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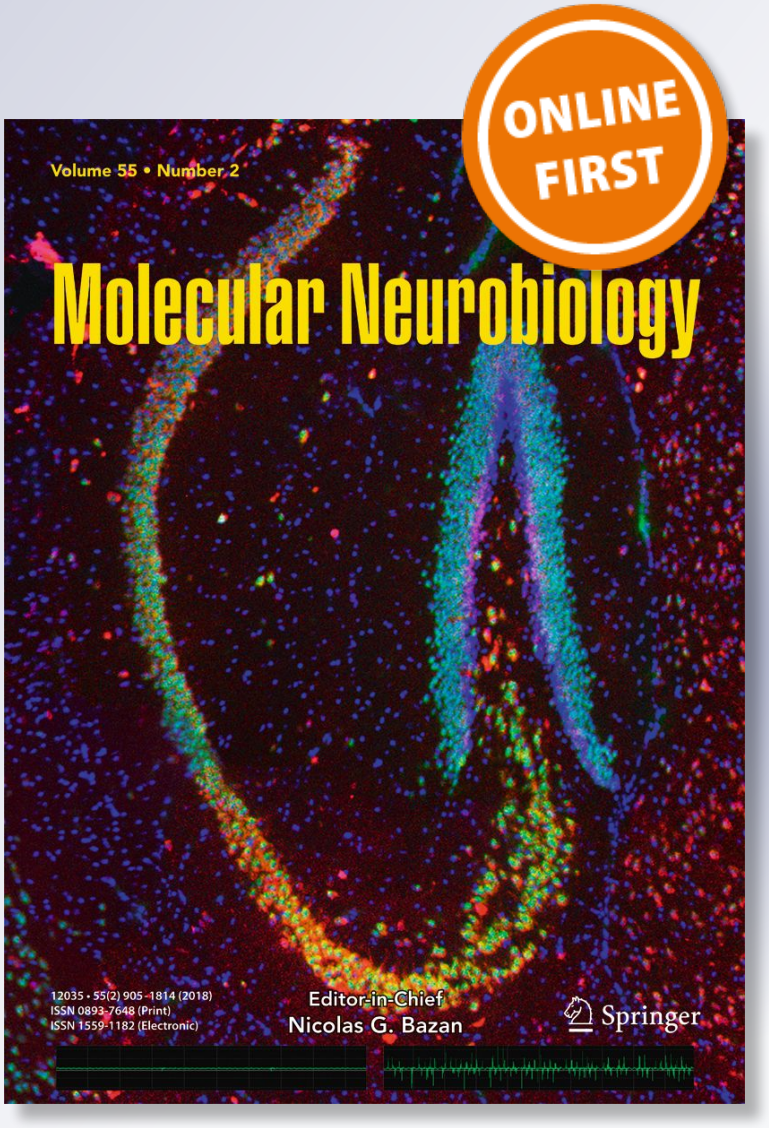

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Endothelial Cell Dysfunction and Injury in Subarachnoid Hemorrhage

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Abstract

In the brain, vascular endothelial cells conserve blood viscosity, control blood flow, and form the interface between central nervous system and circulating blood. Clinical outcome after aneurysmal subarachnoid hemorrhage is linked to early brain injury, cerebral vasospasm, and other causes of delayed cerebral ischemia. The cerebral vasculature remains a unique target for therapies since it becomes rapidly disrupted after subarachnoid hemorrhage, and damage to the blood vessels continues into the delayed injury phase. The current failure of therapies to improve clinical outcome warrants a re-evaluation of current therapeutic approaches. The mechanisms of endothelial cell injury and blood–brain barrier breakdown are critical to the pathway of cerebral injury, and an improved understanding of these mechanisms may lead to novel therapeutic targets. This review provides an update on the current understanding of endothelial cell injury following aneurysmal subarachnoid hemorrhage, including blood–brain barrier dysfunction.

Keywords Endothelial cell · Subarachnoid hemorrhage · Blood–brain barrier · Cerebral vasospasm · Microthrombosis

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) affects 30,000 people per year in the United States, with mortality rates estimated to be as high as two-thirds[1, 2]. Most patients with aSAH are critically ill and require a prolonged intensive care unit stay resulting in significant public health costs. Additionally, aSAH carries a disproportionately high toll in terms of productive life-years lost because it has an earlier mean age of onset and is associated with higher disability and morbidity rates when compared to other types of stroke [3]. Patients who survive the initial bleed are at risk for a multitude of secondary insults including rebleeding, hydrocephalus, and delayed ischemia [4]. Poor outcome after aSAH occurs in 50 to 75% of patients and is credited to secondary ischemia in approximately 30% [5]. This delayed cerebral ischemia (DCI) has been attributed to cerebral vasospasm (CV), microthrombosis, and cortical spreading depolarizations [6–9]. A meta-analysis of seven randomized, double-blind, placebo-controlled trials showed that the L-type calcium channel blocker nimodipine decreased the risk of poor outcome in patients with aSAH by 42% [10]. However, since the adoption of nimodipine, there has been no significant therapeutic breakthrough likely related to the multiple factors that mediate the deleterious effects of aSAH (Table 1) [16, 17]. Advances in understanding the mechanisms underlying the long-term complications of aSAH is a prerequisite for the development of new therapeutic strategies to follow the initial life-saving treatments.

