

# Cerebrovascular pathophysiology of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage

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**Summary.** Aneurysmal subarachnoid hemorrhage (SAH) remains a serious cerebrovascular disease. Even if SAH patients survive the initial insults, delayed cerebral ischemia (DCI) may occur at 4 days or later post-SAH. DCI is characteristic of SAH, and is considered to develop by blood breakdown products and inflammatory reactions, or secondary to early brain injury, acute pathophysiological events that occur in the brain within the first 72 hours of aneurysmal SAH. The pathology underlying DCI may involve large artery vasospasm and/or microcirculatory disturbances by microvasospasm, microthrombosis, dysfunction of venous outflow and compression of microvasculature by vasogenic or cytotoxic tissue edema. Recent clinical evidence has shown that large artery vasospasm is not the only cause of DCI, and that both large artery vasospasm-dependent and -independent cerebral infarction causes poor outcome. Animal studies suggest that mechanisms of vasospasm may differ between large artery and arterioles or capillaries, and that many kinds of cells in the vascular wall and brain parenchyma may be involved in the pathogenesis of microcirculatory disturbances. The impairment of the paravascular and glymphatic systems also may play important roles in the development of DCI. As pathological mediators for DCI, glutamate and several matricellular proteins have been investigated in addition to inflammatory molecules. Glutamate is involved in excitotoxicity contributing to cortical spreading ischemia and epileptic activity-related events. Microvascular dysfunction is an attractive mechanism to explain the cause of poor outcomes independently of large cerebral artery vasospasm, but needs more studies to clarify the pathophysiology or mechanisms and to develop a novel therapeutic strategy.

**Key words:** Cerebral vasospasm, Delayed cerebral ischemia, Early brain injury, Inflammation, Matricellular protein, Microcirculation, Subarachnoid hemorrhage

## Introduction

Subarachnoid hemorrhage (SAH) caused by a rupture of an intracranial aneurysm is associated with not only intracranial pathologies such as a sudden increase in intracranial pressure (ICP) and acute global cerebral ischemia, but also systemic complications including cardiopulmonary dysfunction (Taki et al., 2011; Suzuki et al., 2020a). Thus, the prognosis of aneurysmal SAH remains very poor: 10-15% of SAH patients die before arriving at hospital (Petridis et al., 2017), and thereafter about 25% of those die within 24 - 72 hours of onset (Geraghty et al., 2019). The acute pathophysiological events that occur in the brain within the first 72 hours of aneurysmal SAH are collectively called early brain injury (EBI) (Suzuki, 2015). EBI begins to develop by arterial bleeding-induced ICP elevation, (transient) global cerebral ischemia, mechanical brain injuries due to causes including intracerebral hemorrhage and/or acute hydrocephalus, and extravasated blood components within minutes post-SAH (Suzuki, 2015; Suzuki et al., 2020a). The pathophysiology of EBI include microthrombosis, early vasoconstriction, disturbance of cerebral (blood flow) autoregulation, blood-brain barrier (BBB) disruption, venous drainage dysfunction, brain

**Abbreviations.** ATP, adenosine triphosphate; BBB, blood-brain barrier; CSD, cortical spreading depolarization; CSF, cerebrospinal fluid; DAMP, damage-associated molecular pattern; DCI, delayed cerebral ischemia; EBI, early brain injury; ET, endothelin; HIF, hypoxia-inducible transcription factor; ICP, intracranial pressure; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NF, nuclear factor; NO, nitric oxide; OxyHb, oxyhemoglobin; ROS, reactive oxygen species; SAH, subarachnoid hemorrhage; SUR1, sulfonylurea receptor 1; TLR4, Toll-like receptor 4; TNC, tenascin-C; TRPM4, transient receptor potential melastatin 4; VEGF, vascular endothelial growth factor.

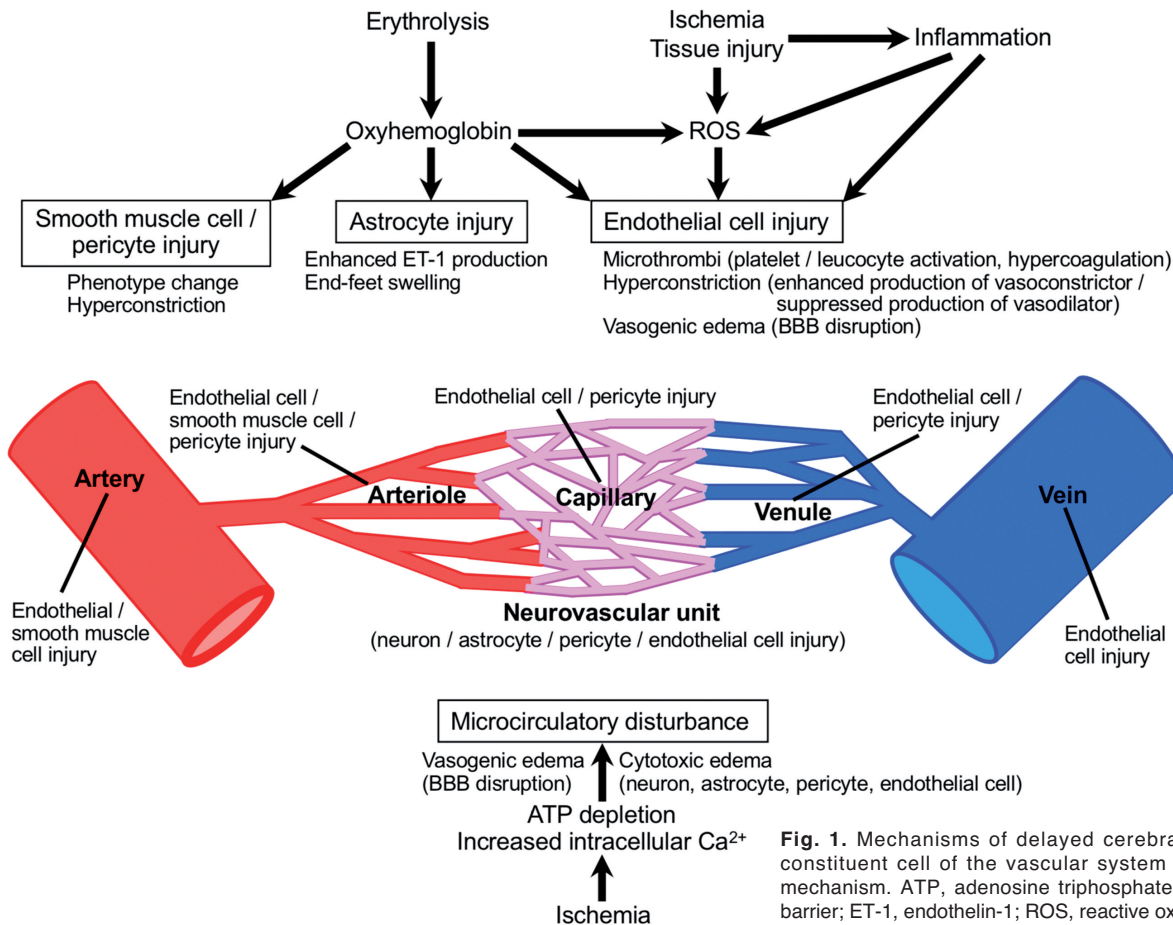
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tissue hypoxia, metabolic derangement (mismatch of metabolic supply and demand, and localized electrolyte imbalance), cortical spreading depolarization (CSD), excitotoxicity, inflammation, free radical reaction, and others (Hasegawa et al., 2015; Xiao et al., 2017; de Oliveira Manoel and Macdonald, 2018; Suzuki et al., 2020a), causing neuronal damage (Nakano et al., 2019b; Okada et al., 2019a). EBI is accompanied by both systemic and intracranial inflammatory reactions (Suzuki, 2019). BBB disruption promotes blood-borne cells including leukocytes and platelets as well as potentially neurotoxic and vasoactive compounds such as prothrombin/thrombin and fibrinogen/fibrin to enter brain parenchyma, and therefore aggravates neuroinflammation and EBI further (Suzuki et al., 2020a). SAH-induced cardiopulmonary or other medical complications also contribute to the development and aggravation of EBI (Suzuki et al., 2010c; Radolf et al., 2014; Suzuki, 2015). EBI may be revealed as high intensity lesions on diffusion-weighted magnetic resonance images or global cerebral edema on computed tomography in a clinical setting, which may reflect cytotoxic edema or a mixture of cytotoxic and vasogenic edema by acute microcirculatory disturbance, respectively (Frontera et

al., 2015; Suzuki et al., 2020a).

