40]. Moreover, these cells portray a dual role in neuroinflammation and perivascular biology, significantly influencing the NGVU. Current research indicates that microglial cells cooperate closely with ECs and pericytes, establishing a triad that is critical for BBB function. This interaction encompasses bi-directional signaling that assists in modulating immune cell trafficking, vascular tone, and responses to injury. For example, microglia-derived factors such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) can regulate the expression of TJ proteins in ECs, thereby affecting BBB permeability[41]. Microglia can react to pathological stimuli according to their different phenotypes, ranging from a protective, anti-inflammatory state to a pro-inflammatory neurotoxic state. Chronic activation of microglia unfolds the release of pro-inflammatory cytokines and reactive oxygen species (ROS), which can annihilate BBB integrity by disrupting ECs and TJ proteins[42,43]. Microglia, which originated from myeloid progenitors, display a distinct profile in comparison with neuroglia like astrocytes, which are derived from neuroprogenitor cells. This distinctness is essential as microglia serve as the predominant immune defense in the CNS by actively inspecting the brain environment, removing debris, and responding to injuries via processes like phagocytosis and cytokine release. Astrocytes, instead, maintain the BBB, act as repair mechanisms post-injury and give nutritional support[44].

Basement membrane

The basement membrane of the BBB controls the transit of substances from the bloodstream to the brain and provides structural support. It produces a specific extracellular matrix composed of laminins, collagen type IV, nidogen, and heparan sulfate proteoglycans (HSPGs). Laminins support movement, adherence of cells, and stability of phenotypes, with unique isoforms contributing to the distinctiveness of the BBB[45]. Apart from that, the structural stability is provided by collagen type IV, whereas laminin and collagen networks are connected by nidogen. HSPGs control cell signaling and barrier permeability. The basement membrane promotes angiogenesis, protects the CNS, and regulates cell transmigration and neuroimmune interactions. It is essential for brain development and injury response because it directs the migration of ECs during vessel creation and repair. Additionally, CBF and BBB functions are regulated by interactions between astrocytes and pericytes, which is crucial for neurophysiology and BBB dynamics. Hence, current knowledge of the basement membrane provides insights into CNS health and disease through BBB physiology and NGVU dynamic interactions[46,47].

BBB DYSFUNCTION AND NEUROLOGICAL DISORDERS: AN OVERVIEW

In physiological conditions, the BBB's integrity, maintained by TJs among ECs, pericytes, and astrocyte endfeet, is paramount for neural function, protecting the brain from toxins and pathogens, while strictly

allowing nutrients and signaling molecules to pass through[48]. Consequently, BBB dysfunction appears to play an important role in the early stages of various neurological disorders such as Alzheimer's disease, Parkinson's disease, traumatic brain injuries (TBI), multiple sclerosis, epilepsy, and CSVD[49-53]. Studies on these conditions provide significant insights into the multifaceted impact of BBB dysfunction, underlining the crucial need for a better understanding of its aberrant mechanisms and translational therapeutic opportunities.

In Alzheimer's disease, BBB breakdown contributes to amyloid-beta (Aβ) accumulation and neurodegeneration[52]. Sweeney *et al.* (2019) demonstrated that disrupting the BBB increases the accumulation and hinders the effective clearance of Aβ[53]. More recently, Custodia *et al.* (2023) emphasized that endothelial dysfunction at the BBB, driven by known vascular risk factors, facilitates the passage of toxic substances to the cerebral parenchyma, leading to neuroinflammation and neuronal degeneration, both of which are central to cognitive deterioration in Alzheimer's disease[54]. As for Parkinson's disease, Joo *et al.* (2023) argued that chronic neuroinflammation induced by activated microglia promotes BBB permeabilization, which is detrimental to dopamine neurons[55]. This process highlights the role of pro-inflammatory cells and cytokines in exacerbating BBB dysfunction in this condition. Additionally, de Rus Jacquet *et al.* (2023) used a brain-chip model to demonstrate how inflammatory astrocytes contribute to BBB dysfunction in Parkinson's disease, providing insights into the disease's pathogenic mechanisms[56].

TBI is another condition where BBB dysfunction plays a critical role. Cash and Theus (2020) investigated the cellular and molecular mechanisms involved in BBB stability regulation following TBI. They emphasized that TBI induces BBB disruption through mechanisms such as oxidative stress, neuroinflammation, and cellular injury, which compromise the BBB's integrity[57]. Huang *et al.* (2022) explored how factors such as increased permeability and ECs damage contribute to BBB disruption, underscoring the important role BBB dysfunction plays in the pathophysiology of TBI[58]. Furthermore, in conditions like multiple sclerosis, BBB dysfunction permits the entry of autoreactive T cells into the CNS, causing the autoimmune attack on myelin that characterizes this disease[59,60]. Meanwhile, in epilepsy, Vezzani (2012) investigated the brain-autonomous mechanisms responsible for BBB dysfunction during seizures[61]. The research emphasizes how seizures can activate inflammatory responses and trigger the Src kinase pathway, which is a downstream effector of receptor tyrosine kinases (RTKs). This activation led to the derangement of TJ proteins and heightened BBB permeability. The Src's pathway's main role in regulating BBB integrity underlines its significance in both pharmacological interventions and neuropathological conditions. Furthermore, the dysfunction of ATP-binding cassette (ABC) transporters at the BBB surface, as detailed by Mohi-ud-Din *et al.* (2022), emphasizes the importance of nanotechnology-enabled drug delivery systems to overcome these barriers in epilepsy treatment[62].

Notwithstanding, BBB dysfunction is increasingly implicated in CSVD manifestation, as characterized by endothelial damage, TJs disruption, and basement membrane alterations, contributing to the leakage of blood constituents into the brain parenchyma and subsequent neuroinflammation and white matter damage[63]. Importantly, vessels in the vicinity of ePVS in older subjects showed arterial wall thickening and tortuosity, venular widening, inflammation, and BBB failure[64]. Emerging evidence underscores the role of the Wingless and Int-1 (WNT)/β-catenin signaling in ECs and oligodendrocyte interactions as a crucial pathway in maintaining BBB integrity, suggesting that dysregulation of this pathway may exacerbate BBB permeability and hinder white matter repair in CSVD[63]. Furthermore, genetic factors such as gap junction alpha-1 (GJA1) gene polymorphisms have been linked to variations in the topographic distribution and severity of CSVD, implying a genetic predisposition to BBB vulnerability and emphasizing the complexity of BBB dysfunction in disease pathogenesis[65].

BBB dysfunction is recognized in numerous neurological disorders, but the extent to which it is causal or merely a consequence of complex pathophysiological processes remains unclear. The evidence signifies that in some conditions, such as Alzheimer's disease and multiple sclerosis, BBB disintegration appears to be an early and potential causative event, facilitating the assemblage of neurotoxic substances and the entrance of immune cells into the CNS[1,2]. On the contrary, in disorders like Parkinson's disease and CSVD, BBB dysfunction frequently results from chronic neuroinflammation, systemic diseases, and oxidative stressors such as hypertension and diabetes, implying it is a secondary consequence[16,56]. Continued research is required to indisputably define these associations, which would benefit novel therapeutic strategies aimed at mitigating and/or averting BBB breakdown[66,67]. Interestingly, the gut-brain axis also emerges as a novel player in CSVD, where gut dysbiosis has been associated with BBB breakdown and neurovascular pathologies, highlighting the interplay between systemic factors and neurovascular health[68].

