



Endothelial Cell Dysfunction and Injury in Subarachnoid Hemorrhage

T. Peeyush Kumar¹ · Devin W. McBride¹ · Pramod K. Dash² · Kanako Matsumura¹ · Alba Rubi¹ · Spiros L. Blackburn¹

Received: 21 February 2018 / Accepted: 27 June 2018 / Published online: 7 July 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

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.

✉ T. Peeyush Kumar
Peeyush.K.Thankamanipandit@uth.tmc.edu

¹ The Vivian L. Smith Department of Neurosurgery, University of Texas Health Science Center, 6431 Fannin St., Houston, TX 77030, USA

² Department of Neurobiology and Anatomy, McGovern Medical School, Houston, TX 77225, USA

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-HT_{1B}), 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

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 , γ , 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].

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-1 β , 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 cause 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 SIP1 to induce SIP-mediated protection of post-SAHHBB
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 SIP1 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 μ g)	Impede loss in body weight, neurological impairment, brain edema, and BBB disruption	Via deactivation of NF- κ B 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- κ B, IL-6, TNF- α , and IL-1 β
Xie et al. [107]	Endovascular perforation	Sprague–Dawley rats	Netrin-1 45 μ g/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

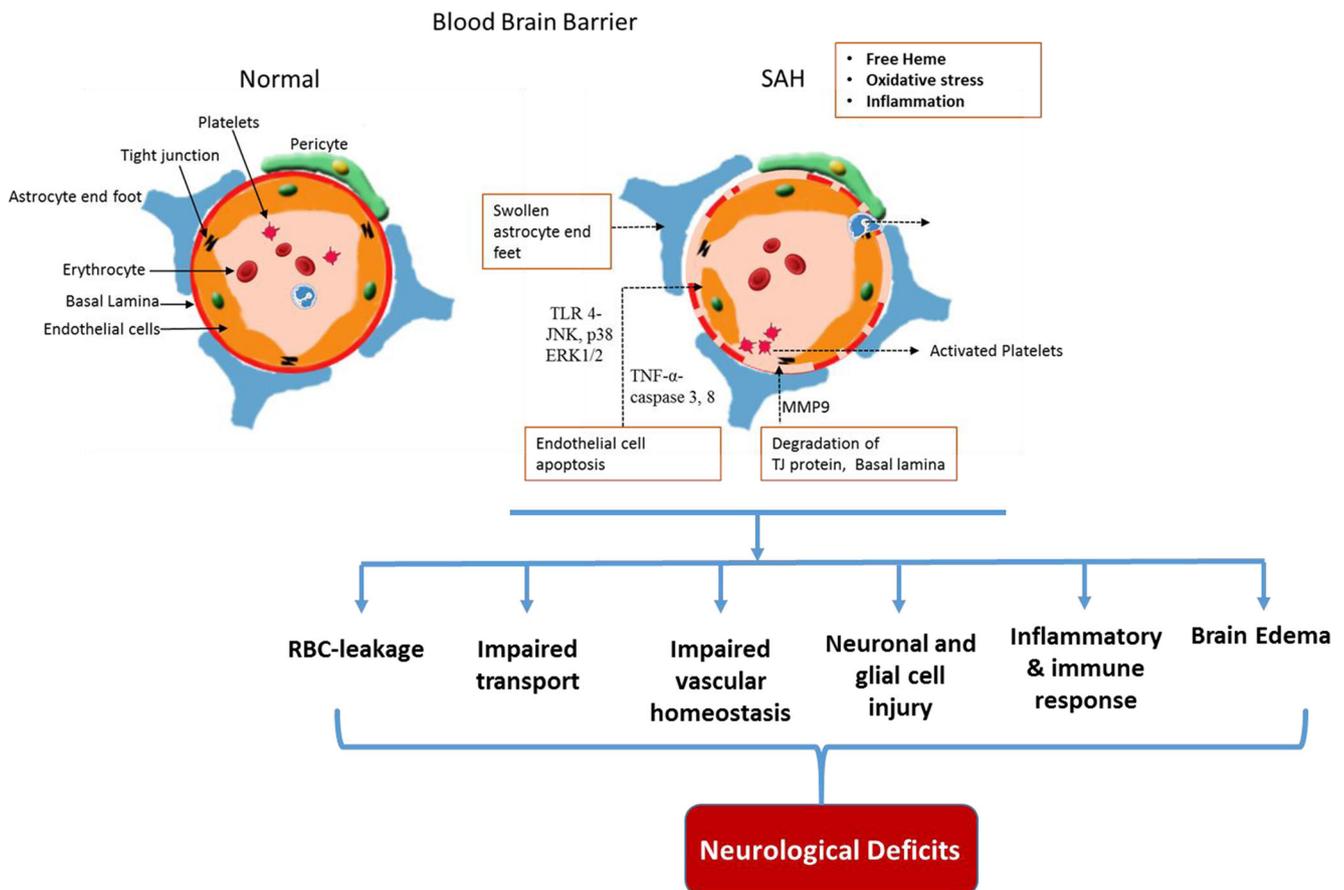


Fig. 1 BBB dysfunction after aSAH. aSAH can damage the BBB and lead to brain edema. aSAH results in RBC lysis and release of heme in the CSF and subarachnoid space. The ensuing oxidative stress and inflammation leads to dysregulation of the neurovascular unit including

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- κ B [164], and Smad [165, 166]

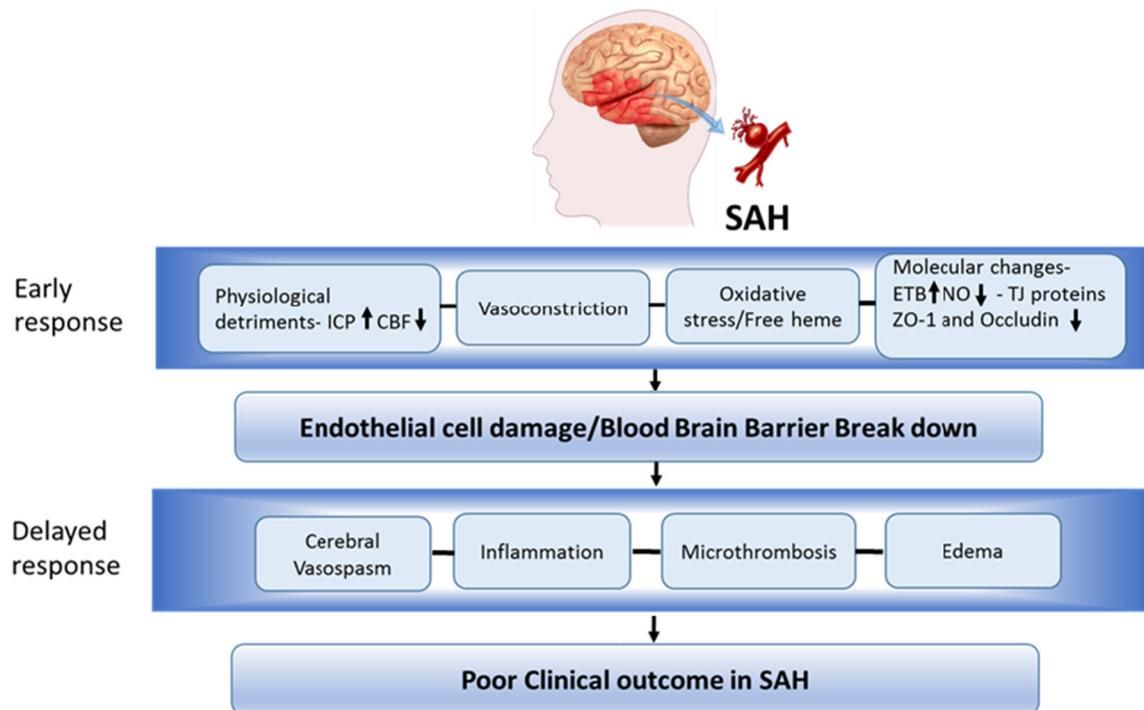


Fig. 2 Pathophysiological events in aSAH. Following aneurysm rupture, EBI occurs due to damage the blood flow irregularities, vasoconstriction, oxidative stress, and molecular changes. This early response initiates EC

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-1 β 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 antiplatelet 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

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.

Acknowledgments This work was supported by the University of Texas Health Science Center and AHA career development grant 18CDA34110036.