Patients surviving the initial insults of an aneurysmal rupture and EBI have a further risk of angiographic cerebral vasospasm in about 70% and delayed cerebral ischemia (DCI) in about 30%, both of which occur at days 4 to 14 or later post-SAH, and are characteristics of SAH (de Oliveira Manoel and Macdonald, 2018; Geraghty et al., 2019). Larger quantities of periarterial hematoma induces more frequent and severer angiographic cerebral vasospasm, while DCI as a whole tends to more frequently develop in patients with poorer admission neurological statuses (de Oliveira Manoel et al., 2015). DCI is considered to occur by cerebral vasospasm and/or microcirculatory disturbances possibly due to microthrombosis, arteriolar constriction, disturbance of cerebral blood flow autoregulation, BBB disruption, CSD, and so on (Xiao et al., 2017). DCI may be caused by blood breakdown products and inflammatory reactions (Xiao et al., 2017). EBI may also be a precursor for cerebral vasospasm and/or delayed microcirculatory disturbances, or at least a contributor to increased tissue vulnerability to the secondary insults (Fujii et al., 2013). Thus, many mechanisms of DCI may be shared or interrelated with those of EBI (Fig. 1),



**Fig. 1.** Mechanisms of delayed cerebral ischemia. Each constituent cell of the vascular system is involved in the mechanism. ATP, adenosine triphosphate; BBB, blood-brain barrier; ET-1, endothelin-1; ROS, reactive oxygen species.

although the details are not clear (Suzuki, 2015). Failure to treat DCI leads to the development of cerebral infarction, an important determinant of poor outcome (Vergouwen et al., 2010; Suzuki et al., 2019). Irrespective of current aggressive treatment, about 65% of SAH patients with Hunt and Hess grade 5, the worst clinical grade, suffered delayed cerebral infarction on computed tomography (Konczalla et al., 2018). The authors recently reported that a matricellular protein tenascin-C (TNC) may be a mediator between EBI and cerebral vasospasm and/or vasospasm-unrelated DCI, that is, delayed microcirculatory disturbances (Suzuki et al., 2020a). Cerebral vasospasm may render brain tissue vulnerable to the development of CSD (Østergaard et al., 2013).

### Cerebral vasospasm

Angiographic vasospasm occurs biphasically: ultra-early (within 48 hours of SAH) and delayed angiographic vasospasm (Phan et al., 2017; de Oliveira Manoel and Macdonald, 2018). Delayed vasospasm is clinically important, and therefore cerebral vasospasm means delayed angiographic vasospasm, which traditionally refers to a prolonged but reversible narrowing of large-capacitance cerebral arteries at the base of the brain beginning several days post-SAH and peaking in severity about one week later. Delayed vasospasm diminishes perfusion in the tissue distal to the narrowing, and causes ischemic symptoms and cerebral infarction, depending on the severity as well as brain conditions, the status of anatomical collateral blood supply and microcirculation, genetic variations such as gene polymorphisms of endothelial nitric oxide (NO) synthase and haptoglobin, and systemic and physiological factors including circulating blood volume, blood pressure, hematocrit, hyponatremia, and gas in arterial blood and cerebrospinal fluid (CSF); and in that case, it is called symptomatic vasospasm or DCI (Ohkuma et al., 2000; Schuiling et al., 2005; Rosalind and Du, 2015; Findlay et al., 2016; Suzuki et al., 2017). Modern SAH management has reduced the combined risks for death and permanent disability from vasospasm alone to less than 10% of SAH patients, but it remains one of the leading causes of preventable poor outcomes after aneurysmal SAH (Findlay et al., 2016). The pathogenesis of cerebral vasospasm basically lies in artery encasement by blood clot and a slower rate of subarachnoid clot clearance, resulting in the most severe vasospasm observed in the main trunk of cerebral artery near a ruptured aneurysm (Findlay et al., 2016). However, vasospasm also develops in intraparenchymal perforating arteries as well as arterioles located on the surface of the brain (Ohkuma and Suzuki, 1999; Findlay et al., 2016): nowadays, arterial narrowing seen on vascular imaging is called as angiographic vasospasm. Ultra-early angiographic vasospasm develops in <10% of SAH cases, possibly by blood products from previous subclinical bleeding (Phan et al., 2017). Ultra-early

vasospasm occurs more likely in cases with increased ICP or intraventricular hemorrhage, and was significantly associated with delayed vasospasm, DCI and poor outcomes (Phan et al., 2017). This may mean that the processes of ultra-early and delayed vasospasm may have a common underlying mechanism, and that ultra-early vasospasm may induce an early harmless phase of vasospasm, which may progress to be symptomatic in a delayed phase (Phan et al., 2017).

Vasospasm is considered to be caused by prolonged vascular smooth muscle contraction and impaired vasorelaxation, associated with remodeling of the arterial wall, consisting of inflammatory mechanisms, modification of extracellular matrix and smooth muscle cell phenotype, injuries or apoptosis of endothelial and smooth muscle cells, and myointimal proliferation (Macdonald and Weir, 1991; Suzuki et al., 2011). Aneurysmal rupture causes EBI, which releases various damage-associated molecular patterns (DAMPs) including reactive oxygen species (ROS) from injured cells or tissues (Suzuki, 2019). Aneurysmal rupture also spreads intravascular blood into the subarachnoid space or the CSF, including fibrinogen and fibrin, both of which are also DAMPs (Suzuki, 2019). Although DAMPs are hidden within cells or in the extracellular matrix under normal conditions, released DAMPs are recognized by pattern recognition receptors such as Toll-like receptor 4 (TLR4) and trigger various inflammatory cascades and microglial cell activation (Kawakita et al., 2017; Okada and Suzuki, 2020), which in turn upregulate cell adhesion molecules on endothelial cells, recruit neutrophils and macrophages into the subarachnoid space and activate them within several hours of onset (Nishikawa and Suzuki, 2018; Kanamaru and Suzuki, 2019; Peeyush Kumar et al., 2019; Suzuki, 2019). The inflammatory cells eat erythrocytes, and promote inflammatory reactions to release cytokines, ROS, vasoactive substances such as endothelin (ET)-1, oxyhemoglobin (OxyHb) and its by-products (Kanamaru and Suzuki, 2019; Khey et al., 2019). By anaerobic glycolysis associated with physiological reactions in ischemic brain and CSF after SAH, lactic acidosis occurs and further induces inflammatory and free radical reactions (Suzuki et al., 2017; Kanamaru and Suzuki, 2019). Erythrolysis begins to occur 2 days after SAH and releases OxyHb, which is also a DAMP (Macdonald and Weir, 1991; Suzuki, 2019). OxyHb can directly induce ET-1 production in endothelial cells and vascular smooth muscle cells via protein kinase C-cyclic adenosine monophosphate signaling (Kanamaru and Suzuki, 2019). Autoxidation of OxyHb produces another DAMP methemoglobin and ROS, causing inflammatory reactions, lipid peroxidation and tissue injuries, as well as producing various vasoactive substances (Findlay et al., 2016; Khey et al., 2019; Peeyush Kumar et al., 2019). Reported substances which cause vasospasm, so-called spasmogens, include OxyHb, hemoglobin degradation products such as bilirubin oxidation products, metabolic products of arachidonic acid, ROS