This section discusses the involvement of BBB dysfunction in a variety of neurological disorders, including Alzheimer's disease, Parkinson's disease, TBI, multiple sclerosis, epilepsy, and CSVD. It reviews how BBB dysfunction leads to disease progression and the significance of extrapolating these mechanisms for therapeutic developments. In essence, it emphasizes the roles of inflammation, oxidative stress, and genetic factors in BBB disruption and the intricate interaction between these components in the pathogenesis of neurological conditions.

EVIDENCE OF ABERRANT BBB DYNAMICS IN CSVD: CLINICOPATHOLOGICAL LESSONS FROM PRECLINICAL MODELS

In vivo models

CSVD is a serious yet frequently disregarded condition that underlies both stroke and dementia, two of the primary causes of illness and death on a global scale[69]. Despite its substantial impact on public health and the well-being of countless individuals, the intricate mechanisms driving CSVD remain largely elusive. This lack of comprehension is partly due to the inherent difficulties in replicating the complex and diverse nature of the disease in experimental conditions.

The examination of BBB dysfunction in the context of CSVD exemplifies these challenges, highlighting the need for advanced animal models that can accurately replicate the subtle nuances of the human pathology associated with this condition[70,71]. Such models are indispensable for delving into the pathophysiological processes underlying BBB disruption in CSVD, offering insights critical for the development of effective interventions. By accurately replicating human CSVD pathology, including the hallmark BBB dysfunction, these models facilitate critical advancements in our understanding, paving the way for innovative therapeutic approaches.

Moreover, the elucidation of BBB dysfunction through these models sheds light on the disease's contribution to the broader spectrum of neurovascular and neurodegenerative disorders, underscoring the interconnectedness of cerebral microvascular health with overall brain function and disease[72,73]. Given the substantial impact of CSVD on the progression of stroke and dementia, unraveling the complexities of BBB dysfunction stands as a cornerstone in mitigating these conditions' burden. This endeavor requires a multidisciplinary approach, integrating insights from animal studies with human clinical observations, to foster a holistic understanding of CSVD. This section aims to explore the central role of animal models in enhancing our comprehension of BBB dysfunction within CSVD, highlighting their contributions to our understanding of the disease.

Aging models

The aging rodent models, particularly mice and rats, have demonstrated that aging is associated with increased permeability of the BBB, modifications in the expression of proteins involved in TJs, and a decrease in CBF. Notably, research on aged rodents revealed a decrease in the levels of TJ proteins such as claudin-5 and occludin, which are integral for maintaining the integrity of BBB[74]. One of the primary mechanisms by which aging contributes to BBB dysfunction is an increase in oxidative stress and inflammation. The accumulation of ROS damages BBB components, while chronic inflammation increases adhesion molecules and the recruitment of immune cells, further compromising BBB integrity[75].

The endothelial nitric oxide synthase-deficient mice, a model for age-dependent CSVD, developed cerebral hypoperfusion, BBB leakage, oxidative stress, astrogliosis, cerebral amyloid angiopathy, microbleeds, microinfarction, and white matter pathology, all of which are characteristic features of CSVD[76]. Furthermore, aging is linked to microvascular degradation, which encompasses a decrease in the number of cerebral capillaries and thickening of the basement membrane. These alterations hinder the transportation of nutrients and oxygen to the brain, thereby contributing to hypoxia and facilitating the breakdown of the BBB dynamics[77]. Aged ECs displayed a decrease in the expression of transporters and receptors required for the proper functioning of the BBB and their ability to repair themselves. Thus, the dysfunction of the ECs is a key factor in the disruption of the BBB associated with aging, and likely to contribute to the onset of CSVD[78]. In addition, the morphology and functionality of astrocytic endfeet change with age, such as swelling and modified potassium buffering, disrupting the neurovascular coupling and the integrity of the BBB[79].

In conclusion, the aging models have enhanced our comprehension of BBB impairment and its role in CSVD. Future investigations should prioritize translating these findings into clinical interventions to prevent or decelerate the progression of BBB dysfunction and CSVD in at-risk individuals across the general population.

Hypertensive models

Hypertension serves as a significant risk factor for CSVD and BBB impairment. The spontaneously hypertensive stroke-prone rat (SHRSP) model is extensively employed to investigate this association because it exhibits the development of severe hypertension, resulting in the occurrence of spontaneous brain lesions similar to those witnessed in the human CSVD, including BBB disruption. This model revealed that hypertension-induced oxidative stress and inflammation play a role in BBB malfunction[80].

The SHRSP model is derived from the spontaneously hypertensive rat (SHR), which is highly vulnerable to stroke and cerebrovascular lesions when exposed to a high-salt diet or other environmental stressors. A recent study used salt-sensitive "Sabra" hypertension-prone rats (SBH/y) and discovered that "Sabra" hypertension-prone deoxycorticosterone acetate (SBH/y-DOCA) rats displayed multiple cerebrovascular pathologies associated with CSVD, including BBB permeability and inflammation[81]. Chronic hypertension causes ECs dysfunction, which manifests as reduced nitric oxide (NO) levels, increased oxidative stress, and an upregulation of adhesion molecules[82]. These alterations disrupt the BBB endothelial barrier, increasing permeability, and facilitating leukocytes to infiltrate the brain parenchyma. Consequently, vascular injury worsens, fostering the development of CSVD pathology[83].

Moreover, hypertension-induced disruption of the BBB is predominantly due to oxidative stress. The occurrence of ROS in hypertensive conditions causes significant damage to various cellular components, particularly the TJ proteins, resulting in increased BBB permeability. Additionally, oxidative stress activates

inflammatory pathways, thereby generating the expression of cytokines and chemokines that further contribute to the disruption of BBB integrity[84]. For instance, in SHRSP models, hypertension causes downregulation or abnormal localization of TJ proteins, namely claudin-5 and occludin. These adjustments undermine the structure of the TJs complex, increasing BBB permeability and subsequently contributing to the development of CSVD[85].

Furthermore, chronic hypertension induces structural and functional changes in cerebral microvessels, including wall thickening, luminal narrowing, and increased vessel tortuosity. These microvascular alterations impair CBF regulation and contribute to hypoxic conditions, further damage the BBB and exacerbate CSVD pathology[86]. Another study that simulated microgravity in rats discovered that it disrupted the BBB, increased oxidative stress levels, and downregulated TJ and AJ proteins expression in the rat brain[87]. Additionally, a study on bilateral common carotid artery ligation (BCCAL) in SHRSP rats revealed alterations in both brain and heart function, including disrupted autonomic functionality and elevated brain-heart coupling, which were associated with the risk of sudden death[88].

While these studies provide insights into BBB dysfunction in CSVD using SHRSP rat models, no specific study focusing solely on BBB dysfunction in CSVD in SHRSP rat models was found. By clarifying the underlying mechanisms, such as endothelial dysfunction, oxidative stress, inflammation, changes in TJ proteins, and microvascular remodeling, this model could emphasize the intricate nature of BBB disruption in the context of hypertension. Targeting these pathways appears to be a promising strategy for the prevention or improvement of CSVD in individuals with hypertension.

Models of chronic cerebral hypoperfusion

Models of chronic cerebral hypoperfusion (CCH) involves a prolonged decrease in CBF, which gradually induces notable changes in both the structure and function of the brain, thereby contributing to the occurrence of CSVD. Animal models for CCH induction have been established in both mice and rats. These models, such as bilateral common carotid artery stenosis (BCAS), offer a means of exploring this specific aspect of cerebral hypoperfusion.