Clinical trials for improving aSAH outcome largely target the cerebral vasculature since it becomes rapidly disrupted, and damage to the blood vessels continues into the delayed

Key Points
- Aneurysmal subarachnoid hemorrhage (aSAH) can cause early and delayed brain injury.
- Few accepted therapeutic strategies are available for improving poor outcome in the treatment of aSAH. Current treatment strategies aimed at rescuing cerebral vasospasm do not improve outcome.
- Endothelial cell injury/dysfunction during early phase injury can play a critical role in the in SAH complications.
- The blood–brain barrier (BBB) protects the central nervous system from neurotoxic plasma components, blood cells, and pathogens present in the systemic circulation.
- Experimental studies have shown that post aSAH, multiple factors contribute to BBB breakdown that can facilitate inflammatory and immune responses contributing to poor outcome.
- Advancement of basic and clinical research directed to rescue endothelial cell injury and protect and restore BBB function following aSAH might provide novel targets for clinical intervention.

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Table 1 Clinical trials for aSAH

Author	Drug	Trial target	Clinical benefit
Allen et al. [3]	Nimodipine	Calcium antagonist—improved neurological deficits due to spasm	(+) Benefit
Haley et al. [11]	Tirilazad	Free radical scavenger—targets vasospasm	(−)
Siironen et al. [12]	Enoxaparin	Anti-coagulating agent—target to prevent vasospasm	(−)
Van den Bergh [13]	Aspirin	Anti-platelet agent—prevents thrombus formation, endothelial injury, and inflammation of the aneurysm wall	(−)
Gomis et al. [14]	Methylprednisolone	Anti-inflammatory—target to prevent vasospasm	(−)
Macdonald et al. [5]	Clozasentan	Endothelin-A receptor antagonist—targets vasospasm after aSAH; reduced vasospasm	(−)
Kirkpatrick et al. [15]	Simvastatin	Attenuates inflammation, oxidation, platelet aggregation, and excitotoxicity—target to reduces vasospasm after aSAH	(−)

injury phase. The brain's ability to regulate systemic and cerebral function depends on blood vessels to supply oxygen and nutrients, form a barrier for toxic substances, and clear waste products [18]. Recent studies recognize that brain endothelial cells (ECs) have additional functions compared to the peripheral vasculature, such as the facilitation of information transfer between neurons and glial cells [19], and maintenance of the blood–brain barrier (BBB) [20–23]. The ECs forming the BBB are distinguished by their lack of fenestrations, minimal pinocytotic activity, and the presence of tight junctions (TJ) [24]. ECs control vascular tone and blood flow via a delicate balance between EC secreted vasoconstrictors such as endothelin-1 (ET-1) and thromboxane (TXA2), and vasodilators such as nitric oxide (NO), prostacyclin (PGI2), and endothelium-derived hyperpolarizing factor (EDHF) [25]. Accumulating evidence indicates that EC dysfunction and increased permeability of the BBB mediates brain injury including the delayed appearance of neuronal dysfunction and death.

Animal studies have shown that aSAH can induce morphological and functional changes in vascular endothelium causing "endothelial dysfunction"—a referral to the failure of ECs to effectively perform basal functions, failure to produce sufficient NO leading to vessel constriction, failure to control coagulation, and failure to control permeability [26–29]. Taken together, we propose that ECs should be considered as the fundamental cell type affected by aSAH pathology and EC injury should be a primary target for therapeutic intervention for aSAH. We further highlight the importance of secondary BBB dysfunction in aSAH during the delayed phase of SAH pathophysiology.

Endothelial Cell Damage in aSAH

Healthy ECs maintain the BBB, regulate thrombus formation, and regulate vascular tone [30–32]. However, early events after aSAH trigger EC dysfunction and apoptosis, which in turn exacerbates the delayed phase of aSAH pathophysiology.

Early Brain Injury

The evolution of parenchymal lesions after aSAH shows a bimodal distribution with an early and delayed peak. Early brain injury (EBI) is the term used to describe the pathophysiological events between bleed day 0 and 3/4 which induce an immediate injury to the brain [33, 34]. Once the aneurysm ruptures, blood extravasates under arterial pressure, damages surrounding tissue, and enters into the subarachnoid space spreading through the CSF around the brain. This acute event causes physical detriments, including rapid rise in intracranial pressure (ICP), decreased cerebral blood flow (CBF), cerebral edema, acute vasospasm, global cerebral ischemia, and dysfunction of autoregulation [35, 36]. These instabilities are thought to play a vital role in aSAH and add significantly to morbidity and outcome [37, 38].