including free radicals, lipid peroxides, cytokines, platelet-derived or epidermal growth factors, ET-1, TNC, sphingosylphosphorylcholine, and adenosine triphosphate (ATP), some of which also belong to DAMPs (Macdonald and Weir, 1991; Suzuki et al., 2011; Shirao et al., 2015; Fujimoto et al., 2018; Kato et al., 2018; Nakano et al., 2019a). The crosstalk among receptors such as TLR4, epidermal growth factor receptors and integrins may also contribute to the development of vasospasm (Nakano et al., 2020; Suzuki et al., 2020a). Conversely, the crosstalk among receptors may explain that for example, an ET receptor antagonist consistently reduced angiographic vasospasm, although the causes of vasospasm are multifactorial (Laban et al., 2015). Arterial smooth muscle cell contraction is initiated by the activation of  $Ca^{2+}$ /calmodulin-dependent myosin light chain kinase, but sustained vasoconstriction, which is  $Ca^{2+}$ -independent and less reversible with pharmacologic vasodilators, may be mediated by other signal transduction pathways, including myosin phosphatase, Rho kinase, protein kinase C, protein tyrosine kinase, mitogen-activated protein kinases (MAPKs), and caldesmon (Tani, 2002; Suzuki et al., 2011, 2020b).

After SAH, disrupted mitochondrial respiration in endothelial cells associated with brain hypoxia or ischemia and autoxidation of extracellular OxyHb produce ROS and lipid peroxides, which permeate the vessel wall and injure endothelial and smooth muscle cells (Findlay et al., 2016; Peeyush Kumar et al., 2019). As a result, endothelial synthesis of vasodilators such as NO, prostacyclin and endothelium-derived hyperpolarization factor and therefore endothelium-dependent vasorelaxation are impaired, while a vasoconstrictor ET-1 is overproduced in the endothelium and the abluminal side of the tunica media (Macdonald and Weir, 1991; Findlay et al., 2016; Peeyush Kumar et al., 2019). OxyHb also scavenges NO (Findlay et al., 2016). Damaged endothelial cells release a number of pro-inflammatory molecules leading to platelet activation (adhesion and aggravation) and the coagulation cascade including fibrin formation, resulting in enhancing inflammatory responses in a feedback loop (Khey et al., 2019). Damaged endothelial cells and activated platelets produce thromboxane A2, causing vasoconstriction (de Oliveira Manoel and Macdonald, 2018; Peeyush Kumar et al., 2019). Pro-inflammatory cytokines like tumor necrosis factor- $\alpha$  and thromboxane A2 also cause endothelial cell apoptosis (Peeyush Kumar et al., 2019). Endothelial cell damage and thrombus formation in spastic artery may contribute to the onset of ischemic symptoms.

Cerebral arteries have receptors for neurotransmitters such as serotonin, and perivascular nerve innervation at the junction of the medial and adventitial layers, which regulate vascular function (constriction and dilatation) as well as phenotypes (growth and differentiation) of smooth muscle cells (Macdonald and Weir, 1991; Zhang et al., 2012). Although SAH, possibly

OxyHb, damages the neural inputs to arterial smooth muscle cells, the significance in vasospasm remains controversial (Macdonald and Weir, 1991). One of the causes of reduced NO production may be the disappearance of neuronal NO synthase from nerve fibers in the arterial adventitia (Østergaard et al., 2013). SAH also damages numerous endothelium-releasing factors, including NO and ET, which regulate the phenotype of adjacent smooth muscle cells (Macdonald and Weir, 1991; Zhang et al., 2012). Another important component is the vasa vasorum, a network of arterioles to supply nutrients to the thick medial layers of large arteries, although cerebral arteries seem to lack a typical vasa vasorum and instead possess a rete vasorum connected to the subarachnoid space, through which CSF and some substances can penetrate to the medial layers (Zhang et al., 2012). Occlusion or damage of the rete vasorum by SAH may compromise the function of cerebral arteries, but the importance of the effect has not been explored (Zhang et al., 2012). Vasospasm of the vasa vasorum was reported in basilar artery spasm after SAH in dogs, and was suggested to affect the vasospasm pathogenesis and the degenerative changes of the spastic basilar artery (Ozoner et al., 2019).

### Microcirculatory disturbance

The failed clazosentan clinical trials, which showed that a selective ET receptor antagonist clazosentan reduced large artery angiographic vasospasm but failed to improve outcomes (Macdonald et al., 2011), have made many SAH researchers think that DCI has less impact on outcomes compared with EBI (Suzuki et al., 2018). However, large artery vasospasm is not the only cause of DCI, and the definition of large artery vasospasm and DCI is separately determined (Vergouwen et al., 2010). In fact, the findings of the trial showed that 1) cerebral infarction developed most commonly in patients with large artery vasospasm and neurological worsening; 2) the majority of patients with moderate to severe large artery vasospasm had neither neurological worsening nor cerebral infarction; and 3) both vasospasm-dependent and -independent cerebral infarction caused poor outcome (Vergouwen et al., 2011). Thus, the authors consider that lessons from the failed clazosentan clinical trials are as follows: 1) the mechanisms of vasospasm may differ between large artery and arterioles or capillaries, and clazosentan may have no effects on “vasospasm” of arterioles or capillaries (Liu et al., 2018a); 2) clazosentan may not prevent the formation of microthrombi (Pisapia et al., 2012); 3) ET-1 is a less potent constrictor in cerebral veins compared with arteries, and therefore clazosentan may fail to alleviate “vasospasm” of cerebral venous system after SAH (Xiao et al., 2017); and 4) clazosentan may not suppress microcirculatory disturbance due to compression of microvasculature and veins by brain edema. In contrast to large conducting arteries such as the arteries of the Circle of Willis providing a conduit



## DCI after SAH

for blood to reach the brain, resistance arteries such as surface pial arteries, penetrating arterioles, and parenchymal arterioles regulate blood flow by dynamically altering vessel caliber. Parenchymal arterioles are morphologically different from surface pial arteries and penetrating arterioles, which are not circumferentially wrapped around by astrocytic foot processes due to the presence of CSF in the Virchow-Robin spaces (Fig. 2). Surface pial arteries and penetrating arterioles have extrinsic innervation from trigeminal and autonomic nerves, whereas parenchymal arterioles have intrinsic innervation (Tso and Macdonald, 2014). Thus, findings obtained from SAH studies targeting large arteries, surface pial arteries, and penetrating arterioles may not be able to be applied to the microcirculatory pathophysiology of parenchymal arterioles, capillaries and venules after SAH. Although most of the following findings have been obtained from experiments of EBI after SAH, EBI may progress to DCI associated with similar mechanisms. In fact, in rat or mouse endovascular perforation models that show acute metabolic changes similar to clinical findings and are most popularly used for studies of EBI, both EBI and cerebral vasospasm occur in the time frame of 72 hours, precluding the dissociation between EBI and DCI pathophysiologies, because cerebral vasospasm is one of important DCI pathologies (Suzuki and Nakano, 2018).