The BCAS models have provided insights into the mechanisms behind BBB dysfunction in CSVD, including oxidative stress, microvascular injury, excitotoxicity, and secondary inflammation[89,90]. The studies have also explored potential therapeutic targets for CSVD, such as drugs that can regulate BBB function and protect against brain damage caused by chronic hypoperfusion[89,90]. CCH has a direct effect on ECs, causing oxidative stress, inflammation, and subsequent harm to the BBB. Decreased CBF reduces the delivery of oxygen and nutrients, exacerbates oxidative stress, and stimulates the secretion of pro-inflammatory cytokines, which disrupt TJs integrity[91].

CCH has been demonstrated to reduce the expression of TJ proteins such as claudin-5, occludin, and ZO-1, leading to increased paracellular permeability and subsequent BBB leakage[92]. CCH-activated microglia lead to the induction of a pro-inflammatory environment within the CNS, inflicting additional harm to the BBB and worsening ECs dysfunction. This inflammatory reaction plays a crucial role in the advancement of BBB disruption in CSVD[85]. At the same time, astrocytes undergo alterations in their cellular structure in response to CCH. The phenomenon of astrocyte endfeet swelling has been consistently observed in various animal models of CCH, providing compelling evidence for the disruption of astrocyte-mediated support and maintenance of the BBB[93].

CCH plays a crucial role in the impairment of BBB function in CSVD, affecting various components such as ECs, TJs integrity, and neurovascular elements. It is imperative to continue conducting research on the intricate interplay between CCH and BBB dysfunction to stimulate the development of efficacious treatment strategies for CSVD and its associated neurological consequences such as vascular dementia, Alzheimer's, and Parkinson's diseases.

Diabetic models

Diabetes mellitus (DM), characterized by persistent hyperglycemia, is a recognized predisposing factor for CSVD, owing to its detrimental impact on the BBB. The streptozotocin (STZ)-treated rodents exhibited hallmark features of DM, including hyperglycemia and insulin deficiency, which parallel to human diabetic conditions. This model demonstrated that hyperglycemia exacerbates BBB permeability, leading to enhanced leakage of plasma constituents into the brain parenchyma, a characteristic feature of CSVD[94].

Moreover, models of type 2 DM, such as db/db mice and Zucker diabetic fatty rats, have shed light on the role of insulin resistance and hyperlipidemia in BBB dysfunction. These models exhibited BBB alterations, including reduced expression of TJ proteins and increased inflammatory marker levels, contributing to CSVD pathology[53]. In diabetic models of CSVD, the disruption of the BBB is increasingly attributed to the interplay between oxidative stress and inflammation as a central mechanism. Hyperglycemia-induced oxidative stress damages ECs, disrupting TJs integrity. Concurrently, diabetes fosters a pro-inflammatory state that further compromises the BBB, emphasizing the role of anti-inflammatory and antioxidant therapies[95].

Apart from that, DM accelerates microvascular complications, including endothelial dysfunction and capillary degeneration, that are critical in CSVD pathogenesis, leading to hypoperfusion and hypoxia, which potentiate BBB dysfunction[96]. Advanced Glycation End (AGEs) products, resulting from prolonged exposure to high glucose levels, interact with RAGE (receptor for AGEs) on ECs of BBB, triggering oxidative stress and inflammatory pathways. This interaction is a key factor in diabetes-associated BBB disruption and CSVD[97]. Understanding the mechanisms by which DM exacerbates BBB dysfunction in CSVD has significant therapeutic implications. Strategies aimed at reducing oxidative stress, inflammation, and hyperglycemia hold promise. Additionally, interventions targeting AGE-RAGE interactions and preserving endothelial function may offer new avenues for mitigating the impact of DM on CSVD[93].

Transgenic models

Transgenic models afford the opportunity to investigate specific gene mutations and their impact on the pathology of CSVD, such as the breakdown of the BBB. Notably, unlike sporadic CSVD, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an inheritable form of CSVD that is caused by genetic mutations in the *NOTCH3* gene. By duplicating various aspects of the human illness, including the deposition of granular osmiophilic material, degeneration of vascular smooth muscle cells, and most importantly, malfunction of the BBB, these mouse models provide significant insights into the mechanisms underlying CADASIL[98].

Transgenic models have revealed that *NOTCH3* gene mutations disrupt NOTCH signaling pathways, which are critical for vascular development and integrity. Altered NOTCH signaling in ECs and pericytes leads to BBB breakdown, highlighting a pathway for therapeutic targeting[99]. Other than that, the degeneration of vascular smooth muscle cells is a hallmark of CADASIL. In transgenic models, vascular smooth muscle cell degeneration contributes to BBB dysfunction through destabilization of the vascular wall, leading to increased permeability and leakage[100].

Additionally, the expression of mutant *NOTCH3* has been associated with an upregulation in pro-inflammatory cytokines and adhesion molecules, indicating the role of inflammation in BBB disruption. This, in turn, promotes leukocyte adhesion and transmigration across the BBB, which ultimately worsens vascular damage[101]. Oxidative stress plays a pivotal role in the disruption of BBB in transgenic models of CSVD. The accumulation of mutant NOTCH3 protein results in an increase in the generation of ROS, thus leading to deleterious effects on the integrity of TJ proteins and ECs. Therefore, the BBB permeability increases, consequently affecting its functionality[102]. Furthermore, the amyloid precursor protein/presenilin 1 (APP/PS1) transgenic mouse model is widely recognized for studying the pathophysiological processes underlying Alzheimer's disease and CSVD. These mice express human genes with familial Alzheimer's disease mutations, leading to overproduction and accumulation of Aβ peptides. Aβ accumulation in cerebral vessels, known as cerebral amyloid angiopathy (CAA), is a critical feature of amyloidal CSVD in APP/PS1 mice. Aβ deposition, particularly in cerebral vessels, mirrors the hallmark features of CSVD and offers a unique window into the disease's vascular components[103]. Aβ deposition compromises vascular integrity, leading to vessel wall thickening, reduced vascular elasticity, and impaired CBF. These changes contribute to hypoperfusion and ischemia, exacerbating BBB dysfunction and neural damage[104].

Recent studies have explored the temporal dynamics of Aβ deposition and BBB dysfunction in APP/PS1 mice, suggesting that BBB breakdown precedes significant Aβ accumulation in the brain parenchyma. This early BBB dysfunction may facilitate Aβ entry from the blood to the brain, highlighting the BBB's critical role in the pathogenesis and progression of amyloidal CSVD and Alzheimer's disease[1]. Specifically, interventions targeting the regulation of β-secretase (BACE1) activity, an enzyme crucial in Aβ formation, have shown promise in decreasing cerebrovascular Aβ accumulation and alleviating BBB impairment in models of APP/PS1[105]. In conclusion, transgenic CSVD models play a crucial role in elucidating the complex interplay between genetic factors, BBB dysfunction, and the development of CSVD. These models provide a powerful platform for dissecting the molecular mechanisms driving the disease and for testing novel therapeutic strategies aimed at mitigating BBB dysfunction and its consequences on brain health.