Animal models demonstrate that constriction of both large and small cerebral vessels occurs immediately after aSAH [33, 39, 40]. Large cerebral vessels go through two phases of constriction accompanied by reduction in CBF and perfusion deficits. The first phase starts as early as 5 min after aSAH and continues for at least 6 h [40–44]. This is followed by constriction of intraparenchymal and pial microvessels (10 to 30 μm) for up to 24 h [33, 40, 45–47]. In the rat aSAH and transient global ischemia models, there is an upregulation of vasoconstriction-mediating EC receptors endothelin B (ET-B) and serotonin receptors (5-HT1B), and downregulation of the vasodilator NO in the cerebral arteries [48, 49]. Arterial samples from aSAH patients who died within 48 h indicate a hyper-responsiveness of ECs to contractile agents like norepinephrine and potassium, and a decreased response to dilatory agents like acetylcholine, thrombin, and bradykinin [50, 51]. Furthermore, it is reported that these hyper-responsive ECs increase activation of smooth muscle cells [52, 53]. Cerebral ischemia post aSAH can induce morphological changes in the vascular endothelium including corrugation of the endothelial membrane and appearance of cytoplasmic flaps or microvilli that extend to the vessel lumen [40, 42, 43]. The underlying molecular changes leading to EC dysfunction after aSAH are

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not completely clear since data from animal models of EC dysfunction are scarce and molecular data from patients are difficult to gather.

EC Apoptosis

Apoptotic damage to the endothelium is a critical event since this compromises BBB integrity, disrupts physiologic vasoregulation, and increases smooth muscle cell proliferation and blood coagulation [54]. Multiple factors can induce EC apoptosis including oxidative stress, oxyhemoglobin (OxyHb), and iron overload. EC apoptosis is reported to occur 24 h after aSAH [55]. Following aneurysm rupture, blood components (and blood breakdown products) lead to pathological events which cause damage to healthy endothelium [42]. OxyHb, the main component of erythrocytes, exerts a direct cytotoxic effect in cultured bovine brain ECs via caspase-8 or -9 [56–58]. Animal studies [59, 60] and postmortem human studies [61] report EC death after aSAH, mediated via OxyHb [62] elevation of intracellular Ca^{2+} [63], matrix metalloproteinase 9 (MMP-9) [59], and generation of free radicals [64]. In rat models, 10 min after aSAH the apoptosis marker cleaved-caspase-3 and tunnel staining colocalize with endothelial staining. Further, the endothelial lining of the parenchymal vessels is disrupted and detaches from the basal lamina layer within 10 min [26, 55]. Apoptosis of neurovascular ECs results in increased diffusion of serum from the vascular lumen into brain causing vasogenic edema.

There are several sources for the excessive generation of free radicals following SAH, including disrupted mitochondrial respiration and extracellular hemoglobin (following RBC lysis). Oxidative stress in aSAH and secondary EC dysfunction has been previously reviewed explaining the production of excessive free radicals in aSAH and their connections to acute brain injury, as well as the importance of antioxidant treatment [65, 66]. Hemoglobin breakdown results in iron overload in the acute phase of aSAH. Iron overload and iron-mediated free radical production causes loss of TJ proteins and degeneration of ECs in transient forebrain ischemia rat model [67]. A better understanding of EC apoptotic pathways after aSAH may foster the development of new therapies.

SAH Induced Blood–Brain Barrier Dysfunction

The BBB has developed as a complex, dynamic, adaptable interface that limits entry of potentially neurotoxic plasma components, blood cells, and pathogens into the central nervous system (CNS) [67]. The BBB is primarily formed by brain microvascular ECs with tight junctions and astrocyte end feet. A number of factors are unique to ECs forming the BBB including endothelial TJ and adherens junction (AJ) proteins, non-selective fenestrae, pinocytosis, bulk-flow transcytosis, and suppression of leukocyte adhesion molecules [23]. Intracellularly, TJ proteins are connected to actin filaments via zona occludens-1 and 2 (ZO-1, ZO-2); adherens junction proteins are connected via catenins (α, β, , and p120) [68]. During normal physiological conditions, a precise equilibrium between endothelial cell–cell adhesion and actin–myosin-based centripetal tension tightly controls the semi-permeability of microvascular barriers. Actomyosin contraction and myosin light chain phosphorylation plays an important role in maintaining TJ regulation [69].

Studies investigating BBB dysfunction associated with aSAH are relatively few compared with the variety of studies on vasospasm. Experimental studies have shown that after aSAH, significant BBB permeability change occurs beginning at 36 h, peaking at 48 h, and normalizing on day 3[70], although the exact time course of BBB dysfunction in humans has not been studied. Doczi et al. has demonstrated BBB damage occurs as early as 3 h after aSAH in some clinical studies and animal models [71, 72]. Multiple factors can contribute to BBB breakdown after aSAH including EC apoptosis (as previously discussed), EC contraction, and disruption of EC TJ proteins [73–75].