## Microvasospasm

After SAH, microvessels consistently show altered vasoreactivity, that is, impaired vasodilation and enhanced vasoconstriction to various topical agents, causing a significant compromise of cerebrovascular reserve and a high risk for the development of cerebral ischemia (Tso and Macdonald, 2014). The heterogeneous luminal narrowing (pearl string-like contraction) of pial and parenchymal arterioles was reported to occur possibly via a mechanism different

from that of vasospasm in a large artery at least in terms of ET-1 and Rho kinase: inhibitors of ET-1 and Rho kinase reversed large artery vasospasm but had fewer inhibitory effects against vasospasm of smaller arterioles (Ohkuma and Suzuki, 1999; Luo et al., 2016; Liu et al., 2018a). Smaller pial and parenchymal arterioles have a greater degree of vasoconstriction than in larger pial and parenchymal arteries or arterioles (Friedrich et al., 2012; Tso and Macdonald, 2014; Luo et al., 2016). Capillaries also show segmental luminal narrowing or constriction after SAH, which is mediated by pericytes and followed by pericyte death (apoptosis): the phenomena were prevented by a free radical scavenger (Fumoto et al., 2019). Pericyte death caused a reduced capillary density and maintained an inactive microcirculation (Xiao et al., 2017). On the other hand, it is still controversial whether the diameter of cerebral venules is reduced after SAH or not (Friedrich et al., 2012; Xiao et al., 2017; Fumoto et al., 2019).

Pericytes cover capillaries and venules as well as arterioles, and may determine the diameter of these vessels. Post-SAH penetration of OxyHb into brain parenchyma possibly via the paravascular and the glymphatic systems (paravascular CSF pathways consisting of influx along arterioles and capillaries, and efflux along para-venous spaces; Fig. 3) suppresses NO/cyclic guanosine monophosphate signaling at multiple levels, and causes phenotypic transformation of pericytes to a hypercontractile form to reduce microvessel diameters (Geraghty et al., 2019; Pu et al., 2019). OxyHb induces the release of ROS and the production of ET-1 in astrocytes to constrict pericytes, and may cause swelling of perivascular astrocyte end-feet to compress the capillary lumen, as well as luminal endothelial protrusions to further compromise blood flow (Østergaard et al., 2013; Tso and Macdonald, 2014; Geraghty et al., 2019). Global ischemia by elevated ICP after SAH also constricts pericytes and reduces blood flow in the microcirculation (Xiao et al., 2017).

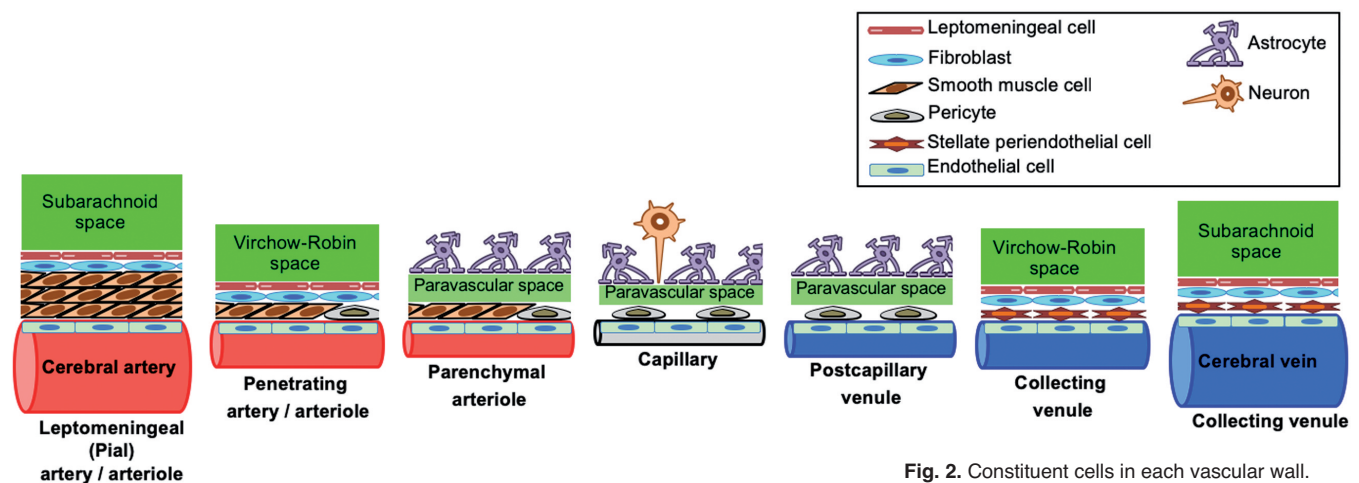
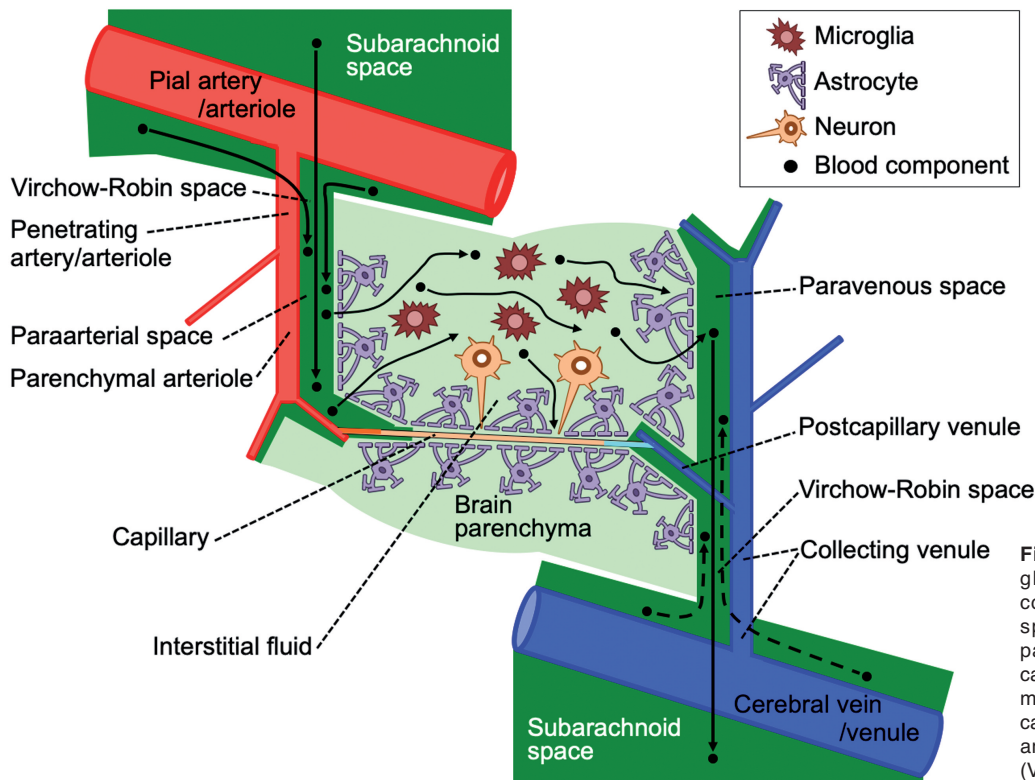


Fig. 2. Constituent cells in each vascular wall.

Capillary flow disturbance and reduced tissue oxygen tension increase the risk of capillary NO depletion, and then may lead to a vicious cycle by causing further tissue hypoxia, because oxygen is the substrate for NO production via NO synthases (Østergaard et al., 2013). NO releasing nerve fibers are known to be present at both arterial and capillary levels, but the significance in capillary levels has not been determined after SAH (Østergaard et al., 2013). Some researchers consider vascular smooth muscle cells as the dominator for microvessel autoregulation (Xiao et al., 2017).