In vitro models

The *in vitro* models have become indispensable tools in the neurological research arsenal, offering a meticulously controlled environment to dissect the multifaceted interactions among key cellular components of the BBB, including ECs, astrocytes, pericytes, and the extracellular matrix. Each of these components plays a pivotal role in maintaining BBB integrity, and their dysfunction contributes to the pathogenesis of CSVD.

In vitro models have been instrumental in elucidating the impact of oxidative stress, inflammation, and the altered cellular crosstalk that characterizes BBB dysfunction in CSVD. For example, studies have shown how oxidative stress can lead to endothelial damage, compromising the barrier function and facilitating the entry of potentially neurotoxic substances into the brain parenchyma[106]. Similarly, *in vitro* models have shed light on the role of inflammation in BBB breakdown, demonstrating how inflammatory mediators can disrupt endothelial TJs and increase permeability[107]. Moreover, the development of advanced *in vitro* models, including three-dimensional (3D) cultures and organ-on-a-chip technologies, has further refined our ability to simulate the physiological and pathological states of BBB. These sophisticated models mimic the BBB's dynamic and complex nature more accurately, enabling detailed investigations into the cellular and molecular mechanisms of CSVD-associated BBB dysfunction[108,109]. They have also facilitated the evaluation of potential therapeutics, offering insights into their ability to restore BBB integrity and function.

The next section of this paper will explore recent advancements in *in vitro* BBB models, highlighting their contributions to our understanding of CSVD pathophysiology. It will delve into the innovative methodologies employed to replicate the BBB's complexity and the insights gained from these models regarding disease mechanisms.

ECs monolayer

ECs monolayers have become a cornerstone in this research, especially in the investigation of CSVD-related BBB dysfunction. ECs monolayers, derived from human or animal cerebral microvessels or established ECs lines, provide a simplified yet powerful model to study BBB properties. These models allow for direct manipulation of the ECs, the innermost layer of the BBB, to investigate their response to various stimuli or conditions associated with CSVD. The studies have focused on the pathological changes in CSVD, including small subcortical infarcts, white matter hyperintensities, lacune, cerebral microbleeds, ePVS, and brain atrophy[110].

Additionally, the studies have discussed the criteria for defining endothelial dysfunction, such as endothelial activation, impaired endothelial mechanotransduction, and reduced NO release[111]. Recent studies have brought to light the dynamic nature of TJ proteins in response to stressors relevant to CSVD by using ECs monolayers. An example of this is hyperglycemia, which has been demonstrated to reduce the expression of claudin-5 and occludin, crucial elements of TJs, thus compromising the integrity of the barrier[112]. Advanced imaging techniques and molecular methods have further clarified the signaling pathways involved in this phenomenon, including the participation of protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) pathways[113].

ECs monolayers have also been utilized for the examination of the endothelium's role in the initiation and propagation of inflammatory responses within the BBB. The exposure to inflammatory cytokines, such as TNF-α and IL-1β, which are commonly elevated in CSVD, triggers the upregulation of adhesion molecules such as vascular cell adhesion molecules (VCAM-1) and intercellular cells adhesion molecules (ICAM-1) on ECs. This upregulation facilitates the adhesion and transmigration of leukocytes, as described by Béguin *et al.* (2019)[114]. The recruitment of leukocytes exacerbates the disruption of the BBB, thus playing a role in a cycle of inflammation and vascular damage that is typical of CSVD. Moreover, under inflammatory circumstances, endothelial cells release matrix metalloproteinases (MMPs), particularly MMP-9, which break down components of the extracellular matrix and proteins of TJs, further compromising the integrity of the BBB[115].

Moreover, studies have indicated that ROS, produced because of hyperglycemia or ischemic circumstances, possess the ability to directly harm ECs, resulting in modifications to the expression of TJ proteins and an increase in permeability[116]. Various antioxidants and pharmacological agents inhibiting ROS generation have been experimentally evaluated on ECs monolayers, revealing potential therapeutic advantages in maintaining BBB functionality[117-119]. In models of ECs monolayers, a diabetic environment characterized by persistent hyperglycemia is efficiently replicated to examine its influence on the properties of the BBB[17,50,120]. Hyperglycemia-induced activation of the polyol pathway and increased formation of AGEs have been implicated in endothelial dysfunction and increased BBB permeability. This process is mediated through the interaction of AGEs-RAGE on ECs, triggering inflammatory and oxidative stress pathways that disrupt BBB integrity[121].

Recent studies have also focused on ECs metabolism, revealing that metabolic shifts within ECs under pathological conditions can influence BBB integrity. For example, alterations in endothelial glycolysis and

mitochondrial function have been linked to changes in TJs protein expression and endothelial barrier function, suggesting novel targets for therapeutic intervention[122]. The utilization of ECs monolayers has made a substantial contribution to the comprehension of BBB dysfunction in CSVD, offering insights into mechanisms and pinpointing possible therapeutic targets. With the progression of this model, there is potential for revealing new dimensions of BBB physiology and pathology, enhancing our capacity to address CSVD and its harmful impacts on brain well-being.

Co-culture models

In neurovascular research, co-culture models are particularly valuable for studying the BBB because they allow the growth of two or more different cell types together in a controlled environment. This method is designed to mimic the interactions between these cell types as closely as possible to how they occur in the body, allowing researchers to study the complex dynamics of cellular communication, behavior, and function within a more physiologically relevant context. Co-culture models of the BBB typically involve the growth of ECs alongside one or more other cell types to investigate how their interactions influence BBB integrity, function, and response to pathological stimuli.

A study by Park *et al.* (2023) developed advanced co-culture models that simulate the BBB by incorporating ECs, astrocytes, pericytes, and neurons[123]. These models were specifically tailored to replicate various neurodegenerative conditions, including those mimicking CSVD. The researchers found that neurodegenerative conditions led to distinct patterns of BBB disruption, highlighting the role of inflammatory mediators, oxidative stress, and the dysregulation of TJ proteins. Specifically, the study underscored the differential impact of Aβ peptides and hyperphosphorylated tau proteins on BBB integrity, reflecting the complex interplay between neurodegenerative disease markers and BBB function[123]. Additionally, recent studies using co-culture models have highlighted the critical role of pericytes in maintaining BBB integrity and their involvement in the pathogenesis of CSVD. Pericytes, through their interactions with ECs, regulate CBF, angiogenesis, and ECs proliferation. In the context of CSVD, pericyte detachment and dysfunction have been associated with BBB leakage, emphasizing the importance of pericyte-ECs signaling in preserving BBB integrity[124].

Thus, co-culture models have revolutionized our understanding of BBB dysfunction in CSVD by providing a more accurate representation of the NGVU. These models enable the study of interactions between ECs, astrocytes, pericytes, and neurons, shedding light on how disruptions in these interactions contribute to BBB breakdown in CSVD. Importantly, co-culture systems have facilitated the identification of potential therapeutic targets and the evaluation of treatment strategies aimed at preserving BBB integrity. As research progresses, these models hold promise for uncovering novel insights into CSVD pathomechanism and accelerating the development of effective therapies.

Organ-on-a-chip models

Organ-on-a-chip models leverage microfluidic platforms to create cell culture environments that simulate the physical and biochemical aspects of human organs, providing a dynamic system to study complex physiological responses[125]. These micro-engineered devices integrate various cell types into a single chip, creating organ-specific models that accurately replicate organ microenvironments and functions. Organ-on-a-chip models stand out for their ability to model human physiology more realistically than traditional 2D cell cultures, offering profound implications for drug discovery, disease modeling, and personalized medicine. By closely mimicking the *in vivo* environment, organ-on-a-chip technology provides a critical bridge between *in vitro* experiments and clinical reality, paving the way for advancements in understanding human biology and developing safer, more effective therapies.