Studies investigating changes in the expression of TJ proteins and BBB permeability show no significant change in Caveolin-1 and Claudin-5 expression in the basement membrane, but do note a significant decrease in the expression of ZO-1 and Occludin at 3 and 72 h [76]. The downregulation of TJ protein ZO-1 and Occludin in ECs facilitates capillary leakage responsible for the increase in BBB permeability [35, 77]. The exact mechanism for the disruption of TJ proteins after aSAH is not clear, and the intracellular signaling events warrant further investigation.

Pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α) and thromboxane A2 cause EC apoptosis and contribute to BBB dysfunction [78]. Tunnel and immunofluorescence staining in ECs demonstrate that activation of TNF-α receptor-1 induce caspase-8 and activates caspase-3 leading to DNA fragmentation and apoptosis [79–81]. Moreover, inflammatory cytokines induce MMP production which disrupts the BBB [82–85]. Accumulating evidence suggests a role for MMP-9 in the early disruption of the BBB after aSAH [86, 87]. MMP-9 degrades the extracellular matrix of the cerebral microvessel basal lamina, which includes collagen IV, laminin, fibronectin, and inter-endothelial TJ proteins such as ZO-1 [88–90]. Tenascin-C (TNC), an extracellular matrix protein, is strongly induced in the spastic cerebral artery wall in rat aSAH [91, 92]. In the murine endovascular perforation model, TNC knockout improves neurological score and brain edema by reducing BBB disruption and degradation of tight junction protein ZO-1. BBB rescue in TNC knockout is reported to be via inactivation of three major MAPKs (JNK, p38, and ERK1/2) in brain capillary ECs, and inhibition of MMP-9 induction [93, 94].

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Systemic heme-induced BBB damage and permeability has also been demonstrated in a study on guinea pig exchange transfusion model [95]. Free intravascular Hb reduced expression of ZO-1, claudin 5 (small to medium size vessels), and increased GFAP in astrocytes (marker of BBB disruption) [95]. Increased iron deposits, oxidative stress, and inflammation damage ECs with subsequent BBB dysfunction in aSAH [65, 67, 96, 97] (Table 2). Together, these studies highlight the critical contribution of endothelial injury in BBB dysfunction, thereby underscoring the central role of endothelium in vascular protection (Figs. 1 and 2).

Endothelial Cell Pathophysiology in the Delayed Phase of aSAH

The major components of aSAH, which comprise the delayed phase of injury, include CV, microthrombosis, and inflammation. Below, we discuss the role of ECs in correlation with known delayed pathological events after aSAH.

Cerebral Vasospasm

Delayed CV typically develops 5 to 12 days after aSAH and continues for approximately 2 weeks; it affects more than one in five surviving patients [108, 109]. Cerebral ischemia, secondary to CV, is a major cause of morbidity and mortality after aSAH [110]. The sequence of events resulting in pathological CV includes EC dysfunction, smooth muscle contraction, inflammation, and changes in vascular responsiveness [27, 111–113]. The risk for development of CV after aSAH is linked to the amount of blood in the subarachnoid space [114, 115], with the primary instigator for this cascade thought to be free hemoglobin within the subarachnoid space [116, 117]. Damage from free heme includes neuronal and EC apoptosis, decreased nitric oxide (NO) production, increased ET-1 levels, lipid peroxidation of cell membranes, and direct oxidative stress on smooth muscle cells [28, 29, 65, 116–120]. This damage contributes to vasospasm through the loss of important vasodilator NO and via increased ET-1, a powerful vasoconstrictor peptide [121].

Nitric oxide is a major vasodilator produced principally by ECs. EC dysfunction resulting in decreased availability of NO may contribute to the development of vasospasm, and several studies support this finding. Blood products in the subarachnoid space are shown to reduce NO bioavailability by a variety of mechanisms including malfunction of NO-generating enzyme [122]. Reactive oxygen species (ROS) oxidize bilirubin to bilirubin oxidation products (BOXes) and inhibit eNOS [122]. Scavenging of NO by the vast amounts of extracellular hemoglobin also acts as an NO "sink" [123, 124]. Further supporting these findings, NO-based therapies reverse aSAH-associated vasospasm [125–127].

ET-1, a major isoform of endothelin molecules, represents the best-studied class of molecules in aSAH therapy. ET-1 acts on vascular smooth muscle via receptors on smooth muscle cells (ET-A and ET-B2) and endothelial cells (ET-B1) [128, 129] and causes profound and sustained vasoconstriction. ET-1 concentration is elevated in the CSF of aSAH patients and correlates with the development of CV [130]. Subsequent experiments have identified a number of plausible mechanisms to explain the ET-1 increase after aSAH. ET-1 production is increased by activated leukocytes in the CSF via IL-1ss, IL-6, and TNF-α [131]. OxyHgb also directly induces ET-1 production in ECs and smooth muscle cells via protein kinase C (PKC)–cAMP [130]. Experimentally, the ET-1 concentration required to induce ischemia is magnitudes higher than those measured in aSAH patients suggesting other factors are involved in the development of vasospasm [132, 133].