Compliance with Ethical Standards

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

Reference

1. Fleegler EW, Lee LK, Monuteaux MC, Hemenway D, Mannix R (2013) Firearm legislation and firearm-related fatalities in the United States. *JAMA Intern Med* 173:732–740. <https://doi.org/10.1001/jamainternmed.2013.1286>
2. Rincon F, Rossenwasser RH, Dumont A (2013) The epidemiology of admissions of nontraumatic subarachnoid hemorrhage in the United States. *Neurosurgery* 73:217–222. <https://doi.org/10.1227/01.neu.0000430290.93304.33>
3. Allen GS, Ahn HS, Preziosi TJ, Battye R, Boone SC, Chou SN, Kelly DL, Weir BK et al (1983a) Cerebral arterial spasm—a controlled trial of nimodipine in patients with subarachnoid hemorrhage. *N Engl J Med* 308:619–624. <https://doi.org/10.1056/NEJM198303173081103>
4. Diringner MN, Bleck TP, Hemphill JC, Menon D, Shutter L, Vespa P, Bruder N, Connolly ES et al (2011) Critical care management of patients following aneurysmal subarachnoid hemorrhage: recommendations from the Neurocritical Care Society’s multidisciplinary consensus conference. *Neurocrit Care*. <https://doi.org/10.1007/s12028-011-9605-9>
5. Macdonald RL, Higashida RT, Keller E, Mayer SA, Molyneux A, Raabe A, Vajkoczy P, Wanke I et al (2011) Clazosentan, an endothelin receptor antagonist, in patients with aneurysmal subarachnoid haemorrhage undergoing surgical clipping: a randomised, double-blind, placebo-controlled phase 3 trial (CONSCIOUS-2). *Lancet Neurol* 10:618–625. [https://doi.org/10.1016/S1474-4422\(11\)70108-9](https://doi.org/10.1016/S1474-4422(11)70108-9)
6. Blackburn SL, Kumar PT, McBride D, Zeineddine HA, Leclerc J, Choi HA, Dash PK, Grotta J et al (2018) Unique contribution of haptoglobin and haptoglobin genotype in aneurysmal subarachnoid hemorrhage. *Front Physiol* 9:592. <https://doi.org/10.3389/fphys.2018.00592>
7. Rowland MJ, Hadjipavlou G, Kelly M, Westbrook J, Pattinson KTS (2012) Delayed cerebral ischaemia after subarachnoid haemorrhage: looking beyond vasospasm. *Br J Anaesth*. <https://doi.org/10.1093/bja/aes264>
8. Vergouwen MDI, Vermeulen M, Coert BA, Stroes ESG, Roos YBWM (2008) Microthrombosis after aneurysmal subarachnoid hemorrhage: an additional explanation for delayed cerebral ischemia. *J Cereb Blood Flow Metab*. <https://doi.org/10.1038/jcbfm.2008.74>
9. Woitzik J, Dreier JP, Hecht N, Fiss I, Sandow N, Major S, Winkler M, Dahlem YA et al (2012) Delayed cerebral ischemia and spreading depolarization in absence of angiographic vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 32:203–212. <https://doi.org/10.1038/jcbfm.2011.169>
10. Tettenborn D, Dyck J (1990) Prevention and treatment of delayed ischemic dysfunction in patients with aneurysmal subarachnoid hemorrhage. *Stroke*
11. Haley EC, Kassell NF, Alves WM, Weir BK, Hansen CA (1995) Phase II trial of tirilazad in aneurysmal subarachnoid hemorrhage. A report of the Cooperative Aneurysm Study. *J Neurosurg* 82:786–790. <https://doi.org/10.3171/jns.1995.82.5.0786>

12. Siironen J, Juvela S, Varis J, Porras M, Poussa K, Ilveskero S, Hernesniemi J, Lassila R (2003) No effect of enoxaparin on outcome of aneurysmal subarachnoid hemorrhage: a randomized, double-blind, placebo-controlled clinical trial. *J Neurosurg* 99: 953–959. <https://doi.org/10.3171/jns.2003.99.6.0953>
13. van den Bergh WM (2006) Randomized controlled trial of acetylsalicylic acid in aneurysmal subarachnoid hemorrhage: the MASH study. *Stroke* 37:2326–2330. <https://doi.org/10.1161/01.STR.0000236841.16055.0f>
14. Gomis P, Graftieaux JP, Sercombe R, Hettler D, Scherpereel B, Rousseaux P (2010) Randomized, double-blind, placebo-controlled, pilot trial of high-dose methylprednisolone in aneurysmal subarachnoid hemorrhage. *J Neurosurg* 112:681–688. <https://doi.org/10.3171/2009.4.JNS081377>
15. Kirkpatrick PJ, Turner CL, Smith C, Hutchinson PJ, Murray GD (2014) Simvastatin in aneurysmal subarachnoid haemorrhage (STASH): a multicentre randomised phase 3 trial. *Lancet Neurol* 13:666–675. [https://doi.org/10.1016/S1474-4422\(14\)70084-5](https://doi.org/10.1016/S1474-4422(14)70084-5)
16. Dreier JP, Sakowitz OW, Harder A, Zimmer C, Dirnagl U, Valdueza JM, Unterberg AW (2002) Focal laminar cortical MR signal abnormalities after subarachnoid hemorrhage. *Ann Neurol* 52:825–829. <https://doi.org/10.1002/ana.10383>
17. Petruk KC, West M, Mohr G, Weir BK, Benoit BG, Gentili F, Disney LB, Khan MI et al (1988) Nimodipine treatment in poor-grade aneurysm patients: results of a multicenter double-blind placebo-controlled trial. *J Neurosurg* 68:505–517. <https://doi.org/10.3171/jns.1988.68.4.0505>
18. Carmeliet P (2003) Blood vessels and nerves: common signals, pathways and diseases. *Nat Rev Genet*. <https://doi.org/10.1038/nrg1158>
19. Wang Y, Wang N, Cai B, Wang GY, Li J, Piao XX (2015) In vitro model of the blood–brain barrier established by co-culture of primary cerebral microvascular endothelial and astrocyte cells. *Neural Regen Res* 10:2011–2017. <https://doi.org/10.4103/1673-5374.172320>
20. Abbott NJ, Rönneback L, Hansson E (2006) Astrocyte–endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci*. <https://doi.org/10.1038/nm1824>