Organ-on-a-chip technology represents a breakthrough in BBB modeling, offering dynamic systems that replicate blood flow and mechanical forces of the cerebral microenvironment. The BBB-on-a-chip (μBBB) technology has been widely used in the study of brain cancer[126]. Additionally, a linked alveolus-BBB organ chip platform has been proposed to investigate the effects of severe acute respiratory syndrome - coronavirus 2 (SARS-CoV-2) on human brains in a multi-organ context[127]. Organ-on-a-chip technology has emerged as a new approach to better recapitulate the highly dynamic *in vivo* human brain microenvironment, including the neural neurovascular unit and the BBB[128].

Furthermore, microfluidic technology and microfabrication have been used to rebuild the BBB *in vitro,* with a focus on drug delivery studies[129]. Human BBB-on-a-chip technologies have also been developed for studying neurodegenerative diseases such as Alzheimer's disease and for drug prescreening[130]. These models have been instrumental in studying the effects of shear stress on BBB integrity and the impact of CSVD risk factors, such as hypertension, on ECs function and barrier properties[131]. Organ-on-a-chip models have shed light on several critical aspects of BBB dysfunction in CSVD, including the role of ECs pathology, the impact of inflammatory mediators, and the consequences of altered blood flow and shear stress on BBB integrity. By simulating the pathological conditions of CSVD, such as chronic hypertension and hyperglycemia, researchers are able to observe the direct effects of these factors on BBB permeability and TJs integrity, advancing our understanding of CSVD pathophysiology[132]. Furthermore, these models have facilitated the study of Aβ accumulation and its effects on BBB function, a hallmark of both Alzheimer's disease and CSVD. Organ-on-a-chip technology has enabled the detailed examination of how Aβ interacts with various components of the neurovascular unit, contributing to endothelial dysfunction and neurovascular uncoupling, critical processes in the development and progression of CSVD[133]. Nevertheless, more focused research is required to elucidate the dysfunction of the BBB in organ-on-a-chip models for CSVD due to the limited number of studies in this particular area.

3D BBB models

The 3D BBB models represent a transformative advance in neurological research, bridging crucial gaps in our understanding of BBB function and dysfunction. These models employ cutting-edge bioengineering techniques to recreate the complex structure and function of the BBB *in vitro*, offering a more physiologically relevant and dynamic system than traditional two-dimensional cultures. By mimicking the 3D architecture and cellular interactions of the BBB, these models provide invaluable insights into the mechanisms of neurovascular coupling, the pathophysiology of neurological diseases, and the evaluation of drug delivery systems across the BBB[134]. The incorporation of various cell types found in the BBB, including ECs, astrocytes, pericytes, and neurons, into a cohesive 3D structure allows for the study of cellular responses to pathological stimuli and therapeutic agents in an environment that closely resembles *in vivo* conditions.

Wei *et al.* (2023) developed a human-cell-based BBB platform with integrated electrodes to monitor barrier tightness in real time[135]. They demonstrated that oxygen-glucose deprivation (OGD) induced rapid remodeling of cellular actin structures and subsequent morphological changes in ECs, leading to barrier breakage. Chen *et al.* (2021) summarized the development of microfluidics-based BBB models derived from human stem cells, which can provide a better platform for high-throughput drug screening and targeted delivery[136]. Potjewyd *et al.* (2021) discussed the use of induced pluripotent stem cells (iPSCs) to generate human NGVU models for studying BBB function and disease mechanisms, including CSVD[137]. Bouhrira *et al.* (2020) investigated the effect of disturbed flow on BBB integrity using a 3D perfusable bifurcation model, showing that disturbed flow caused barrier disruption in the CNS[138].

Therefore, 3D BBB models have shed light on the molecular mechanisms leading to BBB dysfunction in CSVD. These models have also been instrumental in exploring the accumulation of neurotoxic substances, including Aβ, and their impact on BBB integrity and neural health[133]. The 3D BBB models have emerged as a crucial innovation in neurological research, offering a dynamic approach to studying the complexities of the BBB and its role in CSVD. By more accurately mimicking the *in vivo* environment of the BBB, these models provide a platform for in-depth exploration of the pathophysiological changes associated with CSVD, including the breakdown of barrier integrity and the resultant impact on brain health. The incorporation of key cellular components of the neurovascular unit in a 3D matrix allows for a better understanding of the cellular crosstalk and molecular mechanisms that underlie BBB dysfunction. This advancement holds promise for uncovering novel therapeutic targets and enhancing drug screening processes, ultimately contributing to the development of more effective treatments for CSVD and related neurological conditions.

This section elaborates on the preclinical models used to study BBB dysfunction in CSVD, spanning from aging, systemic hypertension, chronic cerebral hypoperfusion, diabetes mellitus, and transgenic to *in vitro* models. Such models illuminate key insights about the pathophysiology of CSVD and possible therapeutic targets. The significance of advanced animal models in mimicking human CSVD pathology is also promising for enhancing our understanding of its natural history and its contribution to neurovascular and neurodegenerative interactions.

MECHANISMS OF ABERRANT BBB DYNAMICS IN CSVD: PRECLINICAL INSIGHTS

The integrity of the BBB is paramount for the normal functioning of the CNS, as it serves as a vital connection between the brain's microenvironment and the systemic circulation. CSVD represents a spectrum of pathological conditions affecting the small vessels of the brain, causing lesions in both the white and grey matter and resulting in significant morbidity through its impact on cognitive decline, stroke, and dementia. A pivotal aspect of CSVD pathology involves the disruption of the BBB, a process mediated by a complex interplay of cellular and molecular mechanisms. Advancements in preclinical models, including transgenic and *in vitro* approaches, have been pivotal in our understanding of CSVD, particularly concerning BBB disruption. By replicating the complex pathology of CSVD and its impact on BBB integrity within controlled environments, these models have revealed key mechanisms at play, such as oxidative stress, inflammation, and endothelial dysfunction[70,71]. This evidence not only deepens our comprehension of the disease's cellular and molecular landscape but also highlights potential therapeutic targets to preserve BBB integrity and counter CSVD progression. This section delves into the preclinical insights into the potential pathomechanisms of BBB dysfunction in CSVD, drawing upon recent advancements in research to elucidate the cellular and molecular processes driving this disruption.

Endothelial dysfunction and CSVD: impacts on cell integrity, TJs, and trans-endothelial transport

Endothelial dysfunction plays a pivotal role in the pathogenesis of cardiovascular diseases. It fundamentally alters the endothelium's ability to maintain vascular homeostasis, leading to impaired regulation of vascular tone, increased permeability, and pro-inflammatory state. This dysfunction is central to the pathophysiology of CSVD, leading to a cascade of vascular abnormalities that compromise cerebral microvascular health. CSVD disrupts ECs integrity by promoting ECs activation and dysfunction. This is characterized by the shift toward a pro-inflammatory and prothrombotic state, vasoconstriction, and cell proliferation, as highlighted by Gallo *et al.* (2022)[139].