Neurons interact with parenchymal microvessels. In normal brain tissues, increased neuronal activity leads to arteriolar vasodilation and functional hyperemia (increased local blood flow): that is, neurons release glutamate, which binds to metabotropic glutamate receptors on astrocytes and triggers an increase in intracellular  $Ca^{2+}$  and then activation of large-conductance  $Ca^{2+}$ -activated  $K^+$  channels for outflow of  $K^+$  at astrocytic end-feet, resulting in overall mild increases in perivascular  $K^+$  concentration (<20 mM) inducing smooth muscle cell hyperpolarization and relaxation (Dreier, 2011; Tso and Macdonald, 2014). However, in the setting of SAH, a subarachnoid blood clot increases a basal perivascular  $K^+$  concentration in relation to hemolysis, and therefore the physiological mechanisms of neurovascular coupling cause excessive perivascular  $K^+$  concentrations (>20mM) and impaired

clearance of perivascular  $K^+$ , resulting in smooth muscle cell depolarization and parenchymal arteriolar constriction (pathological inversion of neurovascular coupling) (Tso and Macdonald, 2014). Impairment or inversion of neurovascular coupling may develop time-dependently up to 96 hours post-SAH in rats or mice, and any neuronal activation including sensory stimulation as well as the resultant changes in metabolic agents such as increased  $CO_2$  and decreased pH may result in vasoconstriction, mismatch of metabolism and blood flow, relative ischemia, and further brain damage in SAH (Balbi et al., 2017). Decreased basal NO and higher amplitude in spontaneous  $Ca^{2+}$  oscillations in hypertrophic astrocyte end-feet surrounding arterioles (resulting in surges of extracellular potassium) caused by the presence of blood degradation products may also contribute to the inverted neurovascular coupling, which forms the basis of spreading ischemia associated with CSD after SAH (Dreier, 2011; Østergaard et al., 2013; Pappas et al., 2015). CSD is defined as a slowly self-propagating wave of sustained neuronal and astroglial depolarization that spreads in all directions from a region of onset, and may be induced by decreased levels of oxygen and glucose associated with causes such as global cerebral ischemia at aneurysmal rupture or the subsequent focal cerebral ischemia, along with decreased levels of NO, increased levels of potassium, OxyHb, ET-1 and glutamate in the subarachnoid space



**Fig. 3.** The paravascular and the glymphatic systems. Blood components in the subarachnoid space enter into the brain parenchyma along arterioles and capillaries, and exit along venules. In massive subarachnoid hemorrhage cases, erythrocytes may regurgitate and infiltrate into the perivascular (Virchow-Robin) spaces of venules.

## DCI after SAH

after SAH (Dreier, 2011; Østergaard et al., 2013; Geraghty and Testai, 2017). More massive SAH facilitated more frequent peri-infarct CSDs and vasoconstrictive hypoperfusion responses in a delayed fashion at around 72 hours in blood-injection SAH models in mice, and the interaction between focal ischemia and SAH may create a vicious cycle leading to DCI (Oka et al., 2017). CSD also raises extracellular  $K^+$  and reduces NO, contributing to spreading ischemia and cortical necrosis (van Lieshout et al., 2018). The pathological vasoconstriction after SAH induces neuronal ischemia and deprives neurons, especially those with increased activity and metabolic demand due to CSD, of oxygen, glucose and then ATP (supply-demand mismatch), potentially leading to cytotoxic edema, cell death or apoptosis (Dreier, 2011; Tso and Macdonald, 2014). Cellular toxicity and vasoconstrictive effects of CSD may act in synergy with direct ischemic effects of extravasated blood to develop cerebral infarction (Hartings et al., 2017).

Recently, it was reported that meningeal lymphatic drainage or sinus-associated lymphatic vessels are blocked possibly by blood clots after SAH (Pu et al., 2019). The impaired lymphatic drainage was associated with increased aquaporin-4 expression and loss of polarized localization of aquaporin-4 at end-foot processes of reactive astrocytes, which suggest the impairment of the glymphatic system (Pu et al., 2019). Brain tissue factor, which is predominantly produced by astrocytes and activated by proinflammatory cytokines, may play a critical role in the localization of blood spread and the disturbance of CSF flow by increasing fibrin formation and deposition (Golanov et al., 2018). The glymphatic system is a specialized brain-wide anatomic structure located at the paravascular space and is ensheathed with astrocytic end-feet expressing water channel aquaporin-4, working as the paravascular CSF pathway consisting of influx along para-arterial spaces and efflux along para-venous spaces via aquaporin-4 water channels (Luo et al., 2016). As a result, blood cells, including T lymphocytes, hemoglobin, other blood components, and toxic metabolites may enter into and accumulate within brain perivascular and paravascular spaces, causing prolonged arteriolar vasospasm (Pu et al., 2019). SAH may increase the permeability of the paravascular space to perfuse more subarachnoid blood and blood-related molecules into the brain parenchyma: the paravascular pathway consists of the Virchow-Robin space (perivascular space), where the subarachnoid space meets the paravascular space, and the paravascular space, and plays an important role in mediating cerebral arterial and arteriolar spasm, parenchymal arteriolar inflammation, widespread perivascular neuroinflammation, formation of microthrombi in the capillary network and microinfarction independently of glymphatic control (Luo et al., 2016) (Fig. 3). Perivascular pathways for the drainage of interstitial fluid and solutes, including amyloid- $\beta$ , from the brain have been also reported, although the perivascular

drainage pathways along vascular basement membranes do not seem to have the capacity for the drainage of cells: injected tracers diffuse through the extracellular spaces of the brain and enter basement membranes of capillaries between the endothelial layer and the surrounding astrocytes to drain out of the brain centrifugally along basement membranes surrounding smooth muscle cells in the tunica media of arterioles, then through the adventitia of pial and larger cerebral arteries to cervical lymph nodes (Weller et al., 2008). The failure of the perivascular drainage pathways along vascular basement membranes is involved in cerebral amyloid angiopathy and Alzheimer's disease (Weller et al., 2008), but its significance is unknown in post-SAH pathology.

### Microthrombosis

Microthrombosis is observed in brain parenchymal microvessels, pial arterioles as well as large cerebral arteries after SAH (Fumoto et al., 2019; Khey et al., 2019). Microthrombi may be not merely emboli resulting from endothelial injury at the site of aneurysmal rupture, because they are observed globally in both cerebral hemispheres (Ishikawa et al., 2009). Microthrombosis may be caused by hypercoagulability, endothelial damage (apoptosis) and microvascular narrowing (stasis or turbulence of blood flow), which were at least partly suppressed by a free radical scavenger (Fumoto et al., 2019). A study reported that approximately 30% of constricted arterioles were occluded by microthrombi and that the frequency of arteriolar microthrombosis correlated with the degree of arteriolar constriction in endovascular perforation mice models (Friedrich et al., 2012). Despite the fact that arteriolar constriction was reversible, persistent vasoconstriction of arterioles could become refractory, and may even cause diffuse thrombosis (Wang et al., 2018).

SAH induced rolling and adhesion of leukocytes and platelets on the endothelium (platelet-leukocyte-endothelial cell interactions) particularly in the postcapillary venules, but to a small degree in arterioles at the surface of the brain (Ishikawa et al., 2009). The activation of inflammatory cells and platelets in the cerebral microvasculature may depend on endothelial cell damage by ROS and proinflammatory cytokines associated with tissue hypoxia due to ICP elevation or secondary focal ischemia as well as massive SAH, and lead to microthrombus formation and microvascular stasis throughout the entire cerebral hemisphere, even distant from the aneurysm rupture site (Ishikawa et al., 2009; McBride et al., 2017). Activated platelets also display P-selectin and release chemokines or cytokines, which promote leukocyte adherence and transmigration at sites of platelet deposition, leading to further endothelial cell damage and microthrombus formation (Smyth et al., 2009). Endothelial cell-mediated thrombosis further promotes inflammation via activation



of circulating leukocytes through protease activated receptor-1 and TLR4 signaling, inducing a positive feed-forward loop to activate platelets and to form microthrombi, associated with low activity of ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13) that prevents platelet adherence (McBride et al., 2017). In addition, platelet aggregates were observed to be extravasated into the brain parenchyma and likely propagated pro-inflammatory signaling, further suggesting cross talk between inflammation and thrombosis after SAH (McBride et al., 2017). The presence of microthrombi correlated with the degree of microvascular vasoconstriction as well as spatially with regions of neuronal apoptosis (Tso and Macdonald, 2014). Moreover, platelet-mediated microthrombosis releases glutamate, which mediates excitotoxic brain injury and neuronal dysfunction after SAH (Bell et al., 2014). Impairment of the fibrinolytic cascade is also suggested after SAH in terms of higher activity of plasminogen activator inhibitor-1 (Kawakita et al., 2019). Furthermore, peroxynitrite that is formed by the reaction of ROS with NO inactivates tissue plasminogen activator to increase thrombogenicity, leading to the risk of further brain damage (Østergaard et al., 2013).