21. Engelhardt B (2003) Development of the blood–brain barrier. *Cell Tissue Res*. <https://doi.org/10.1007/s00441-003-0751-z>
22. Engelhardt B, Liebner S (2014) Novel insights into the development and maintenance of the blood–brain barrier. *Cell Tissue Res*. <https://doi.org/10.1007/s00441-014-1811-2>
23. Obermeier B, Daneman R, Ransohoff RM (2013a) Development, maintenance and disruption of the blood–brain barrier. *Nat Med*. <https://doi.org/10.1038/nm.3407>
24. Hawkins BT (2005) The blood–brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* 57:173–185. <https://doi.org/10.1124/pr.57.2.4>
25. Sandoo A, Veldhuijzen van Zanten JJCS, Metsios GS, Carroll D, Kitas GD (2010a) The endothelium and its role in regulating vascular tone. *Open Cardiovasc Med J* 4:302–312. <https://doi.org/10.2174/1874192401004010302>
26. Friedrich V, Flores R, Muller A, Sehba FA (2010a) Escape of intraluminal platelets into brain parenchyma after subarachnoid hemorrhage. *Neuroscience* 165:968–975. <https://doi.org/10.1016/j.neuroscience.2009.10.038>
27. Iuliano BA, Pluta RM, Jung C, Oldfield EH (2004) Endothelial dysfunction in a primate model of cerebral vasospasm. *J Neurosurg* 100:287–294. <https://doi.org/10.3171/jns.2004.100.2.0287>
28. Pluta RM (2008a) Dysfunction of nitric oxide synthases as a cause and therapeutic target in delayed cerebral vasospasm after SAH. *Acta Neurochirurgica Supplementum*. pp. 139–147. doi:<https://doi.org/10.1007/978-3-211-75718-5-28>
29. Pluta RM (2008b) Dysfunction of nitric oxide synthases as a cause and therapeutic target in delayed cerebral vasospasm after SAH. *Acta Neurochir* 104:139–147
30. Findlay JM, Weir BK, Kanamaru K, Espinosa F (1989) Arterial wall changes in cerebral vasospasm. *Neurosurgery* 25:736–745 discussion 745–6
31. Sasaki T, Kassell NF, Zuccarello M, Nakagomi T, Fujiwara S, Colohan AR, Lehman M (1986) Barrier disruption in the major cerebral arteries during the acute stage after experimental subarachnoid hemorrhage. *Neurosurgery* 19:177–184. <https://doi.org/10.1227/00006123-198608000-00002>
32. Zuccarello M, Kassell NF, Sasaki T, Fujiwara S, Nakagomi T, Lehman RM (1987) Barrier disruption in the major cerebral arteries after experimental subarachnoid hemorrhage in spontaneously hypertensive and normotensive rats. *Neurosurgery* 21:515–522. <https://doi.org/10.1227/00006123-198710000-00013>
33. Bederson JB, Levy AL, Ding WH, Kahn R, DiPerna CA, Jenkins AL, Vallabhajosyula P (1998) Acute vasoconstriction after subarachnoid hemorrhage. *Neurosurgery* 42:352–362. <https://doi.org/10.1097/00006123-199802000-00091>
34. Kusaka G, Ishikawa M, Nanda A, Granger DN, Zhang JH (2004) Signaling pathways for early brain injury after subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 24:916–925. <https://doi.org/10.1097/01.WCB.0000125886.48838.7E>
35. Fujii M, Duris K, Altay O, Soejima Y, Sherchan P, Zhang JH (2012) Inhibition of rho kinase by hydroxyfasudil attenuates brain edema after subarachnoid hemorrhage in rats. *Neurochem Int* 60: 327–333. <https://doi.org/10.1016/j.neuint.2011.12.014>
36. Plesnila N (2013) Pathophysiological role of global cerebral ischemia following subarachnoid hemorrhage: the current experimental evidence. *Stroke Res Treat*. <https://doi.org/10.1155/2013/651958>
37. Fujii M, Yan J, Rolland WB, Soejima Y, Caner B, Zhang JH (n.d.) Early brain injury, an evolving frontier in subarachnoid hemorrhage research. <https://doi.org/10.1007/s12975-013-0257-2>
38. Guo ZD, Sun XC, Zhang JH, (2011) Mechanisms of early brain injury after SAH: matrix metalloproteinase 9. *Acta Neurochirurgica Supplementum* pp 63–65. doi:<https://doi.org/10.1007/978-3-7091-0353-1-11>
39. Sehba F a, Flores R, Muller A, Friedrich V, Chen J-F, Britz GW, Winn HR, Bederson JB (2010) Adenosine A(2A) receptors in early ischemic vascular injury after subarachnoid hemorrhage. Laboratory investigation. *J Neurosurg* 113:826–834. <https://doi.org/10.3171/2009.9.JNS09802>
40. Sehba F a, Friedrich V, Makonnen G, Bederson JB (2007) Acute cerebral vascular injury after subarachnoid hemorrhage and its prevention by administration of a nitric oxide donor. *J Neurosurg* 106:321–329. <https://doi.org/10.3171/jns.2007.106.2.321>
41. Alkan T, Tureyen K, Ulutas M, Kahveci N, Goren B, Korfali E, Ozluk K (2001) Acute and delayed vasoconstriction after subarachnoid hemorrhage: local cerebral blood flow, histopathology, and morphology in the rat basilar artery. *Arch Physiol Biochem* 109:145–153. <https://doi.org/10.1076/apab.109.2.145.4267>
42. Clower BR, Yamamoto Y, Cain L, Haines DE, Smith RR (1994) Endothelial injury following experimental subarachnoid hemorrhage in rats: effects on brain blood flow. *Anat Rec* 240:104–114. <https://doi.org/10.1002/ar.1092400110>
43. Ono S, Date I, Nakajima M, Onoda K, Ogihara K, Shiota T, Asari S, Ninomiya Y et al (1997) Three-dimensional analysis of vasospastic major cerebral arteries in rats with the corrosion cast technique. *Stroke* 28:1631–1637. <https://doi.org/10.1161/01.STR.28.8.1631>
44. Ono S, Date I, Onoda K, Ohmoto T (2003) Time course of the diameter of the major cerebral arteries after subarachnoid hemorrhage using corrosion cast technique. *Neurol Res* 25:383–389. <https://doi.org/10.1179/016164103101201535>