Nevertheless, ET-1 antagonists or ET-1 inhibitors can attenuate vasoconstriction in the experimental model and in clinical trials [134–136]. Apart from vasoconstriction, ET-1 can causes inflammation and smooth muscle cell proliferation in the vessel. The binding of ET-1 to ET-A receptors activates macrophages, increases neutrophil–vessel wall interactions, and elevates free radical concentrations, all of which lead to EC dysfunction [25, 137]. A phase IIa clinical trial using clazosentan, a selective ET-A receptor antagonist, significantly reduced angiographic vasospasm by 48% compared to placebo group. A phase IIb clinical trial (CONSCIOUS-1) showed that intravenous clazosentan significantly and dose-dependently reduced moderate or severe angiographic vasospasm when compared to placebo with a trend toward reduction in clinically relevant vasospasm-related events [54]. CONSCIOUS-2, a phase III clinical trial in patients undergoing surgical clipping revealed clazosentan at 5 mg/h reduced vasospasm but had no significant effect on mortality and vasospasm-related morbidity or functional outcome [5].

aSAH also contributes to the development of vasospasm by remodeling of the vascular wall structure. This remodeling is primarily the result of EC apoptosis and smooth muscle proliferation [138–141]. aSAH-induced apoptosis in ECs leads to destruction of the BBB, eventually exposing smooth muscle cells to vasoconstrictors in the blood. ET-1 can induce smooth muscle cell proliferation by binding to endothelin receptors [142] or activating other growth factors such as platelet-derived growth factor (PDGF) [143]. Smooth muscle proliferation combined with PDGF at the site of thrombus contributes to vessel wall thickening and vascular stiffening leading to delayed CV [144]. Prolonged arterial vasoconstriction also contributes to ultrastructural damage to the vessel wall layer, including vacuolization of ECs and loss of tight junctions, breakage of the internal elastic lamina, and patchy myonecrosis in the tunica media [61, 145].

Table 2 Studies on BBB rescue after aSAH

Author	SAH model	Species	Treatment	Level of rescue	Proposed mechanism
Ersahin et al. [98]	Blood injection	Male Wistar albino rats	Melatonin (10 mg/kg)	Treatment protects BBB integrity and reduced brain edema; improved neurological symptoms	Melatonin can easily cross BBB, and authors claim the neuroprotection may be due to its free radical scavenging properties
Chen et al. [99]	Endovascular perforation	Sprague–Dawley rats	Norrin (25 ng)	Increased expression of TJs occludin, VE-cadherin, and ZO-1; improved neurological outcome	Norrin via Frizzled-4 receptors promotes β-catenin nuclear translocation and thereby increased TJ protein expression
Ying et al. [100]	Endovascular perforation	Sprague–Dawley rats	Valproic acid (300 mg/kg)	Prevented TJ protein degradation, brain edema, and neural apoptosis; improved neurological outcome	HSP70/MMP-9 and the HSP70/Akt pathway
Altay et al. [101]	Endovascular perforation	CD-1 mice	2% Isoflurane	Improved neurological score, brain edema, and BBB permeability	Activate SphK1 and S1P1 to induce S1P-mediated protection of post-SAH BBB
Yuan et al. [102]	Endovascular perforation	C57BL/6 mice	Curcumin (100 mg/kg)	Improved neurological score, brain edema, BBB permeability, and TJ protein	Act via suppressing MMP-9 expression and activating microglia
Zuo et al. [103]	Endovascular perforation	Sprague–Dawley rats	Artesunate (200 mg/kg)	Treatment improved neurological score, brain edema, BBB permeability, and TJ protein	Via S1P1 signal activate PI3K/Akt pathway and stabilizing B-catenin via GSK3b inhibition
Suzuki et al. [104]	Endovascular perforation	Sprague–Dawley rats	r-osteopontin (0.1 μg)	Impede loss in body weight, neurological impairment, brain edema, and BBB disruption	Via deactivation of NF-κB activity, thereby improving the balance between proteolytic (MMP-9) and matrix stabilizing factors (TIMP-1)
Enkhjargal et al. [105]	Endovascular perforation	Sprague–Dawley rats	Vitamin D 30 ng/kg	Improve BBB permeability, brain edema, and neurological score	Through endogenous upregulation of OPN and subsequent CD44 and P-gp glycosylation signals in brain endothelial cells
Pang et al. [106]	Endovascular perforation	C57BL/6J mice	Apolipoprotein E	Reduce BBB permeability, neuron and EC apoptosis, TJ protein degradation	Inhibited proinflammatory activators of MMP-9 including CypA, NF-κB, IL-6, TNF-α, and IL-1β
Xie et al. [107]	Endovascular perforation	Sprague–Dawley rats	Netrin-1 45 μg/kg	Reduce neurological impairment, reduce brain edema, preserve BBB integrity, and increase expression of TJ protein	Via phosphorylated focal adhesion kinase activation and inhibition of RhoA activity