Studies on animal models, like the smooth muscle knockout mice with specific deletion of Myosin Phosphatase Target Subunit 1 ($MYPT1^{SMKO}$), a novel spontaneous model for age- and hypertension-

dependent CSVD, have demonstrated how genetic and environmental factors contribute to endothelial dysfunction. This model unraveled the complex interplay between hypertension, a common risk factor for CSVD, and endothelial health, highlighting the role of oxidative stress and inflammation in damaging ECs[17]. Disruption in TJs, often observed in CSVD, leads to increased BBB permeability, allowing harmful substances to enter the brain parenchyma. Studies have shown that certain pathogens can exploit TJs to invade intestinal epithelial cells, suggesting a similar vulnerability might exist in cerebral ECs, potentially contributing to CSVD pathogenesis[140].

Research on models of sterile corneal inflammation revealed that inflammation, a key feature of CSVD, leads to a decrease in ZO-1 expression, suggesting a direct link between inflammatory processes and the compromise of TJs integrity[141]. This has significant implications for CSVD, as it underscores the importance of managing inflammatory pathways to protect TJs and maintain BBB integrity. Moreover, the trans-endothelial transport in CSVD is significantly affected, as the disease compromises the endothelium's selective permeability. The CSVD-induced endothelial dysfunction allowed the unregulated transport of lipoproteins and inflammatory cells into the brain tissue, contributing to the disease's progression and severity[115]. A pioneering study demonstrated that sphingosine-1-phosphate receptor 3 (S1P3) regulates the trans-endothelial transport of high-density lipoproteins (HDL) and high-density lipoproteins (LDL) in opposite ways, indicating that lipid transport across the endothelium is finely regulated and that disruption can contribute to CSVD pathology[142]. These findings suggest that targeting specific receptors and pathways involved in trans-endothelial transport may offer new therapeutic avenues for managing CSVD.

Inflammatory responses: the role of inflammation in BBB breakdown in CSVD, including the involvement of cytokines and leukocyte trafficking

CSVD is a pathology characterized by the disruption of this essential BBB, mediated by complex inflammatory responses that include the pivotal roles of cytokines and leukocyte trafficking. The roles of cytokines and leukocyte trafficking in disrupting the BBB have been extensively studied through preclinical models, offering invaluable insights into the underlying mechanisms. These studies not only illuminate the intricacies of BBB dysfunction but also pave the way for novel therapeutic strategies aimed at mitigating inflammation and preserving BBB integrity in the context of CSVD. The integrity of the BBB is compromised in CSVD through the action of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-17 (IL-17), and TNF-α, which orchestrate a cascade of events leading to endothelial dysfunction and barrier permeability. These cytokines not only disrupt TJs between ECs, but they also facilitate the recruitment and adhesion of immune cells to the BBB, exacerbating its breakdown[143].

The inflammatory milieu is further compounded by astrocytes and microglia, which, upon activation, release additional cytokines and chemokines, perpetuating the cycle of inflammation and barrier disruption. Research using BBB-on-a-chip models has offered a dynamic platform for assessing the real-time effects of cytokine exposure on BBB function, revealing concentration-dependent disruptions in barrier integrity[144]. Microglia, the resident immune cells of the CNS, also act as a double-edged sword in CSVD. While they are essential for maintaining CNS homeostasis and responding to injury, their chronic activation can exacerbate BBB disruption by releasing pro-inflammatory cytokines and ROS. In addition to the resident microglia, the brain's immune response to injury involves the employment of peripheral immune cells across the BBB. This recruitment depicts a key component of the brain's ability to react to injury, encompassing a complex interaction between resident and recruited immune cells that manage reparative, inflammatory, and regenerative mechanisms[145]. Preclinical models have highlighted the contribution of microglial activation in the pathogenesis of BBB dysfunction in CSVD, suggesting that modulating microglial responses could be beneficial[146]. Though their classical markers such as the fractalkine receptor CX3CR1, cluster of differentiation receptors (CD68, CD11b, CD14, CD45, CD80 and CD115), and ionized calcium-binding

adaptor molecule (Iba1) have been identified, it is worth mentioning that microglial are a heterogenous population and their activation is dynamic; therefore, recent advances in single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq) technologies have been approached to comprehensively characterize microglia in neurodegenerative diseases[147].

Oxidative stress: evidence linking oxidative stress to BBB disruption in CSVD

Oxidative stress arises when there is an imbalance between the generation of ROS and the body's capacity to neutralize their adverse effects, playing a key role in the compromise of the BBB. High levels of ROS not only initiate inflammatory reactions but also affect autophagic activities, together impairing the BBB. This interaction highlights the critical need to regulate ROS levels to safeguard against BBB impairment. Proper management of ROS production and clearance is essential, as elevated ROS levels can prompt inflammation and alter autophagic functions, further compromising the integrity of the BBB[148]. This process is mediated through several molecular pathways, leading to endothelial dysfunction, TJs alterations, and increased barrier permeability[148].

Dysregulated autophagy, which is influenced by oxidative stress in ECs, can lead to the accumulation of damaged proteins and organelles, contributing to endothelial dysfunction and BBB disruption[149]. A crucial antioxidant defense mechanism within endothelial cells involves the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. Activation of Nrf2 leads to the upregulation of antioxidant response element (ARE)-driven genes, which encode several antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). This mechanism is pivotal in counteracting ROS-induced endothelial damage and preserving TJs integrity[110]. Other than that, the study by Sun *et al.* (2023) provides evidence that lanthanum-induced activation of MMP9, facilitated by oxidative stress, can lead to the degradation of TJ proteins such as occludin and ZO-1[150]. This process is mediated by a reduction in Nrf2 expression, a key transcription factor involved in cellular antioxidant responses, underscoring the critical role of the Nrf2 pathway in protecting TJs integrity against oxidative stress-induced damage[151].

Oxidative stress also can cause mitochondrial damage, contributing to the disruption of ECs and TJs. Mitochondrial dysfunction in ECs leads to decreased ATP production, increased ROS generation, and the release of pro-apoptotic factors. These events contribute to ECs apoptosis and TJs disruption, emphasizing the role of mitochondria as targets for therapeutic intervention[152]. Additionally, ATN-161, a synthetic peptide derived from fibronectin specifically stands for the amino acid sequence Ac-PHSCN-NH2, where "Ac" represents an acetyl group and "NH2" represents an amide group, ameliorates ischemia/reperfusion-induced oxidative stress, fibro-inflammation, mitochondrial damage, and apoptosis-mediated tight junction disruption, suggesting mitochondrial pathways as targets for preserving BBB integrity[153]. This study demonstrates the multifaceted role of ATN-161 in counteracting oxidative stress and its downstream effects on the BBB, emphasizing the interconnection between oxidative stress, mitochondrial dysfunction, and BBB integrity.

The evidence from preclinical studies underscores the significant role of oxidative stress in BBB disruption within the context of CSVD[148,154,155]. By elucidating the molecular pathways affected by oxidative stress, these studies offer valuable insights into potential therapeutic targets. Continued exploration of these mechanisms is essential for developing effective treatments aimed at preserving BBB integrity and mitigating the progression of CSVD.

Blood flow alteration: changes in CBF in CSVD models affect BBB integrity

In CSVD, alterations in CBF are not merely symptoms but act as catalysts exacerbating the disease's underlying mechanisms. The BBB's compromised integrity due to fluctuating CBF underscores a critical

vulnerability in CSVD, leading to the disruption of cerebral homeostasis and contributing to the pathology's progression. Preclinical investigations have played a crucial role in elucidating these mechanisms, demonstrating how even minor alterations in blood circulation can initiate a series of harmful consequences within the vascular system of the brain. These insights underscore the pivotal role of maintaining hemodynamic stability, not just for preserving vascular health but also for safeguarding the brain against the insidious advances of CSVD.