45. Sehba F a, Ding WH, Chereshnev I, Bederson JB (1999) Effects of S-nitrosoglutathione on acute vasoconstriction and glutamate release after subarachnoid hemorrhage. *Stroke* 30:1955–1961. <https://doi.org/10.1161/01.STR.30.9.1955>
46. Sehba F a, Mostafa G, Friedrich V, Bederson JB (2005) Acute microvascular platelet aggregation after subarachnoid hemorrhage. *J Neurosurg* 102:1094–1100. <https://doi.org/10.3171/jns.2005.102.6.1094>
47. Sun BL, Zheng CB, Yang MF, Yuan H, Zhang SM, Wang LX (2009) Dynamic alterations of cerebral pial microcirculation during experimental subarachnoid hemorrhage. *Cell Mol Neurobiol* 29:235–241. <https://doi.org/10.1007/s10571-008-9316-8>
48. Hansen-Schwartz J, Hoel NL, Xu C-B, Svendgaard N-A, Edvinsson L (2003a) Subarachnoid hemorrhage-induced upregulation of the 5-HT1B receptor in cerebral arteries in rats. *J Neurosurg* 99:115–120. <https://doi.org/10.3171/jns.2003.99.1.0115>
49. Hansen-Schwartz J, Hoel NL, Zhou M, Xu C-B, Svendgaard NA, Edvinsson L (2003b) Subarachnoid hemorrhage enhances endothelin receptor expression and function in rat cerebral arteries. *Neurosurgery* 52(1188–1194):1194–1195. <https://doi.org/10.1227/01.NEU.0000058467.82442.64>
50. Hongo K, Kassell NF, Nakagomi T, Sasaki T, Tsukahara T, Ogawa H, Vollmer DG, Lehman RM (1988) Subarachnoid hemorrhage inhibition of endothelium-derived relaxing factor in rabbit basilar artery. *J Neurosurg* 69:247–253. <https://doi.org/10.3171/jns.1988.69.2.0247>
51. Nakagomi T, Kassell NF, Sasaki T, Fujiwara S, Lehman RM, Johshita H, Nazar GB, Torner JC (1987) Effect of subarachnoid hemorrhage on endothelium-dependent vasodilation. *J Neurosurg* 66:915–923. <https://doi.org/10.3171/jns.1987.66.6.0915>
52. Bevan JA, Bevan RD, Walters CL, Wellman T (1998) Functional changes in human pial arteries (300 to 900 micrometer ID) within 48 hours of aneurysmal subarachnoid hemorrhage. *Stroke* 29:2575–2579
53. Hatake K, Wakabayashi I, Kakishita E, Hishida S (1992) Impairment of endothelium-dependent relaxation in human basilar artery after subarachnoid hemorrhage. *Stroke* 23:1111–1116 discussion 1116-7
54. Choy JC, Granville DJ, Hunt DWC, McManus BM (2001) Endothelial cell apoptosis: biochemical characteristics and potential implications for atherosclerosis. *J Mol Cell Cardiol* 33:1673–1690. <https://doi.org/10.1006/jmcc.2001.1419>
55. Friedrich V, Flores R, Sehba FA (2012) Cell death starts early after subarachnoid hemorrhage. *Neurosci Lett* 512:6–11. <https://doi.org/10.1016/j.neulet.2012.01.036>
56. Comair YG, Schipper HM, Brem S (1993) The prevention of oxyhemoglobin-induced endothelial and smooth muscle cytoskeletal injury by deferoxamine. *Neurosurgery* 32:58–64 discussion 64–5
57. Foley PL, Takenaka K, Kassell NF, Lee KS (1994b) Cytotoxic effects of bloody cerebrospinal fluid on cerebral endothelial cells in culture. *J Neurosurg* 81:87–92. <https://doi.org/10.3171/jns.1994.81.1.0087>
58. Takenaka K, Kassell NF, Foley PL, Lee KS (1993) Oxyhemoglobin-induced cytotoxicity and arachidonic acid release in cultured bovine endothelial cells. *Stroke* 24:839–845 **discussion 845–6**
59. Guo Z, Xu L, Wang X, Sun X (2015) MMP-9 expression and activity is concurrent with endothelial cell apoptosis in the basilar artery after subarachnoid hemorrhaging in rats. *Neurol Sci* 36:1241–1245. <https://doi.org/10.1007/s10072-015-2092-6>
60. Suzuki H, Sozen T, Hasegawa Y, Chen W, Kanamaru K, Taki W, Zhang JH (2011) Subarachnoid hemorrhage causes pulmonary endothelial cell apoptosis and neurogenic pulmonary edema in mice. *Acta Neurochir* 111:129–132. https://doi.org/10.1007/978-3-7091-0693-8_21
61. Zubkov AY, Ogihara K, Bernanke DH, Parent AD, Zhang J (2000) Apoptosis of endothelial cells in vessels affected by cerebral vasospasm. *Surg Neurol* 53:260–266. [https://doi.org/10.1016/S0090-3019\(99\)00187-1](https://doi.org/10.1016/S0090-3019(99)00187-1)
62. Meguro T, Chen B, Lancon J, Zhang JH (2001) Oxyhemoglobin induces caspase-mediated cell death in cerebral endothelial cells. *J Neurochem* 77:1128–1135. <https://doi.org/10.1046/j.1471-4159.2001.00313.x>
63. Zhang H, Weir BK, Macdonald RL, Marton LS, Solenski NJ, Kwan AL, Lee KS (1996) Mechanisms of [Ca⁺⁺]i elevation induced by erythrocyte components in endothelial cells. *J Pharmacol Exp Ther* 277:1501–1509
64. Cook DA, Vollrath B (1995) Free radicals and intracellular events associated with cerebrovascular spasm. *Cardiovasc Res*. [https://doi.org/10.1016/S0008-6363\(95\)00087-9](https://doi.org/10.1016/S0008-6363(95)00087-9)
65. Ayer RE, Zhang JH (2008a) Oxidative stress in subarachnoid haemorrhage: significance in acute brain injury and vasospasm. *Acta Neurochir* 104:33–41
66. Lum H, Roebuck KA (2001) Oxidant stress and endothelial cell dysfunction. *Am J Physiol Cell Physiol* 280:C719–C741. <https://doi.org/10.1152/ajpcell.2001.280.4.C719>
67. Won SM, Lee JH, Park UJ, Gwag J, Gwag BJ, Lee YB (2011) Iron mediates endothelial cell damage and blood–brain barrier opening in the hippocampus after transient forebrain ischemia in rats. *Exp Mol Med* 43:121–128. <https://doi.org/10.3858/emm.2011.43.2.020>
68. Hartsock A, Nelson WJ (2008) Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta Biomembr*. <https://doi.org/10.1016/j.bbmem.2007.07.012>
69. Shen Q, Rigor RR, Pivetti CD, Wu MH, Yuan SY (2010) Myosin light chain kinase in microvascular endothelial barrier function. *Cardiovasc Res*. <https://doi.org/10.1093/cvr/cvq144>
70. Germanò a, d’Avella D, Imperatore C, Caruso G, Tomasello F (2000) Time-course of blood–brain barrier permeability changes after experimental subarachnoid haemorrhage. *Acta Neurochir* 142:575–580; discussion 580–1. <https://doi.org/10.1007/s007010050472>
71. Dóczy T (1985) The pathogenetic and prognostic significance of blood–brain barrier damage at the acute stage of aneurysmal subarachnoid haemorrhage. Clinical and experimental studies. *Acta Neurochir (Wien)* 77:110–132. <https://doi.org/10.1007/BF01476215>
72. Doczi T, Joo F, Adam G, Bozóky B, Szerdahelyi P (1986) Blood–brain barrier damage during the acute stage of subarachnoid hemorrhage, as exemplified by a new animal model. *Neurosurgery* 18:733–739. <https://doi.org/10.1227/0006123-198606000-00010>
73. Gules I, Satoh M, Nanda A, Zhang JH (2003) Apoptosis, blood–brain barrier, and subarachnoid hemorrhage. *Acta Neurochir* 86:483–487
74. Kahles T, Luedike P, Endres M, Galla H-J, Steinmetz H, Busse R, Neumann-Haefelin T, Brandes RP (2007) NADPH oxidase plays a central role in blood–brain barrier damage in experimental stroke. *Stroke* 38:3000–3006. <https://doi.org/10.1161/STROKEAHA.107.489765>
75. Li Y, Yang H, Ni W, Gu Y (2017) Effects of deferoxamine on blood–brain barrier disruption after subarachnoid hemorrhage. *PLoS One* 12:e0172784. <https://doi.org/10.1371/journal.pone.0172784>
76. Li Z, Liang G, Ma T, Li J, Wang P, Liu L, Yu B, Liu Y et al (2015) Blood–brain barrier permeability change and regulation mechanism after subarachnoid hemorrhage. *Metab Brain Dis* 30:597–603. <https://doi.org/10.1007/s11011-014-9609-1>