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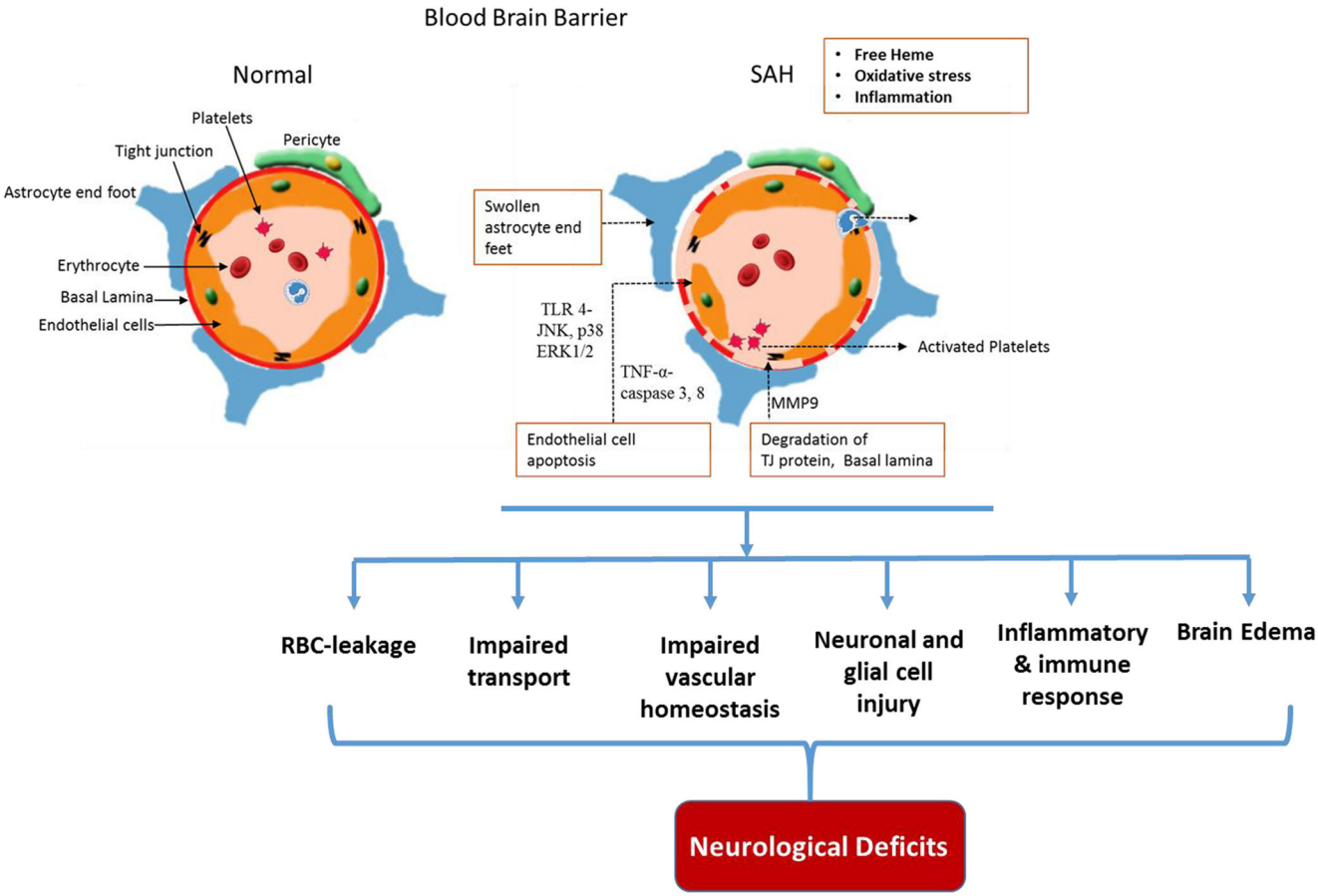

Fig. 1 BBB dysfunction after aSAH. aSAH can damage the BBB and lead to brain edema. aSAH results in RBC lysis and release of heme in the CSF and subarachnoid space. The ensuing oxidative stress and inflammation leads to dysregulation of the neurovascular unit including impaired vascular tone, swollen astrocyte end feet, loss of TJ integrity, disruption of the basement membrane, EC apoptosis, and leukocyte infiltration

Inflammation

The first study showing the connection between inflammation and aSAH was in 1964 [146]. Cerebral arteries from patients who died after aSAH were examined to find the accumulation of mononuclear leukocytes below the endothelium of the arteries near the ruptured aneurysm. Subsequently, the presence of macrophages in the tunica media and adventitia of the vessels were linked to angiographic vasospasm [147]. Later, additional studies in aSAH patients showed the existence of inflammatory cytokines and immunological proteins in the endothelium of spastic arteries [148, 149]. Clinical trials have confirmed that inflammation is linked to poor neurological outcome after aSAH [150–152].