#### *Dysfunction of venous outflow*

Postcapillary venules are covered with pericytes, whereas collecting venules and cerebral veins have stellate periendothelial cells forming a basket-like network around the vessel wall, which have some characteristics similar to vascular smooth muscle cells and probably regulate blood volume by mildly changing the caliber of the vessels (Takahashi et al., 1994) (Fig. 2). However, even the superficial cerebral veins possess neither smooth muscle cells nor valves to prevent the back flow of venous blood unlike veins in the periphery (Takahashi et al., 1994). The whole cerebral venous system is surrounded by adrenergic nerve fibers (Chen et al., 2015). Veins, venules as well as capillaries have thinner walls compared with arteries, and therefore may be easily compressed and even collapsed by elevated ICP or the surrounding tissue edema including swollen astrocyte end-feet and adherent leukocytes, leading to an increase in the hydrostatic pressure of upstream vessels and the resultant development or aggravation of tissue edema as well as a decrease in blood outflow (Chen et al., 2015). In swine models of SAH by a blood injection or clot placement into the subarachnoid space between frontal lobe sulci, erythrocyte infiltration into the perivascular (Virchow-Robin) spaces of arterioles and venules were more prominently observed within the superficial cortex and caused complete vessel collapse in some cases, whereas congestion of the capillaries, microvasculature and small venules was present in the region of edema or in the deeper aspect (Hartings et al., 2017) (Fig. 3). Magnetic resonance venography demonstrated significantly increased venous volume

within the cortex at 3 hours and one day after SAH by a blood injection into the cisterna magna in rats (Sun et al., 2016). Recent studies found that vasospasm in deep cerebral veins or collapse of the veins by compression with the surrounding edematous tissues developed from 1 day and peaked at 5-7 days after SAH in rabbits (Xiao et al., 2017). In addition, oxidative stress and inflammation may damage venous endothelial cells and trigger clot formation in the cerebral venous system after SAH: the risk of thrombosis is much higher in veins because of lower blood pressure and slower blood flow compared with arteries (Chen et al., 2015).

#### *Compression of vasculature by tissue edema*

Delayed onset of cerebral edema develops within two weeks of onset in 12% of patients with aneurysmal SAH (Suzuki et al., 2020a). Both cytotoxic and vasogenic edema can cause DCI by compression of microvasculature and veins.

Post-SAH ischemia, tissue injuries and extravasated blood components including heme, thrombin, fibrinogen, platelets and leukocytes activate microglia as well as various cells including astrocytes, neurons, vascular endothelial cells, vascular smooth muscle cells, neutrophils, macrophages, and platelets through TLR4, initiating inflammatory cascades to produce pro-inflammatory cytokines and mediators including tumor necrosis factor- $\alpha$ , interleukins-1 $\beta$ , -6, -8, and -12, matrix metalloproteinase (MMP)-9, ROS and matricellular proteins such as TNC and periostin (Kanamaru and Suzuki, 2019; Kawakita et al., 2019; Khey et al., 2019; Suzuki et al., 2020a). BBB disruption is an underlying mechanism of vasogenic edema after aneurysmal SAH, and may be developed by multiple mechanisms including endothelial cell apoptosis and disruption of tight junctions (Okada et al., 2020; Peeyush Kumar et al., 2019). After aneurysmal SAH, multiple factors including ROS, OxyHb, iron overload, pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$ , as well as thromboxane A2 can induce endothelial cell apoptosis and BBB dysfunction (Kanamaru and Suzuki, 2019; Peeyush Kumar et al., 2019). Tissue hypoxia is known to damage mitochondria, which in turn exacerbates the energy crisis by reducing the amount of ATP, and amplifies the production of ROS (Østergaard et al., 2013). In addition to disrupted mitochondrial respiration, extracellular OxyHb following erythrolysis and the subsequent iron overload can be sources for the excessive generation of ROS after SAH, and cerebral microvascular endothelial cells with increased mitochondrial content are known to be susceptible to oxidative stress (Tso and Macdonald, 2014; Kanamaru and Suzuki, 2019). Activation of the contractile apparatus of the endothelial cell skeleton through myosin light chain kinases also contributes to BBB dysfunction after SAH by reducing endothelial cell-to-cell contact and tightness of the BBB integrity without modulation of tight junction protein levels (Luh et al.,



2019). Tissue hypoxia is considered to activate myosin light chain kinases (Luh et al., 2019).

Tissue ischemia and hypoxia after SAH not only induce energy storage loss, metabolic failure, and disturbed ionic hemostasis, but also cause an excessive release of neurotransmitters such as glutamates (Frontera et al., 2015; Kanamaru and Suzuki, 2019; van Lieshout et al., 2018). ATP depletion and excess intracellular calcium lead to persistent opening of sulfonyleurea receptor 1 (SUR1)-transient receptor potential melastatin 4 (TRPM4) channels, resulting in cytotoxic edema due to massive sodium influx into neurons and astrocytes as well as vasogenic edema due to endothelial cell swelling or inflammation and subsequent disruption of the interendothelial tight junctions (Hayman et al., 2017). An intraparenchymal concentration of glutamate was elevated at 1 to 7 days post-SAH, and was independently associated with DCI and 12-month poor outcomes in a clinical setting (Helbok et al., 2017).

Glutamate, a major excitatory transmitter, is synthesized by activated astrocytes and microglia, and mediates massive  $\text{Ca}^{2+}$  influx and subsequent mitochondrial dysfunction, leading to apoptotic cell death and necrosis via excessive activation of 4 different receptors, including ionotropic glutamate receptors (N-methyl-D-aspartate,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and kainate receptors) and a metabotropic glutamate receptor (Dreier, 2011; Østergaard et al., 2013; Geraghty and Testai, 2017; Zhang et al., 2018). That is, a derangement in the neurotransmitter release and the inhibition of reuptake lead to excitotoxicity, CSD, and loss of energy stores causing ionic imbalance, resulting in the induction of apoptosis of cells constituting the neurovascular unit (neurons, astrocytes, pericytes and vascular endothelial cells), and cytotoxic and vasogenic cerebral edema (Tso and Macdonald, 2014; Frontera et al., 2015; Kanamaru and Suzuki, 2019; van Lieshout et al., 2018). There is a positive feedback loop between glutamate receptor stimulation and glutamate release (Zhang et al., 2018). The onset of persistent CSD is accompanied by abrupt ion translocation between intracellular and extracellular spaces (disruption in ion homeostasis: an increase in extracellular  $\text{K}^{+}$  ion, but decreases in  $\text{Na}^{+}$ ,  $\text{Ca}^{2+}$  ions and pH), which results in an osmotic imbalance to cause swelling (cytotoxic edema) of neurons and astrocytic end-feet, distortion of neuronal dendritic architecture, and spreading depression of electrocorticographic activity, as well as increased releases of neurotransmitters such as glutamate, leading to glutamate-induced excessive stimulation and neurotoxicity (Dreier, 2011; Østergaard et al., 2013; Geraghty and Testai, 2017; Ashayeri Ahmadabad et al., 2020). Both CSD and ictal epileptic events occur after SAH, but the relationships between them are uncertain. CSD may lower the seizure threshold (Ashayeri Ahmadabad et al., 2020). Although CSD and epilepsy are distinct entities, they have similar toxic effects including increased metabolic demand, inverse

neurovascular coupling, BBB dysfunction and cell death (Geraghty and Testai, 2017). In addition to CSD, seizure and some kind of epileptiform discharges may be related to inflammation, vasospasm, microthrombosis and DCI; however, it remains possible that they are all parallel processes, because all of them more likely occur in more severely injured brain (Kim et al., 2017; Geraghty et al., 2019). TLR4 is a common signaling pathway in CSD-induced neuroinflammation in microglia, astrocytes and neurons, leading to neuronal damage (Ashayeri Ahmadabad et al., 2020).