77. Kondo T, Hafezi-Moghadam A, Thomas K, Wagner DD, Kahn CR (2004) Mice lacking insulin or insulin-like growth factor 1 receptors in vascular endothelial cells maintain normal blood–brain barrier. *Biochem Biophys Res Commun* 317:315–320. <https://doi.org/10.1016/j.bbrc.2004.03.043>
78. Ansar S, Larsen C, Maddahi A, Edvinsson L (2010) Subarachnoid hemorrhage induces enhanced expression of thromboxane A2 receptors in rat cerebral arteries. *Brain Res* 1316:163–172. <https://doi.org/10.1016/j.brainres.2009.12.031>
79. Victor FC, Gottlieb AB (2002) TNF-alpha and apoptosis: implications for the pathogenesis and treatment of psoriasis. *J Drugs Dermatol* 1:264–275
80. Zhou C, Yamaguchi M, Colohan ART, Zhang JH (2005) Role of p53 and apoptosis in cerebral vasospasm after experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 25:572–582. <https://doi.org/10.1038/sj.jcbfm.9600069>
81. Zhou C, Yamaguchi M, Kusaka G, Schonholz C, Nanda A, Zhang JH (2004) Caspase inhibitors prevent endothelial apoptosis and cerebral vasospasm in dog model of experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 24:419–431
82. Lakhani SE, Kirchgessner A, Tepper D, Leonard A (2013) Matrix metalloproteinases and blood–brain barrier disruption in acute ischemic stroke. *Front Neurol* 4:32. <https://doi.org/10.3389/fneur.2013.00032>
83. Leib SL, Leppert D, Clements J, Täuber MG (2000) Matrix metalloproteinases contribute to brain damage in experimental pneumococcal meningitis. *Infect Immun* 68:615–620
84. Rosenberg GA, Estrada EY, Dencoff JE, Stetler-Stevenson WG (1995) Tumor necrosis factor- α -induced gelatinase B causes delayed opening of the blood–brain barrier: an expanded therapeutic window. *Brain Res* 703:151–155. [https://doi.org/10.1016/0006-8993\(95\)01089-0](https://doi.org/10.1016/0006-8993(95)01089-0)
85. Seo JH, Guo S, Lok J, Navaratna D, Whalen MJ, Kim K-W, Lo EH (2012) Neurovascular matrix metalloproteinases and the blood–brain barrier. *Curr Pharm Des* 18:3645–3648 doi:CPD-EPUB-20120511-002 [pii]
86. Egashira Y, Zhao H, Hua Y, Keep RF, Xi G (2015) White matter injury after subarachnoid hemorrhage: role of blood–brain barrier disruption and matrix metalloproteinase-9. *Stroke* 46:2909–2915. <https://doi.org/10.1161/STROKEAHA.115.010351>
87. Turner RJ, Sharp FR (2016) Implications of MMP9 for blood brain barrier disruption and hemorrhagic transformation following ischemic stroke. *Front Cell Neurosci* 10:56. <https://doi.org/10.3389/fncel.2016.00056>
88. Guo Z, Sun X, He Z, Jiang Y, Zhang X, Zhang JH (2010) Matrix metalloproteinase-9 potentiates early brain injury after subarachnoid hemorrhage. *Neurol Res* 32:715–720. <https://doi.org/10.1179/016164109X12478302362491>
89. Lo EH, Wang X, Louise Cuzner M (2002) Extracellular proteolysis in brain injury and inflammation: role for plasminogen activators and matrix metalloproteinases. *J Neurosci Res*. <https://doi.org/10.1002/jnr.10270>
90. Lu P, Takai K, Weaver VM, Werb Z (2011) Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb Perspect Biol* 3:1–24. <https://doi.org/10.1101/cshperspect.a005058>
91. Shiba M, Fujimoto M, Imanaka-Yoshida K, Yoshida T, Taki W, Suzuki H (2014) Tenascin-C causes neuronal apoptosis after subarachnoid hemorrhage in rats. *Transl Stroke Res* 5:238–247. <https://doi.org/10.1007/s12975-014-0333-2>
92. Suzuki H, Kanamaru K, Suzuki Y, Aimi Y, Matsubara N, Araki T, Takayasu M, Kinoshita N et al (2010b) Tenascin-C is induced in cerebral vasospasm after subarachnoid hemorrhage in rats and humans: a pilot study. *Neurol Res* 32:179–184. <https://doi.org/10.1179/174313208X355495>
93. Fujimoto M, Shiba M, Kawakita F, Liu L, Shimojo N, Imanaka-Yoshida K, Yoshida T, Suzuki H (2017) Effects of tenascin-C knockout on cerebral vasospasm after experimental subarachnoid hemorrhage in mice. *Mol Neurobiol* 1–8. <https://doi.org/10.1007/s12035-017-0466-x>
94. Shiba M, Fujimoto M, Kawakita F, Imanaka-Yoshida K, Yoshida T, Kanamaru K, Taki W, Suzuki H (2015) Effects of tenascin-C on early brain injury after subarachnoid hemorrhage in rats. In: *Neurovascular events after subarachnoid hemorrhage*. Springer International Publishing, Cham, pp. 69–73. https://doi.org/10.1007/978-3-319-04981-6_12
95. Butt OI, Buehler PW, D’Agnillo F (2011) Blood–brain barrier disruption and oxidative stress in Guinea pig after systemic exposure to modified cell-free hemoglobin. *Am J Pathol* 178:1316–1328. <https://doi.org/10.1016/j.ajpath.2010.12.006>
96. Chen J, Chen G, Li J, Qian C, Mo H, Gu C, Yan F, Yan W et al (2014) Melatonin attenuates inflammatory response-induced brain edema in early brain injury following a subarachnoid hemorrhage: a possible role for the regulation of pro-inflammatory cytokines. *J Pineal Res* 57:340–347. <https://doi.org/10.1111/jpi.12173>
97. Fan L f, He P y, Peng Y c, Du Q h, Ma Y j, Jin J x, Xu H z, Li J r et al (2017) Mdivi-1 ameliorates early brain injury after subarachnoid hemorrhage via the suppression of inflammation-related blood–brain barrier disruption and endoplasmic reticulum stress-based apoptosis. *Free Radic Biol Med* 112:336–349. <https://doi.org/10.1016/j.freeradbiomed.2017.08.003>
98. Ersahin M, Toklu HZ, Çetinel Ş, Yüksel M, Yèen BÇ, Şener G (2009) Melatonin reduces experimental subarachnoid hemorrhage-induced oxidative brain damage and neurological symptoms. *J Pineal Res* 46:324–332. <https://doi.org/10.1111/j.1600-079X.2009.00664.x>
99. Chen Y, Zhang Y, Tang J, Liu F, Hu Q, Luo C, Tang J, Feng H et al (2015) Norrin protected blood–brain barrier via frizzled-4/ β -catenin pathway after subarachnoid hemorrhage in rats. *Stroke* 46:529–536. <https://doi.org/10.1161/STROKEAHA.114.007265>
100. Ying G-y, Jing C-h, Li J-r, Wu C, Feng Y, Jing-yin Chen MD, L. W, Brandon J et al (2016) Neuroprotective effects of valproic acid on blood–brain barrier disruption and apoptosis-related early brain injury in rats subjected to subarachnoid hemorrhage are modulated by heat shock protein 70/matrix metalloproteinases and heat shock protein 70/AKT. *Neurosurgery* 79:286–295. <https://doi.org/10.1227/NEU.0000000000001264>
101. Altay O, Suzuki H, Hasegawa Y, Caner B, Krafft PR, Fujii M, Tang J, Zhang JH (2012) Isoflurane attenuates blood–brain barrier disruption in ipsilateral hemisphere after subarachnoid hemorrhage in mice. *Stroke* 43:2513–2516. <https://doi.org/10.1161/STROKEAHA.112.661728>
102. Yuan J, Liu W, Zhu H, Zhang X, Feng Y, Chen Y, Feng H, Lin J (2017) Curcumin attenuates blood–brain barrier disruption after subarachnoid hemorrhage in mice. *J Surg Res* 207:85–91. <https://doi.org/10.1016/j.jss.2016.08.090>
103. Zuo S, Ge H, Li Q, Zhang X, Hu R, Hu S, Liu X, Zhang JH et al (2017) Artesunate protected blood–brain barrier via sphingosine 1 phosphate receptor 1/phosphatidylinositol 3 kinase pathway after subarachnoid hemorrhage in rats. *Mol Neurobiol* 54:1213–1228. <https://doi.org/10.1007/s12035-016-9732-6>
104. Suzuki H, Ayer R, Sugawara T, Chen W, Sozen T, Hasegawa Y, Kanamaru K, Zhang JH (2010a) Protective effects of recombinant osteopontin on early brain injury after subarachnoid hemorrhage in rats. *Crit Care Med* 38:612–618. <https://doi.org/10.1097/CCM.0b013e3181c027ae>
105. Enkhjargal B, McBride DW, Manaenko A, Reis C, Sakai Y, Tang J, Zhang JH (2016) Intranasal administration of vitamin D attenuates blood–brain barrier disruption through endogenous upregulation of osteopontin and activation of CD44/P-gp glycosylation signaling