Inflammation in blood vessels is described as the "leukocyte–endothelial cell interaction" and is a root cause of CV in aSAH [152, 153]. Products from erythrocyte lysis including methemoglobin, heme, and hemin can activate microglial toll-like receptor 4 (TLR4), which initiates the inflammatory cascade that can damage surrounding tissues including neurons and ECs [113, 154]. In murine models, microglial activation is reported on the first day of aSAH, which histologically correlates well with the presence of vasospasm and behavioral deficits [155, 156]. Following TLR4 binding, microglia release TNF-α, which in turn triggers the upregulation of specific cell adhesion molecules (CAMs) on the luminal surface of ECs. Endothelial CAM expression consequently allows macrophages and neutrophils to bind to the ECs and migrate into the subarachnoid space, where they phagocytose extravasated RBCs via Hp–Hgb complexes [153, 157–160]. While these immune cells help in clearing degraded blood, the immune cells can become trapped in the subarachnoid space due to alterations in CSF flow and the restoration of the endothelial tight junction barrier. Inside the subarachnoid space, the trapped macrophages and neutrophils degranulate and release a multitude of inflammatory factors into the CSF including endothelins, oxidative radicals, and toxic intermediates [161]. These inflammatory factors can contribute to EC damage, vasoconstriction, arterial narrowing, chemical meningitis, and cerebritis [162]. The cytokines generated by macrophages and neutrophils can induce activation of JAK-STAT [163], NF-κB [164], and Smad [165, 166]

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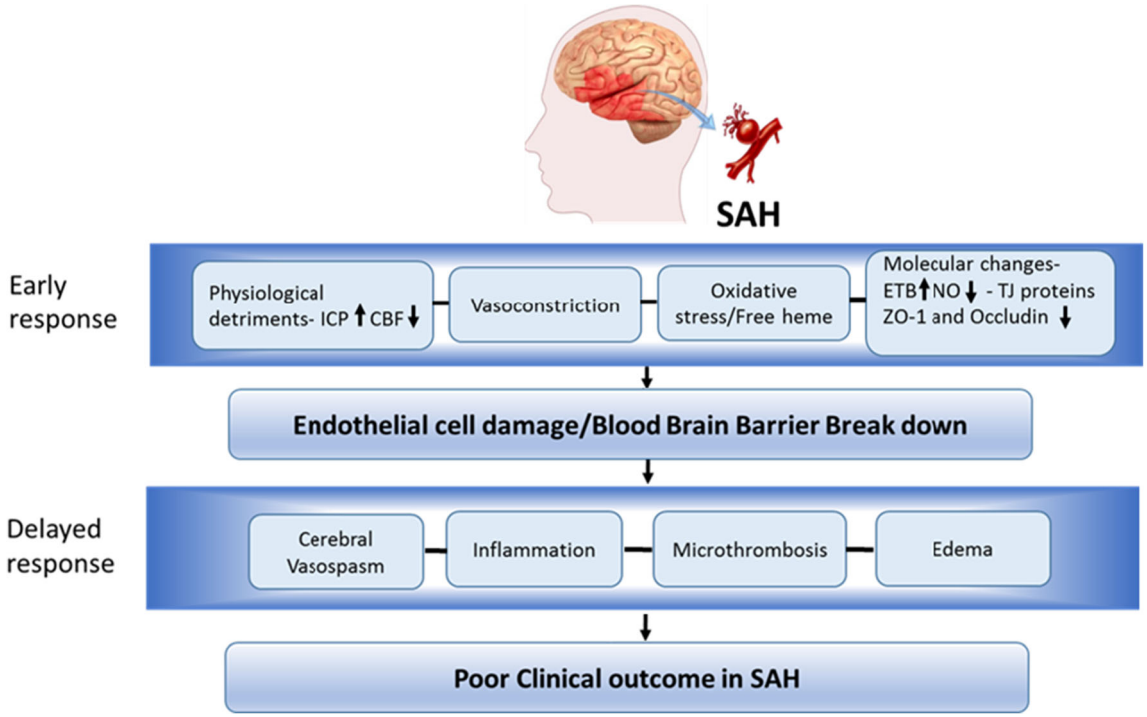

Fig. 2 Pathophysiological events in aSAH. Following aneurysm rupture, EBI occurs due to damage the blood flow irregularities, vasoconstriction, oxidative stress, and molecular changes. This early response initiates EC damage and BBB breakdown leading to delayed responses such as CV, inflammation, microthrombosis, and brain edema leading to poor outcome

signaling pathways leading to an inflammatory response involving cell adhesion, permeability, and apoptosis in ECs [167, 168]. Further, these pathways modify the production/activity of vasodilatory mediators such as NO, PGI2, EDHF, and bradykinin, as well as vasoconstrictive mediators such as ET-1 and angiotensin II ([169–171]). In addition, ROS, TNF-α, and IL-1β produced by activated microglia can disrupt the BBB integrity by altering the expression of ZO-1, claudin-5, occludin, and P-glycoprotein [172].