Zhang *et al.* (2022) highlighted that decreased CBF and delayed arterial transit are independently associated with WMHs, suggesting contributions to CSVD pathology through BBB compromise[16]. The independent associations of reduced CBF and delayed arterial transit with WMHs suggest that alterations in blood flow not only reflect underlying vascular pathology but actively contribute to the disease process by compromising the BBB. The study by Wang *et al.* (2020) underscores the compounded impact of aging and CSVD on CBF autoregulation and cognitive function in diabetic rats' model, highlighting the interconnectedness of these factors in contributing to BBB disruption[156]. Aging exacerbates the diabetic-induced impairments in CBF autoregulation, leading to unstable cerebral perfusion that can damage the BBB and affect cognitive health.

Besides that, the research conducted by Ali *et al.* (2022) emphasizes the crucial role of vascular endothelial growth factor (VEGF) signaling, particularly VEGF-A, in modulating CBF and BBB integrity, drawing parallels between Alzheimer's disease and CSVD[157]. In both conditions, the adhesion of leukocytes to the brain microvascular endothelium, leading to stalled capillary blood flow and a consequent reduction in CBF, is identified as a key pathological mechanism exacerbating cognitive decline. Targeting VEGF signaling is a potential therapeutic approach to enhance CBF and maintain BBB integrity in CSVD, similar to observations in Alzheimer's disease models. Advanced multi-layer Monte Carlo modeling has improved the accuracy of CBF quantification in the presence of systemic physiology crosstalk, offering new insights into the relationship between CBF and tissue microstructural integrity. This approach may provide a more accurate assessment of how changes in CBF impact BBB integrity and, consequently, the progression of CSVD[158].

Apart from that, Ling *et al.* (2023) highlighted the critical link between the ability of the brain to regulate its blood flow dynamically and the integrity of the BBB in reversible cerebral vasoconstriction syndrome (RCVS)[159]. Their research demonstrated that disruptions in the brain's capacity to adjust blood flow in response to changes in blood pressure are significantly associated with BBB disturbances. Such disturbances are central to the pathology of RCVS, potentially allowing harmful substances to penetrate the brain and intensify neurological manifestations. This study emphasizes the protective role of dynamic cerebral autoregulation in safeguarding the BBB and suggests focusing on therapies that enhance this regulatory function as a strategy to preserve BBB integrity and alleviate the adverse neurological outcomes linked to RCVS. In summary, CSVD presents a complex challenge where alterations in CBF not only signal the disease's presence but actively exacerbate its mechanisms, impacting the critical functionality and integrity of the BBB. As summarized in Figure 2, these preclinical insights collectively provide a deeper understanding of the mechanisms underpinning CSVD and underscore the importance of maintaining hemodynamic stability and BBB health.

This section highlights the specific mechanism by which BBB dysfunction occurs in CSVD, focusing on endothelial dysfunction, inflammatory responses, oxidative stress, and blood flow alterations. It emphasizes how these mechanisms impair BBB integrity, resulting in increased permeability and subsequent neurovascular damage. The discernment obtained from preclinical models highlights the importance of

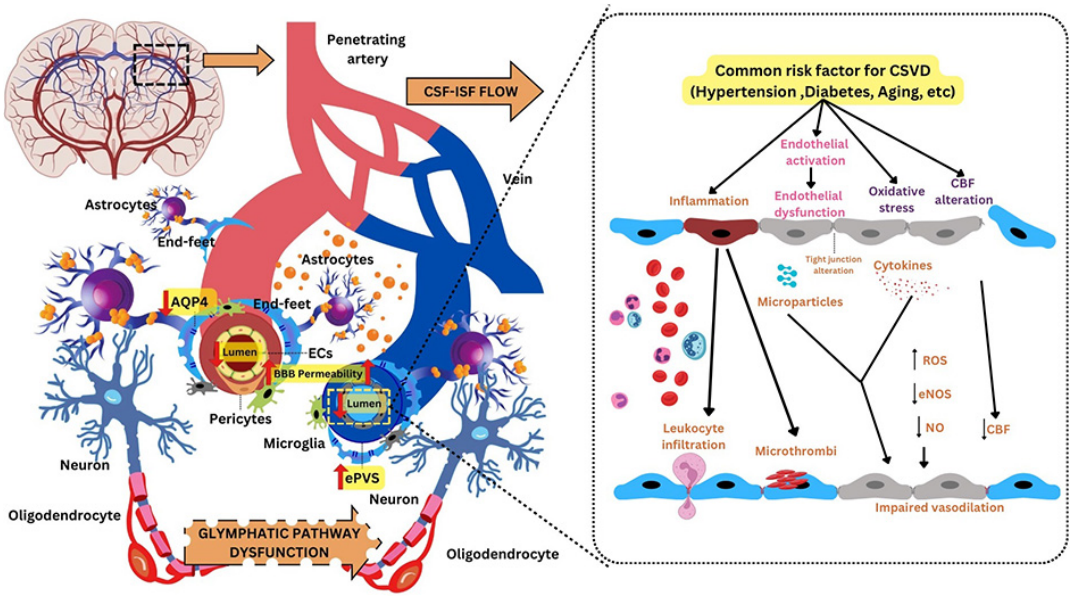

Figure 2. Mechanisms of aberrant BBB dynamics in CSVD. This illustration portrays the correlation between the BBB and the glymphatic system within the framework of common risk factors for CSVD, like hypertension, diabetes, and aging. These factors may result in the dysfunction of the NGVU and the dynamics of the glymphatic system, such as reduced polarization of AQP4, ePVS, and increased BBB permeability. The right panel connects these alterations with endothelial cells and the decrease of the lumen size, depicting the mechanism including endothelial activation, endothelial dysfunction, oxidative stress, and CBF alteration, which collectively lead to a cascade of inflammatory and thrombotic responses, culminating in impaired vasodilation and potentially contributing to CSVD disease progression.

understanding this process to develop targeted interventions aimed at conserving BBB function and mitigating the development of CSVD.

CHALLENGES AND FUTURE DIRECTIONS IN PRECLINICAL RESEARCH

Translational challenges

Preclinical research plays a crucial role in unveiling the fundamental mechanisms of CSVD, with a specific focus on significant factors such as alterations in CBF, disruptions in the integrity of the BBB, and abnormalities in vascular signaling pathways. Despite the tremendous relevance of these discoveries, a variety of obstacles can hinder their direct implementation in clinical contexts. The most recent study showed a significant alteration in the BBB leakage rate of gadolinium chelates (K^{trans}) and BBB water exchange rate (k_w) between subtypes of CSVD (sporadic CSVD, CADASIL and HTRA-1-related CSVD), showing the heterogeneity of BBB dysfunction[160]. Another major hurdle is the sex discrepancies in CSVD and the BBB. According to a systematic review and meta-analysis, CSVD is more prevalent and/or severe in men than in women, especially among those presenting with stroke. Nonetheless, further research is necessary to identify sex-specific biological and social disparities and understand their roles in these sex differences[161]. In contrast, women but not men were likely to have more severe and arteriosclerosis/lipohyalinosis asymptomatic SVD with increased levels of circulatory homocysteine[162]. Although beyond the scope of this review, it is particularly important to emphasize that sex hormones play a role in the BBB integrity[163]. Steroid hormones - especially sex hormones - can easily cross the BBB bidirectionally through the process of transmembrane protein because of their small size and lipid solubility[164]. A study reported that the administration of estrogen protected the brain from the immunological response during inflammation by increasing BBB functionality and decreasing leukocyte extravasation across the BBB[165]. The influence of sex in BBB disruption and neurodegenerative diseases is still understudied[166].