- after subarachnoid hemorrhage in rats. *J Cereb Blood Flow Metab* 37:2555–2566. <https://doi.org/10.1177/0271678X16671147>
106. Pang J, Chen Y, Kuai L, Yang P, Peng J, Wu Y, Chen Y, Vitek MP et al (2017) Inhibition of blood–brain barrier disruption by an apolipoprotein E-mimetic peptide ameliorates early brain injury in experimental subarachnoid hemorrhage. *Transl Stroke Res* 8: 257–272. <https://doi.org/10.1007/s12975-016-0507-1>
 107. Xie, Z., Enkhjargal, B., Reis, C., Huang, L., Wan, W., Tang, J., Cheng, Y., Zhang, J.H., 2017. Netrin-1 preserves blood–brain barrier integrity through deleted in colorectal cancer/focal adhesion kinase/RhoA signaling pathway following subarachnoid hemorrhage in rats. *J. Am. Heart Assoc.* 6. doi:<https://doi.org/10.1161/JAHA.116.005198>
 108. Bendok BR, Getch CC, Malisch TW, Batjer HH (1998) Treatment of aneurysmal subarachnoid hemorrhage. *Semin Neurol* 18:521–531. <https://doi.org/10.1055/s-2008-1040905>
 109. Dorsch NW (1995) Cerebral arterial spasm—a clinical review. *Br J Neurosurg* 9:403–412
 110. Burrell C, Avalon NE, Siegel J, Pizzi M, Dutta T, Charlesworth MC, Freeman WD (2016) Precision medicine of aneurysmal subarachnoid hemorrhage, vasospasm and delayed cerebral ischemia. *Expert Rev Neurother.* <https://doi.org/10.1080/14737175.2016.1203257>
 111. Chyatte D (1990) Anti-inflammatory agents and cerebral vasospasm. *Neurosurg Clin N Am* 1:433–450
 112. Matsui T, Takuwa Y, Johshita H, Yamashita K, Asano T (1991) Possible role of protein kinase C-dependent smooth muscle contraction in the pathogenesis of chronic cerebral vasospasm. *J Cereb Blood Flow Metab* 11:143–149. <https://doi.org/10.1038/jcbfm.1991.17>
 113. Pradilla G, Chaichana KL, Hoang S, Huang J, Tamargo RJ (2010) Inflammation and cerebral vasospasm after subarachnoid hemorrhage. *Neurosurg Clin N Am.* <https://doi.org/10.1016/j.nec.2009.10.008>
 114. Crowley RW, Medel R, Dumont AS, Ilodigwe D, Kassell NF, Mayer SA, Ruefenacht D, Schmiedek P et al (2011) Angiographic vasospasm is strongly correlated with cerebral infarction after subarachnoid hemorrhage. *Stroke* 42:919–923. <https://doi.org/10.1161/STROKEAHA.110.597005>
 115. Jung S-W, Lee C-Y, Yim M-B (2012) The relationship between subarachnoid hemorrhage volume and development of cerebral vasospasm. *J Cerebrovasc Endovasc Neurosurg* 14:186–191. <https://doi.org/10.7461/jcen.2012.14.3.186>
 116. Macdonald RL, Weir BK (1991) A review of hemoglobin and the pathogenesis of cerebral vasospasm. *Stroke* 22:971–982. <https://doi.org/10.1161/01.STR.22.8.971>
 117. Suzuki H, Muramatsu M, Kojima T, Taki W (2003) Intracranial heme metabolism and cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Stroke* 34:2796–2800. <https://doi.org/10.1161/01.STR.0000103743.62248.12>
 118. Caner H, Oruçkaptan H, Bolay H, Kilinç K, Senaati S, Benli K, Ayhan A (1991) The role of lipid peroxidation in the genesis of vasospasm secondary to subarachnoid hemorrhage. *Kobe J Med Sci* 37:13–20
 119. Chen Z, Gao C, Hua Y, Keep RF, Muraszko K, Xi G (2011) Role of iron in brain injury after intraventricular hemorrhage. *Stroke* 42: 465–470. <https://doi.org/10.1161/STROKEAHA.110.602755>
 120. Lin G, Macdonald RL, Marton LS, Kowalczyk A, Solenski NJ, Weir BK (2001) Hemoglobin increases endothelin-1 in endothelial cells by decreasing nitric oxide. *Biochem Biophys Res Commun* 280:824–830. <https://doi.org/10.1006/BBRC.2000.4167>
 121. Alabadi JA, Torregrosa G, Miranda FJ, Salom JB, Centeno JM, Alborch E (1997) Impairment of the modulatory role of nitric oxide on the endothelin-1-elicited contraction of cerebral arteries: a pathogenetic factor in cerebral vasospasm after subarachnoid hemorrhage? *Neurosurgery* 41:245–252
 122. Sabri M, Ai J, Knight B, Tariq A, Jeon H, Shang X, Marsden PA, MacDonald RL (2011) Uncoupling of endothelial nitric oxide synthase after experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 31:190–199. <https://doi.org/10.1038/jcbfm.2010.76>
 123. Olsen SB, Tang DB, Jackson MR, Gomez ER, Ayala B, Alving BM (1996) Enhancement of platelet deposition by cross-linked hemoglobin in a rat carotid endarterectomy model. *Circulation* 93:327–332. <https://doi.org/10.1161/01.CIR.93.2.327>
 124. Pluta R (2005) Delayed cerebral vasospasm and nitric oxide: review, new hypothesis, and proposed treatment. *Pharmacol Ther* 105:23–56. <https://doi.org/10.1016/j.pharmthera.2004.10.002>
 125. Gabikian P, Clatterbuck RE, Eberhart CG, Tyler BM, Tierney TS, Tamargo RJ (2002) Prevention of experimental cerebral vasospasm by intracranial delivery of a nitric oxide donor from a controlled-release polymer: toxicity and efficacy studies in rabbits and rats. *Stroke* 33:2681–2686. <https://doi.org/10.1161/01.STR.0000033931.62992.B1>
 126. Pluta RM, Oldfield EH, Boock RJ (1997) Reversal and prevention of cerebral vasospasm by intracarotid infusions of nitric oxide donors in a primate model of subarachnoid hemorrhage. *J Neurosurg.* <https://doi.org/10.3171/jns.1997.87.5.0746>
 127. Thomas JE, Rosenwasser RH (1999) Reversal of severe cerebral vasospasm in three patients after aneurysmal subarachnoid hemorrhage: initial observations regarding the use of intraventricular sodium nitroprusside in humans. *Neurosurgery* 44:48. <https://doi.org/10.1097/00006123-199901000-00026>
 128. Bacon CR, Cary NR, Davenport AP (1995) Distribution of endothelin receptors in atherosclerotic human coronary arteries. *J Cardiovasc Pharmacol* 26(Suppl 3):S439–S441
 129. Davenport AP, Kuc RE, Maguire JJ, Harland SP (1995) ETA receptors predominate in the human vasculature and mediate constriction. *J Cardiovasc Pharmacol* 26(Suppl 3):S265–S267. <https://doi.org/10.1097/00005344-199526003-00080>
 130. Seifert V, Löffler BM, Zimmermann M, Roux S, Stolke D (1995) Endothelin concentrations in patients with aneurysmal subarachnoid hemorrhage. Correlation with cerebral vasospasm, delayed ischemic neurological deficits, and volume of hematoma. *J Neurosurg* 82:55–62. <https://doi.org/10.3171/jns.1995.82.1.0055>
 131. Boscolo E, Pavesi G, Zampieri P, Conconi MT, Calore C, Scienza R, Parnigotto PP, Folin M (2006) Endothelial cells from human cerebral aneurysm and arteriovenous malformation release ET-1 in response to vessel rupture. *Int J Mol Med* 18:813–819
 132. Gaetanu P, Rodriguez Baena YR, Grignani G, Spanu G, Pacchiarini L, Paoletti P, Gaetani P, Rodriguez y Baena R et al (1994) Endothelin and aneurysmal subarachnoid haemorrhage: a study of subarachnoid cisternal cerebrospinal fluid. *J Neurol Neurosurg Psychiatry* 57:66–72
 133. Sharkey J, Butcher SP, Kelly JS (1994) Endothelin-1 induced middle cerebral artery occlusion: pathological consequences and neuroprotective effects of MK801. *J Auton Nerv Syst* 49:177–185. [https://doi.org/10.1016/0165-1838\(94\)90109-0](https://doi.org/10.1016/0165-1838(94)90109-0)
 134. Edvinsson L, Povlsen GK, Ahnstedt H, Waldsee R (2014) CaMKII inhibition with KN93 attenuates endothelin and serotonin receptor-mediated vasoconstriction and prevents subarachnoid hemorrhage-induced deficits in sensorimotor function. *J. Neuroinflammation* 11. doi:<https://doi.org/10.1186/s12974-014-0207-2>
 135. Foley PL, Caner HH, Kassell NF, Lee KS (1994a) Reversal of subarachnoid hemorrhage-induced vasoconstriction with an endothelin receptor antagonist. *Neurosurgery* 34:103–108
 136. He GW, Liu MH, Yang Q, Fumary A, Yim APC (2007) Role of endothelin-1 receptor antagonists in vasoconstriction mediated by endothelin and other vasoconstrictors in human internal mammary artery. *Ann Thorac Surg* 84:1522–1527. <https://doi.org/10.1016/j.athoracsur.2007.05.064>