Microthrombosis

In 1983, the presence of microthrombi in patients with cerebral infarction after aSAH was reported [173]. This was further confirmed in patients and animal models [174, 175]. Further clinical evidence suggests that hypercoagulability and platelet activation may correlate with the development of DCI and cerebral infarction [176–180]. Investigation of the microvessel structure in the aSAH animal model demonstrates intimal convolutions and intraluminal thrombi in the constricted vessels, along with thickening of endothelial and sub-endothelial layers [181].

Healthy ECs counteract coagulation via expressing anti-platelet and anticoagulant agents. Damaged ECs can trigger fibrin formation in addition to platelet adhesion and aggregation. Apoptotic ECs become pro-coagulant by increased expression of phosphatidylserine and loss of anti-coagulant membrane components [182]. Further, exposure of sub-endothelial basement membrane collagen by contracted or desquamated ECs supports the adhesion and activation of platelets [183]. Cytokines TNF-α and IL-1 also induce the synthesis of tissue factor, the principal initiator of coagulation [184]. Apoptotic ECs can release tissue factor into the bloodstream as a component of microparticles that are shed from the cell surface and facilitate coagulation [184]. Finally, increase in the cell adhesion molecule P-selectin in the microvessels and decreased NO is suggested as a mechanism for microthrombosis after aSAH [181]. Together, these results provide substantial evidence on the role of EC injury in microthrombosis.

Future Directions

New therapeutic targets should mitigate EC and BBB damage/dysfunction since they are central players mediating aSAH pathophysiology. However, further understanding of the cell signaling mechanisms is necessary for novel therapeutics to have a chance of success in clinical trials. The way forward for aSAH drug development may lie in new genomic [185–187] and proteomic [188, 189] technologies that will contribute to understand the effects of pathological stimuli and the mechanisms that regulate vascular dysfunction. Emerging studies on EC formation during brain development reveal a heterogeneous population of ECs in the brain. ECs of the periventricular vascular network have molecular identities

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and functions distinct from those of the pial network, and this may affect therapeutic strategies [190].

The discovery of erythropoietin in the CNS has directed research into the neuroprotective effect of endogenous and recombinant erythropoietin [191]. In ECs, erythropoietin induces expression of ET-1 [192], eNOS expression and NO production, angiogenesis, and prevents apoptosis [191, 193, 194]. Recombinant erythropoietin administration significantly reduces vasospasm, prevents brain damage, and improves neurological outcome in animal aSAH [195, 196]. Clinical trials of erythropoietin in patients with aSAH show that it significantly reduced the incidence of vasospasm [197, 198] and prevents delayed hemodynamic dysfunction [199]. Future experimental research and clinical studies are warranted in this area to determine the beneficial effects of erythropoietin in aSAH patients.

Wnt signaling in EC holds considerable promise for future vascular research in this area. When Wnt pathway signal transducer β-catenin is disrupted in the adult mouse EC, it leads to BBB breakdown, downregulation of TJ proteins (claudin-1 and claudin-3), neuronal injury, multiple brain petechial hemorrhages, and CNS inflammation [200]. Further constitutive activation of Wnt–β-catenin signaling attenuates BBB disruption and hemorrhage defects of G protein coupled receptor-124-conditional knock out from EC mice by rescuing the TJ proteins, pericyte coverage, and extracellular-matrix deficits [201]. In a multiple sclerosis mouse model, Wnt/β-catenin pathway is upregulated in CNS ECs, and Wnt pathway inhibition exacerbates BBB dysfunction including increased $CD4^+$ T-cell infiltration and endothelial transcytosis [202]. These studies indicate that Wnt pathway manipulation holds a promising target to limit BBB damage and maintain the vessel integrity in aSAH.

Conclusion

Patients who experience DCI after aSAH have an increased risk of poor outcome. It has been presumed that CV is the principal mediator of DCI; however, research now shows that multiple pathways are involved. Because ECs interact with all cascades of brain injury following aSAH, they are a potent target for therapeutic intervention and rescue. In the acute phase, stabilizing EC function may mitigate cerebral edema by minimizing BBB dysfunction. Protection in the delayed phase has the potential to reduce EC apoptosis along with microthrombosis and CV. Research into mechanisms that specifically affect ECs as well as the outcome of those mechanisms remains limited. As a result, EC pathophysiology after aSAH serves as fertile area for knowledge growth in the field. We encourage the development of therapeutic approaches that directly focus on vascular EC dysfunction, as this seems to be a central mediator of both early and delayed pathology.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interests for this manuscript.

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Review criteria

This review was based on searches of the PubMed database using each of the terms "Subarachnoid Hemorrhage" and "SAH," in combination with the terms "endothelial cell," "early brain injury" or "EBI," "apoptosis," "blood–brain barrier" or "BBB," "cerebral vasospasm" or "CV," "Inflammation," and "microthrombosis." No time limit was set with regard to publication date. Only English-language articles were retrieved. Appropriate articles were selected based on abstract review. Full articles were subsequently acquired and their references were searched for further appropriate material.