While *in vivo* models and *in vitro* models are crucial for understanding disease mechanisms, they frequently fail to capture the full array of genetic, environmental, and lifestyle factors influencing CSVD in humans, potentially skewing therapeutic efficacy assessments. As a result, translating preclinical findings into clinical applications for the treatment of CSVD requires a multitude of complexities that underscore the significant difference between experimental models and the complex characteristics of human pathology. The disease's intricate nature, characterized by vascular and neurological alterations, demands comprehensive treatment strategies that the preclinical model's reductionist approaches might not address. Additionally, the variability in symptoms and disease progression among patients necessitates personalized treatment approaches, further complicating the development of universal therapies. Ethical and safety considerations add to the complexity of translating research into practice because interventions deemed safe in animal models may pose risks to humans. Therefore, a nuanced and multidisciplinary approach is crucial for developing effective, personalized treatments that can navigate the intricate nature of CSVD and the ethical implications of translating research into practice.

Emerging technologies

Overcoming these challenges to improve patient care requires innovative research methods, cross-disciplinary collaboration, and a commitment to translating scientific discoveries into meaningful clinical interventions, fostering hope for significant advancements in CSVD treatment[51,86,104]. The potential of new technologies, particularly advanced imaging techniques, in advancing BBB research in CSVD cannot be overstated. These emerging tools are bridging the gap from preclinical insights to clinical applications, offering unprecedented opportunities to visualize and understand the intricate dynamics at play in BBB integrity and dysfunction.

One such advancement is the utilization of high-resolution MRI techniques, including dynamic contrast-enhanced MRI (DCE-MRI) and magnetic resonance spectroscopy (MRS). These techniques provide detailed insights into BBB permeability and the biochemical changes occurring within the brain's microenvironment. For instance, Montagne *et al.* (2015) employed DCE-MRI to quantify BBB leakage in aging and dementia, providing a direct link between BBB integrity and neurodegenerative processes[74]. Similarly, the use of ultra-high-field MRI offers enhanced resolution and sensitivity, enabling the detection of microvascular changes and subtle BBB disruptions that precede overt CSVD symptoms[167,168]. Emerging non-invasive imaging technologies, such as near-infrared spectroscopy (NIRS) and transcranial Doppler sonography (TCD), also hold promise for BBB research in CSVD. NIRS, for example, can assess cerebral oxygenation and hemodynamics, indirectly reflecting BBB function, while TCD measures cerebral blood flow velocity, offering insights into hemodynamic impairments associated with BBB disruption[169-172].

Additionally, molecular imaging techniques that employ specific biomarkers for BBB integrity, such as radiolabeled tracers in positron emission tomography (PET), are being explored. These tracers can target and visualize molecular processes associated with BBB disruption, enabling a more targeted approach to understanding CSVD pathology and evaluating therapeutic interventions[2].

Future research priorities

A critical avenue for future research is the identification and exploration of novel molecular pathways contributing to BBB dysfunction in CSVD. Recent studies have emphasized the role of inflammation and oxidative stress in BBB compromise; however, the intricate signaling cascades within these broad categories remain to be fully delineated. For instance, the contributions of microRNAs in regulating ECs functions and their potential as therapeutic targets need further exploration[173]. Additionally, the role of autophagy in BBB integrity presents another promising area for investigation, offering the potential for therapeutic modulation[174]. Enhancing the fidelity of preclinical models to human CSVD pathology is paramount.

While traditional animal models have provided invaluable insights, there is a pressing need for models that more accurately replicate the complex interplay of genetic, environmental, and pathological factors characteristic of CSVD. Human-induced pluripotent stem cell (hiPSC) technologies and organ-on-a-chip models represent cutting-edge advancements in this direction, enabling the study of human BBB characteristics *in vitro* and offering new opportunities for drug screening and mechanistic studies[109,132]. Longitudinal studies using advanced imaging and biomarker analyses are needed to track BBB integrity over time and correlate these findings with cognitive outcomes[50]. Such studies will provide insights into the temporal relationship between BBB disruption and neurodegeneration, potentially identifying early intervention points to halt or slow disease progression.

The development and validation of biomarkers for CSVD and BBB integrity are essential for advancing the field. Biomarkers found in blood, CSF, or detected through non-invasive imaging techniques could provide a means for early diagnosis, monitoring disease progression, and assessing the efficacy of therapeutic interventions[107]. Efforts should also be directed toward identifying genetic markers that predispose individuals to CSVD and BBB dysfunction, offering opportunities for personalized medicine approaches. Emerging technologies, such as single-cell RNA sequencing and organ-on-a-chip models, should be integrated into CSVD research. These technologies can offer unprecedented insights into the cellular and molecular heterogeneity of the BBB in CSVD and enable high-throughput screening of potential therapeutic agents[132,175].

CONCLUSION

In view of the current complex and elusive pathogenesis of CSVD, this review attempts to highlight the crucial role of BBB in sustaining CNS homeostasis and its potential involvement in the CSVD heterogeneous clinical manifestations. The BBB disruption in CSVD leads to the leakage of harmful substances into the brain parenchyma, initiating a cascade of deleterious effects such as inflammation, oxidative stress, and white matter damage. We outlined the issues and opportunities in translating preclinical discoveries into clinical practice, particularly potential therapeutic targets to conserve BBB function and/or alleviate CSVD manifestation that is vulnerable with aging. Such targets may enhance BBB tight junctions, reduce endothelial inflammation, or improve the clearance of toxic metabolites that may collectively halt the progression of CSVD, as well as promoting research on specific BBB integrity biomarkers. Of note, recent scRNA-seq could be employed to examine the molecular diversity of BBB elements, disclosing specific cell populations and their involvements in BBB integrity and dysfunction[176]. Spatial transcriptomics can map gene expression alterations within the BBB, deepening our comprehension of disease pathology[177]. Predictive computational models can spur research on genetic mutations and environmental stressors that can affect BBB integrity and offer hope for viable treatments[53]. Moreover, investigating the effects of environmental factors like drugs and pollutants on BBB function can furnish the understanding of their role in neurodegenerative diseases and CSVD, guiding plans to alleviate these impacts[178]. Here, we reiterated the association of BBB integrity and its dysfunction with that of key CSVD-centric pathomechanisms which include endothelial damage, inflammation, oxidative stress, and altered cerebral blood flow, based on the latest evidence from related preclinical models. These findings underscore the importance of understanding the role of BBB as a novel and critical target in combating CSVD and its consequences, given the notable rapidly expanding world's aging demographics.

DECLARATIONS

Authors' contributions

Drafted, prepared the figures, and revised the manuscript: Zul Ramli SMA

Conceived the original idea, drafted, reviewed, and critically revised the manuscript: Abdul Hamid H, Mustapha M, Mehat MZ
Critically revised the manuscript for important intellectual content: Che Mohd Nassir CMN, A Rahaman SN, Kumar J, Lee SY
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All authors declared that there are no conflicts of interest.

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Not applicable.

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