137. Singhal AK, Symons JD, Boudina S, Jaishy B, Shiu YE (2010) Role of endothelial cells in myocardial ischemia–reperfusion injury. *Vasc Dis Prev* 7:1–14. <https://doi.org/10.2174/1874120701007010001>
138. Dumont AS, Dumont RJ, Chow MM, Lin C-L, Calisaneller T, Ley KF, Kassell NF, Lee KS (2003) Cerebral vasospasm after subarachnoid hemorrhage: putative role of inflammation. *Neurosurgery* 53:123–135. <https://doi.org/10.1227/01.NEU.0000068863.37133.9E>
139. Findlay JM, Macdonald RL, Weir BK (1991) Current concepts of pathophysiology and management of cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *Cerebrovasc Brain Metab Rev* 3:336–361
140. Mayberg MR (1998) Cerebral vasospasm. *Neurosurg Clin N Am* 9:615–627
141. Zhang J, Lewis A, Bernanke D, Zubkov A, Glower B (1998) Stroke: anatomy of a catastrophic event. *Anat Rec*. [https://doi.org/10.1002/\(SICI\)1097-0185\(199804\)253:2<58::AID-AR9>3.0.CO;2-A](https://doi.org/10.1002/(SICI)1097-0185(199804)253:2<58::AID-AR9>3.0.CO;2-A)
142. Davie N, Haleen SJ, Upton PD, Polak JM, Yacoub MH, Morrell NW, Wharton J (2002) ET(a) and ET(B) receptors modulate the proliferation of human pulmonary artery smooth muscle cells. *Am J Respir Crit Care Med* 165:398–405. <https://doi.org/10.1164/ajrccm.165.3.2104059>
143. Yahiaoui L, Villeneuve A, Valderrama-Carvajal H, Burke F, Fixman ED (2006) Endothelin-1 regulates proliferative responses, both alone and synergistically with PDGF, in rat tracheal smooth muscle cells. *Cell Physiol Biochem* 17:37–46. <https://doi.org/10.1159/000091462>
144. Borel CO, McKee A, Parra A, Haglund MM, Solan A, Prabhakar V, Sheng H, Warner DS et al (2003) Possible role for vascular cell proliferation in cerebral vasospasm after subarachnoid hemorrhage. *Stroke* 34:427–432. <https://doi.org/10.1161/01.STR.0000053848.06436.AB>
145. Pickard JD, Graham DI, Mearns E, MacPherson P, Tamura A, Fitch W (1985) Ultrastructure of cerebral arteries following experimental subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry* 48:256–262. <https://doi.org/10.1136/jnnp.48.3.256>
146. Crompton MR (1964) Hypothalamic lesions following the rupture of cerebral berry aneurysms. *Brain* 86:301–314. <https://doi.org/10.1093/brain/86.2.301>
147. Hughes JT, Schianchi PM (1978) Cerebral artery spasm. *J Neurosurg* 48:515–525. <https://doi.org/10.3171/jns.1978.48.4.0515>
148. Ryba M, Jarzabek-Chorzelska M, Chorzelski T, Pastuszko M (1992) Is vascular angiopathy following intracranial aneurysm rupture immunologically mediated? *Acta Neurochir* 117:34–37. <https://doi.org/10.1007/BF01400632>
149. Shimizu T, Kito K, Hoshi T, Yamazaki N, Takahashi K, Takahashi M, Yamane K, Sim C et al (1982) Immunological study of late cerebral vasospasm in subarachnoid hemorrhage. *Neurol Med Chir* 22:613–619. <https://doi.org/10.2176/nmc.22.613>
150. Chaudhry SR, Stoffel-Wagner B, Kinfel TM, Güresir E, Vatter H, Dietrich D, Lamprecht A, Muhammad S (2017) Elevated systemic IL-6 levels in patients with aneurysmal subarachnoid hemorrhage is an unspecific marker for post-SAH complications. *Int J Mol Sci* 18:2580. <https://doi.org/10.3390/ijms18122580>
151. Dhar R, Diringer MN (2008) The burden of the systemic inflammatory response predicts vasospasm and outcome after subarachnoid hemorrhage. *Neurocrit Care* 8:404–412. <https://doi.org/10.1007/s12028-008-9054-2>
152. Provencio JJ (2013) Inflammation in subarachnoid hemorrhage and delayed deterioration associated with vasospasm: a review. *Acta Neurochirurgica, Supplementum*. NIH public access, pp. 233–238. doi:https://doi.org/10.1007/978-3-7091-1192-5_42
153. Gallia GL, Tamargo RJ (2006a) Leukocyte–endothelial cell interactions in chronic vasospasm after subarachnoid hemorrhage. *Neurol Res* 28:750–758. <https://doi.org/10.1179/016164106X152025>
154. Ascenzi P, Bocedi A, Visca P, Altruda F, Tolosano E, Beringhelli T, Fasano M (2005) Hemoglobin and heme scavenging. *IUBMB Life*. <https://doi.org/10.1080/15216540500380871>
155. Provencio JJ, Altay T, Smithason S, Moore SK, Ransohoff RM (2011) Depletion of Ly6G/C+ cells ameliorates delayed cerebral vasospasm in subarachnoid hemorrhage. *J Neuroimmunol* 232:94–100. <https://doi.org/10.1016/j.jneuroim.2010.10.016>
156. Smithason S, Moore SK, Provencio JJ (2012) Systemic administration of LPS worsens delayed deterioration associated with vasospasm after subarachnoid hemorrhage through a myeloid cell-dependent mechanism. *Neurocrit Care* 16:327–334. <https://doi.org/10.1007/s12028-011-9651-3>
157. Hailer NP, Bechmann I, Heizmann S, Nitsch R (1997) Adhesion molecule expression on phagocytic microglial cells following anterograde degeneration of perforant path axons. *Hippocampus* 7:341–349. [https://doi.org/10.1002/\(SICI\)1098-1063\(1997\)7:3<341::AID-HIPO8>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1098-1063(1997)7:3<341::AID-HIPO8>3.0.CO;2-N)
158. Mackay F, Loetscher H, Stueber D, Gehr G, Lesslauer W (1993) Tumor necrosis factor alpha (TNF-alpha)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. *J Exp Med* 177:1277–1286
159. Okada T, Suzuki H (2017) Toll-like receptor 4 as a possible therapeutic target for delayed brain injuries after aneurysmal subarachnoid hemorrhage. *Neural Regen Res* 12:193–196. <https://doi.org/10.4103/1673-5374.200795>
160. Zheng VZ, Wong GKC (2017) Neuroinflammation responses after subarachnoid hemorrhage: a review. *J Clin Neurosci* 42:7–11. <https://doi.org/10.1016/j.jocn.2017.02.001>
161. Springer TA, Anderson DC, Springer TA, Arfors K-E, Lundberg C, Lindbom L, Lundberg K, Beatty PG et al (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multi-step paradigm. *Cell* 76:301–314. [https://doi.org/10.1016/0092-8674\(94\)90337-9](https://doi.org/10.1016/0092-8674(94)90337-9)
162. Dietrich HH, Dacey RG (2000) Molecular keys to the problems of cerebral vasospasm. *Neurosurgery* 46:517–530. <https://doi.org/10.1097/00006123-200003000-00001>
163. Ihle JN (2001) The Stat family in cytokine signaling. *Curr Opin Cell Biol*. [https://doi.org/10.1016/S0955-0674\(00\)00199-X](https://doi.org/10.1016/S0955-0674(00)00199-X)
164. Bond M, Chase AJ, Baker AH, Newby AC (2001) Inhibition of transcription factor NF- κ B reduces matrix metalloproteinase-1, -3 and -9 production by vascular smooth muscle cells. *Cardiovasc Res* 50:556–565. [https://doi.org/10.1016/S0008-6363\(01\)00220-6](https://doi.org/10.1016/S0008-6363(01)00220-6)
165. Lu Q, Harrington EO, Jackson H, Morin N, Shannon C, Rounds S (2006) Transforming growth factor-beta1-induced endothelial barrier dysfunction involves Smad2-dependent p38 activation and subsequent RhoA activation. *J Appl Physiol* 101:375–384. <https://doi.org/10.1152/jappphysiol.01515.2005>
166. Tedgui A (2006) Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* 86:515–581. <https://doi.org/10.1152/physrev.00024.2005>
167. Madge LA, Pober JS (2001) TNF signaling in vascular endothelial cells. *Exp Mol Pathol* 70:317–325. <https://doi.org/10.1006/exmp.2001.2368>
168. Paria BC, Vogel SM, Ahmmed GU, Alamgir S, Shroff J, Malik AB, Tiruppathi C (2004) Tumor necrosis factor-alpha-induced TRPC1 expression amplifies store-operated Ca²⁺ influx and endothelial permeability. *Am J Physiol Lung Cell Mol Physiol* 287:L1303–L1313. <https://doi.org/10.1152/ajplung.00240.2004>
169. Griendling KK, Sorescu D, Lassegue B, Ushio-Fukai M (2000) Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. *Arterioscler Thromb Vasc Biol* 20:2175–2183. <https://doi.org/10.1161/01.ATV.20.10.2175>