MMP-9 is induced by inflammatory cytokines, ROS, matricellular proteins such as TNC, periostin and galectin-3, and other DAMPs, and degrades the extracellular matrix proteins of cerebral microvessel basal lamina and inter-endothelial tight junction proteins, causing BBB disruption (Nishikawa and Suzuki, 2018; Nishikawa et al., 2018a; Peeyush Kumar et al., 2019; Shiba and Suzuki, 2019; Kawakita and Suzuki, 2020). BBB impairment is associated with abluminal and intraparenchymal platelet aggregates (Tso and Macdonald, 2014). Although pericytes help maintain the BBB by inducing the polarization of astrocytic end-feet surrounding microvessels in normal brain (Tso and Macdonald, 2014), pericytes secrete MMP-9 to degrade the BBB, and may have a fundamental role in the disruption of the endothelial matrix in post-SAH venules but not arterioles and capillaries (Xiao et al., 2017). Post-SAH BBB disruption may occur due to the imbalance between destructive molecules including inflammatory cytokines, ROS, TNC, periostin, galectin-3, vascular endothelial growth factor (VEGF)-A, angiopoietin-2, MAPKs, and MMP-9 and protective molecules including another matricellular protein osteopontin, VEGF-B, angiopoietin-1, MAPK phosphatase-1, and tissue inhibitor of MMP-1 (Nakatsuka et al., 2018; Nishikawa et al., 2018b; Kanamaru et al., 2019a, 2019b; Kanamaru and Suzuki, 2019; Tanioka et al., 2019). TLR4 signaling may mediate the upregulation or downregulation of these molecules after SAH (Okada et al., 2019b).

Aging, comorbidities or vascular risk factors may also influence SAH-induced injuries to BBB and the neurovascular unit (Suzuki and Nakano, 2018; Kanamaru et al., 2020). Moreover, severe SAH-induced ICP elevation and brain injuries activate the sympathetic nervous system and induce an excessive release of catecholamines, resulting in systemic inflammation, hyponatremia and various extracerebral organs injury, including neurogenic pulmonary edema, which impairs brain oxygenation and further aggravates neurogenic injuries (Nakamura et al., 2009; Suzuki et al., 2009; Nakatsuka et al., 2017; Suzuki, 2019). The spleen is the most important reservoir of immunocytes, and releases multiple immune cells including monocytes, neutrophils and lymphocytes into peripheral blood, causing systemic inflammation as well as neuroinflammation (Suzuki, 2019; Li et al., 2020). Systemic inflammation triggers platelet adhesion, activation of coagulation cascades and

formation of microthrombi, potentially leading to multiple organ failure (de Oliveira Manoel and Macdonald, 2018). Leukocytosis after SAH is thought to reflect endogenous catecholamine releases. Increased number and endothelial adhesion of leukocytes (leukocyte-endothelial interactions) disturb capillary flow patterns, and cause shunting of erythrocytes through the capillary bed and therefore the reduction of tissue oxygen tension (Østergaard et al., 2013). Cerebral hypoxia may contribute to brain damage by increasing the probability of CSD, and activating hypoxia-inducible transcription factor (HIF)-1 (Østergaard et al., 2013). HIF-1 induces neuroinflammation, BBB dysfunction and edema formation leading to the vicious cycle of further capillary flow disturbances and hypoxia (Østergaard et al., 2013). HIF-1 also upregulates nicotinamide adenine dinucleotide phosphate oxidase 2 and thereby ROS (Østergaard et al., 2013), as well as SUR1-TRPM4 channels and VEGF-A, contributing to the formation of brain edema (Hayman et al., 2017). Post-SAH hyponatremia may be associated with intravascular hypovolemia and therefore increase the risk of DCI (Findlay et al., 2016). In addition, hyponatremia induces both brain swelling and capillary lumen compression by profound swelling of astrocytic end-feet, causing microcirculatory disturbance (Østergaard et al., 2013).

#### Possible involvement of matricellular proteins in DCI

Matricellular proteins are pleiotropic and secretable extracellular matrix proteins, which are controlled by many stimuli, and are transiently upregulated in almost any tissue and cell type with restricted occurrence in space and time, being a key mediator of various physiological and pathological processes (Suzuki et al., 2020a). There are many kinds of matricellular proteins known to be involved in stroke pathology (Kawakita et al., 2019), and at least some matricellular proteins such as TNC and periostin are products of TLR4 pathways (Okada et al., 2019b). TLR4 can be expressed and upregulated in almost all kinds of cells in the central nervous system including microglia, astrocytes, neurons, pericytes, vascular (arterial, capillary and venous) endothelial cells, arterial smooth muscle cells, leukocytes, macrophages, and platelets (Liu et al., 2018b; Okada et al., 2019b; Famakin and Vemuganti, 2020; Nakano et al., 2020). After aneurysmal SAH, extravasated blood and tissue injuries cause a release of many endogenous ligands and DAMPs, including heme, fibrinogen, and matricellular proteins (TNC and galectin-3), which activate TLR4 (Suzuki, 2019). Thus, matricellular proteins can be activators and products of TLR4, forming a positive feedback loop to induce DCI. In fact, many matricellular proteins including TNC, osteopontin, galectin-3, periostin, thrombospondin-1 and plasminogen activator inhibitor-1 have been reported to be related to the development of post-SAH DCI in clinical settings (Nakatsuka et al., 2018; Nishikawa et al., 2018b; Kanamaru et al., 2019b; Kawakita et al.,

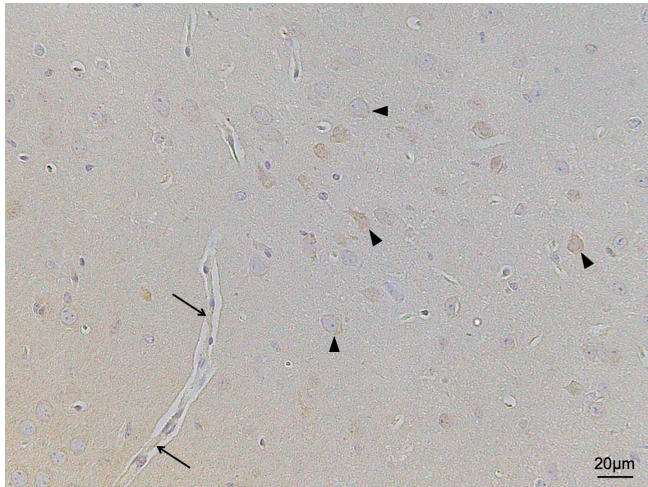
2019; Suzuki et al., 2019; Tanioka et al., 2019), although the mechanisms are not completely clarified.

TNC was upregulated in inflammatory cells in the subarachnoid space and various cells in cerebral arteries (endothelial, smooth muscle, and adventitial cells) and brains (possibly astrocytes, neurons and brain capillary endothelial cells) after SAH by endovascular perforation in rats and mice (Shiba and Suzuki, 2019). Experimental studies using TNC-knockout mice revealed that TNC causes cerebral vasospasm associated with the promotion of periarterial inflammatory cell infiltration and the activation of MAPKs in the arterial smooth muscle cells (Fujimoto et al., 2018), BBB disruption and brain edema formation via the MAPKs-mediated MMP-9 activation (Fujimoto et al., 2016), and neuroinflammation and caspase-dependent neuronal apoptosis by the upregulation and activation of TLR4 and the activation of nuclear factor (NF)- $\kappa$ B (Liu et al., 2018b). TNC may induce DCI via TLR4-mediated MAPK and NF- $\kappa$ B signaling (Okada and Suzuki, 2017). TNC may also contribute to the development of DCI by intravascular or microvascular thrombosis formation, because platelets are activated by the adhesion of platelets to TNC through integrins or a direct interaction between von Willebrand factors and TNC (Midwood et al., 2011). In addition, TNC can facilitate fibrin deposition by negative transcriptional control of tissue plasminogen activator (Midwood et al., 2011), causing the impairment of circulation of CSF and interstitial fluids in the subarachnoid space and the glymphatic system, which may also contribute to the development of DCI.

TNC and other matricellular proteins such as periostin and galectin-3 can interact and activate each other, which may form positive feedback mechanisms to develop DCI (Suzuki et al., 2020a). Periostin was upregulated in neurons and capillary endothelial cells and caused at least BBB disruption via MAPK-MMP-9 signaling pathways possibly via integrins and/or induction of TNC in experimental SAH models by endovascular perforation in mice (Liu et al., 2017) (Fig. 4). Our preliminary studies also showed periostin upregulation in the wall of cerebral artery and parenchymal arteriole, suggesting the involvement of periostin in vasospasm of both large artery and arteriole (Figs. 5, 6). In the same model, galectin-3 was upregulated in brain capillary endothelial cells to cause at least BBB disruption by the activation of TLR4, MAPK, signal transducer and activator of transcription-3 and MMP-9 (Nishikawa et al., 2018a). In a rat model of SAH by endovascular perforation, osteopontin was induced in neurons, astrocytes, and microglia at 24 hours associated with neurological deterioration (Sun et al., 2019) and in endothelial and smooth muscle cells in the cerebral artery wall, reactive astrocytes and capillary endothelial cells at 72 hours post-SAH when rat's neurological status recovers (Suzuki et al., 2010b; Enkhjargal et al., 2019; Wang et al., 2019). Osteopontin's expression peaked at 24 hours in neurons



and at 72 hours in other cells, and osteopontin exerted neuroprotective effects against cerebral vasospasm, autoregulatory or microcirculatory dysfunction, BBB disruption and neuronal apoptosis (Suzuki et al., 2010a, 2010b; Wu et al., 2016; Enkhjargal et al., 2019; Sun et al., 2019; Wang et al., 2019). The expression of osteopontin may be upregulated at 24 hours to exert neuroprotective effects against neuronal apoptosis by global cerebral ischemia at the onset of SAH.



**Fig. 4.** Immunohistochemical staining of periostin counterstained with hematoxylin in the brain parenchyma at 24 hours after subarachnoid hemorrhage by endovascular perforation in mice. Capillary endothelial cells (arrows) and neurons (arrowheads) are immunopositive for periostin.

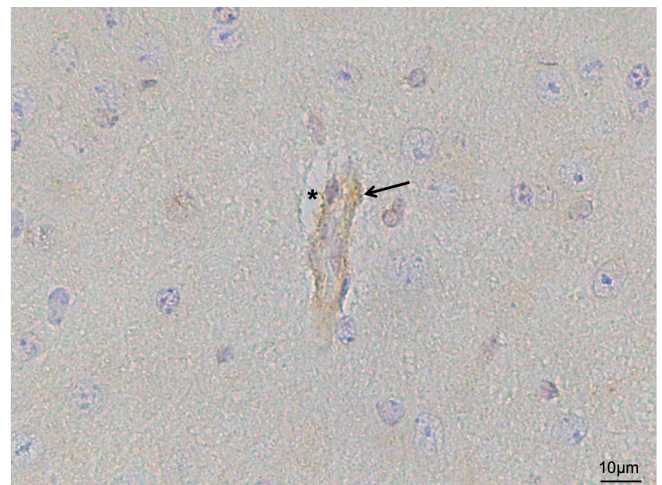


**Fig. 5.** Immunohistochemical staining of periostin counterstained with hematoxylin in the middle cerebral artery at 24 hours after subarachnoid hemorrhage by endovascular perforation in mice. The artery shows vasospasm associated with periostin-positive endothelial and smooth muscle cells as well as adventitial layers (arrow).

Osteopontin may antagonize TNC's effects by inactivating NF- $\kappa$ B, activating an endogenous MAPK inhibitor, MAPK phosphatase-1, and/or inhibiting TNC's binding to the receptors competitively, because osteopontin and TNC may share some receptors (Suzuki et al., 2020a). There are a lot of matricellular proteins that have never been examined in the context of DCI or pathophysiological processes after SAH. It is also interesting to investigate the possible interactions among matricellular proteins in the extracellular space or inside the cells, because they may provide each other with crosslinking functions (Suzuki et al., 2020a). Future studies will clarify the involvement and functional roles of matricellular proteins in post-SAH DCI, and determine how each matricellular protein orchestrates the various phases of EBI and DCI after SAH.

### Perspective

The pathophysiology of cerebral vasospasm is not completely unveiled, but some mechanisms of cerebral vasospasm may be shared or interrelated with that of microcirculatory disturbance. If it is true that microcirculatory disturbance is a more important outcome determinant than cerebral vasospasm, we should consider why anti-vasospasm drugs have failed to improve microcirculatory disturbance. As one plausible explanation, a limited therapeutic time window might be considered. EBI begins to develop within minutes after onset of SAH, while cerebral vasospasm generally develops after 72 hours of SAH in humans. If EBI causes DCI due to microcirculatory disturbance unrelated to cerebral vasospasm, early administration of a drug even within 24 to 72 hours post-SAH might be



**Fig. 6.** Immunohistochemical staining of periostin counterstained with hematoxylin in the brain parenchyma at 24 hours after subarachnoid hemorrhage by endovascular perforation in mice. A constricted parenchymal arteriole (arrow) is immunopositive for periostin, associated with increased paravascular space (asterisk).

too late to prevent the microcirculatory disturbance, although it could be enough for the prevention of cerebral vasospasm. Another possibility is that previously tested drugs reach vascular wall cells such as endothelial and smooth muscle cells, but not effectively parenchymal cells such as pericytes, astrocytes, microglia and neurons due to BBB. It appears that intraparenchymal vessel-related pathologies of DCI involve several cell types within the neurovascular unit and other cells collectively, which do not exist in the walls of pial and perforating arteries, driving microvascular dysfunction after SAH via different pathological pathways or cascades like clusters of CSD from cerebral vasospasm. However, we should remember that most findings regarding microvascular dysfunction after SAH have been provided based on experimental studies using rats or mice. In most SAH models in rats and mice, EBI and cerebral vasospasm as well as microvascular dysfunction have been examined in the time frame of 72 hours, although the time frame of 72 hours for EBI occurrence derives from pre-vasospasm period of human SAH (Suzuki, 2015). Therefore, it is not clear whether microvascular dysfunction observed in the study is an underlying mechanism of EBI or DCI. Microvascular dysfunction is an attractive mechanism to explain the cause of poor outcomes independently of cerebral vasospasm, but needs to be studied more to clarify the pathophysiology or mechanisms and to develop novel therapeutic strategies against it. As pathologies underlying microvascular dysfunction, microvasospasm, microthrombosis, dysfunction of venous outflow and compression of microvasculature by vasogenic or cytotoxic tissue edema are considered, and excitotoxicity may also contribute to DCI via cortical spreading ischemia and epileptic activity-related events. Many kinds of cells in the vascular wall and the brain parenchyma as well as the impairment of the paravascular and glymphatic systems may be involved in the pathogenesis of DCI. As molecular targets, matricellular proteins, TLR4 and glutamate may be promising in addition to some kind of inflammatory molecules, because they may be related to multiple underlying mechanisms of EBI and DCI. We hope that the information in this article will help to advance SAH research.

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**Conflict of Interest.** The authors declare that they have no conflict of interest.

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