170. Kofler S, Nickel T, Weis M (2005) Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. *Clin Sci* 108:205–213. <https://doi.org/10.1042/CS20040174>
171. Winegar RA, Catherine Land M, Morgan WF (1989) Increased chromosomal radiosensitivity of a Chinese hamster ovary cell line that inducibly expresses the *eco* RI restriction endonuclease. *Biochem Biophys Res Commun* 160:1079–1084. [https://doi.org/10.1016/S0006-291X\(89\)80113-5](https://doi.org/10.1016/S0006-291X(89)80113-5)
172. da Fonseca ACC, Matias D, Garcia C, Amaral R, Geraldo LH, Freitas C, Lima FRS (2014) The impact of microglial activation on blood–brain barrier in brain diseases. *Front Cell Neurosci* 8:362. <https://doi.org/10.3389/fncel.2014.00362>
173. Suzuki S, Suzuki M, Iwabuchi T, Kamata Y (1983) Role of multiple cerebral microthrombosis in symptomatic cerebral vasospasm: with a case report. *Neurosurgery* 13:199–203. <https://doi.org/10.1227/00006123-198308000-00018>
174. Stein SC, Browne KD, Chen X-H, Smith DH, Graham DI (2006) Thromboembolism and delayed cerebral ischemia after subarachnoid hemorrhage: an autopsy study. *Neurosurgery* 59:781–788. <https://doi.org/10.1227/01.NEU.0000227519.27569.45>
175. Suzuki S, Kimura M, Souma M, Ohkima H, Shimizu T, Iwabuchi T (1990) Cerebral microthrombosis in symptomatic cerebral vasospasm—a quantitative histological study in autopsy cases. *Neurol Med Chir (Tokyo)* 30:309–316. <https://doi.org/10.2176/nmc.30.309>
176. Frijns CJM, Fijnheer R, Algra A, Van Mourik JA, Van Gijn J, Rinkel GJE (2006) Early circulating levels of endothelial cell activation markers in aneurysmal subarachnoid haemorrhage: associations with cerebral ischaemic events and outcome. *J Neurol Neurosurg Psychiatry* 77:77–83. <https://doi.org/10.1136/jnnp.2005.064956>
177. Hirashima Y, Nakamura S, Endo S, Kuwayama N, Naruse Y, Takaku A (1997) Elevation of platelet activating factor, inflammatory cytokines, and coagulation factors in the internal jugular vein of patients with subarachnoid hemorrhage. *Neurochem Res* 22:1249–1255. <https://doi.org/10.1023/A:1021985030331>
178. Ohkuma H, Suzuki S, Kimura M, Sobata E (1991) Role of platelet function in symptomatic cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *Stroke* 22:854–859
179. Peltonen S, Juvela S, Kaste M, Lassila R (1997) Hemostasis and fibrinolysis activation after subarachnoid hemorrhage. *J Neurosurg* 87:207–214. <https://doi.org/10.3171/jns.1997.87.2.0207>
180. Suzuki M, Kudo A, Otawara Y, Hirashima Y, Takaku A, Ogawa A (1999) Extrinsic pathway of blood coagulation and thrombin in the cerebrospinal fluid after subarachnoid hemorrhage. *Neurosurgery* 44:487–494
181. Sabri M, Ai J, Lakovic K, Macdonald RL (2013) Mechanisms of microthrombosis and microcirculatory constriction after experimental subarachnoid hemorrhage. In: *Acta Neurochirurgica*, Supplementum. Springer, Vienna, pp. 185–192. doi:<https://doi.org/10.1007/978-3-7091-1192-5-35>
182. Bombeli T, Karsan A, Tait JF, Harlan JM (1997) Apoptotic vascular endothelial cells become procoagulant. *Blood* 89:2429–2442. [https://doi.org/10.1016/S0887-7963\(97\)80117-4](https://doi.org/10.1016/S0887-7963(97)80117-4)
183. Yoshizumi M, Perrella M a, Burnett JC, Lee ME (1993) Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. *Circ Res* 73:205–209. <https://doi.org/10.1161/01.RES.73.1.205>
184. Bevilacqua MP, Pober JS, Majeau GR, Fierst W, Cotran RS, Gimbrone MA (1986) Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1 (inflammation/coagulation/tissue factor/monokine/endotoxin). *Med Sci* 83:4533–4537. <https://doi.org/10.1073/pnas.83.12.4533>
185. Calabria AR, Shusta EV (2008) A genomic comparison of in vivo and in vitro brain microvascular endothelial cells. *J Cereb Blood Flow Metab* 28:135–148. <https://doi.org/10.1038/sj.jcbfm.9600518>
186. Huntley MA, Bien-Ly N, Daneman R, Watts RJ (2014) Dissecting gene expression at the blood–brain barrier. *Front Neurosci* 8. <https://doi.org/10.3389/fnins.2014.00355>
187. Hupe M, Li MX, Kneitz S, Davydova D, Yokota C, Kele-Olovsson J, Hot B, Stenman JM et al (2017) Gene expression profiles of brain endothelial cells during embryonic development at bulk and single-cell levels. *Sci Signal* 10. <https://doi.org/10.1126/scisignal.aag2476>
188. González-Cabrero J, Pozo M, Durán MC, De Nicolás R, Egidio J, Vivanco F (2007) The proteome of endothelial cells. *Methods Mol Biol* 357:181–198. <https://doi.org/10.1385/1-59745-214-9:181>
189. Haqqani AS, Kelly J, Baumann E, Haseloff RF, Blasig IE, Stanimirovic DB (2007) Protein markers of ischemic insult in brain endothelial cells identified using 2D gel electrophoresis and ICAT-based quantitative proteomics. *J Proteome Res* 6:226–239. <https://doi.org/10.1021/pr0603811>
190. Won C, Lin Z, Kumar T, Li S, Ding L, Elkhail A, Szabó G, Vasudevan A (2013) Autonomous vascular networks synchronize GABA neuron migration in the embryonic forebrain. *Nat Commun* 4. <https://doi.org/10.1038/ncomms3149>
191. Buemi M, Cavallaro E, Floccari F, Sturiale A, Aloisi C, Trimarchi M, Corica F, Frisina N (2003) The pleiotropic effects of erythropoietin in the central nervous system. *J Neuropathol Exp Neurol*
192. Haller H, Christel C, Dannenberg L, Thiele P, Lindschau C, Luft FC (1996) Signal transduction of erythropoietin in endothelial cells. *Kidney Int*. <https://doi.org/10.1038/ki.1996.339>
193. Banerjee D, Rodriguez M, Nag M, Adamson JW (2000) Exposure of endothelial cells to recombinant human erythropoietin induces nitric oxide synthase activity. *Kidney Int*. <https://doi.org/10.1046/j.1523-1755.2000.00039.x>
194. Beleslin-Cokic BB, Cokic VP, Yu X, Weksler BB, Schechter AN, Noguchi CT (2004) Erythropoietin and hypoxia stimulate erythropoietin receptor and nitric oxide production by endothelial cells. *Blood*. <https://doi.org/10.1182/blood-2004-02-0744>
195. Grasso G, Buemi M, Alafaci C, Sfacteria A, Passalacqua M, Sturiale A, Calapai G, De Vico G et al (2002a) Beneficial effects of systemic administration of recombinant human erythropoietin in rabbits subjected to subarachnoid hemorrhage. *Proc Natl Acad Sci U S A*. <https://doi.org/10.1073/pnas.082097299>
196. Grasso G, Passalacqua M, Sfacteria A, Conti A, Morabito A, Mazzullo G, De VG, Buemi M et al (2002b) Does administration of recombinant human erythropoietin attenuate the increase of S-100 protein observed in cerebrospinal fluid after experimental subarachnoid hemorrhage? *J Neurosurg*. <https://doi.org/10.3171/jns.2002.96.3.0565>
197. Grasso G, Buemi M, Giambardino F (2014) The role of erythropoietin in aneurysmal subarachnoid haemorrhage: from bench to bedside. *Acta Neurochir*. https://doi.org/10.1007/978-3-319-04981-6_13
198. Springborg JB, Møller C, Gideon P, Jørgensen OS, Juhler M, Olsen NV (2007) Erythropoietin in patients with aneurysmal subarachnoid haemorrhage: a double blind randomised clinical trial. *Acta Neurochir*. <https://doi.org/10.1007/s00701-007-1284-z>
199. Güresir E, Vasiliadis N, Konczalla J, Raab P, Hattingen E, Seifert V, Vatter H (2013) Erythropoietin prevents delayed hemodynamic dysfunction after subarachnoid hemorrhage in a randomized controlled experimental setting. *J Neurol Sci*. <https://doi.org/10.1016/j.jns.2013.07.004>
200. Tran KA, Zhang X, Predescu D, Huang X, MacHado RF, Göthert JR, Malik AB, Valyi-Nagy T et al (2016) Endothelial β -catenin signaling is required for maintaining adult blood–brain barrier integrity and central nervous system homeostasis. *Circulation*

- 133:177–186. <https://doi.org/10.1161/CIRCULATIONAHA.115.015982>
201. Chang J, Mancuso MR, Maier C, Liang X, Yuki K, Yang L, Kwong JW, Wang J et al (2017) Gpr124 is essential for blood–brain barrier integrity in central nervous system disease. *Nat Med* 23:450–460. <https://doi.org/10.1038/nm.4309>
202. Lengfeld JE, Lutz SE, Smith JR, Diaconu C, Scott C, Kofman SB, Choi C, Walsh CM et al (2017) Endothelial Wnt/ β -catenin signaling reduces immune cell infiltration in multiple sclerosis. *Proc Natl Acad Sci* 114:E1168–E1177. <https://doi.org/10.1073/pnas.1609905114>

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.