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REVIEW ARTICLE

The role of the microcirculation in delayed cerebral ischemia and chronic degenerative changes after subarachnoid hemorrhage

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The mortality after aneurysmal subarachnoid hemorrhage (SAH) is 50%, and most survivors suffer severe functional and cognitive deficits. Half of SAH patients deteriorate 5 to 14 days after the initial bleeding, so-called delayed cerebral ischemia (DCI). Although often attributed to vasospasms, DCI may develop in the absence of angiographic vasospasms, and therapeutic reversal of angiographic vasospasms fails to improve patient outcome. The etiology of chronic neurodegenerative changes after SAH remains poorly understood. Brain oxygenation depends on both cerebral blood flow (CBF) and its microscopic distribution, the so-called capillary transit time heterogeneity (CTH). In theory, increased CTH can therefore lead to tissue hypoxia in the absence of severe CBF reductions, whereas reductions in CBF, paradoxically, improve brain oxygenation if CTH is critically elevated. We review potential sources of elevated CTH after SAH. Pericyte constrictions in relation to the initial ischemic episode and subsequent oxidative stress, nitric oxide depletion during the pericapillary clearance of oxyhemoglobin, vasogenic edema, leukocytosis, and astrocytic endfeet swelling are identified as potential sources of elevated CTH, and hence of metabolic derangement, after SAH. Irreversible changes in capillary morphology and function are predicted to contribute to long-term relative tissue hypoxia, inflammation, and neurodegeneration. We discuss diagnostic and therapeutic implications of these predictions.

INTRODUCTION

Aneurysmal subarachnoid hemorrhage (SAH) is caused by the rupture of a cerebral aneurysm, and the subsequent accumulation of blood in the subarachnoid space.[1] The age-standardized incidence of SAH is 6 to 7 per 100,000 citizens per year in most countries, but 20 per 100,000 citizens per year in Finland and Japan.[1,2] Half of the patients are below the age of 55 at the time of their SAH,[1] and only half of the SAH patients are alive one month after the bleeding.[3] Less than 40% of those who survive a SAH are able to return to their previous occupation, and 44% to 93% of the survivors experience restrictions in instrumental activities of daily living.[4] The majority of patients experience impaired memory, executive function, and language function in the months, and in some cases years, after their SAH.[4] More importantly, permanent neurocognitive symptoms such as fatigue, depression, anxiety, and sleep disorders affect the quality of life of the majority of SAH survivors.[4]

Early Brain Injury

The majority of deaths after SAH occur within 2 days of the bleeding.[5] The release of arterial blood into the subarachnoid space is accompanied by intense headache and an acute increase in intracranial pressure, often causing intracranial circulatory arrest and loss of consciousness.[6,7] The mechanisms of the resulting early brain injury are dominated by cell death, blood–brain barrier (BBB) disruption, and brain edema—see ref. 8 for a comprehensive review. The brain edema is predominantly caused by extravasation of plasma across a leaking BBB (vasogenic edema): Animal models show BBB disruption as early as 30 minutes after cortical SAH,[9] and the leakage of large molecules remains high within the first 48 hours of the bleeding, after which it normalizes in some species— see ref. 10 and Table 2 therein. In humans, increased blood–brain barrier permeability to diagnostic contrast agents[9,11] and radiographic signs of global edema[12] can be observed within 5 to 6 days of the SAH. Studies using diffusion-weighted MRI confirm the formation of diffuse edema acutely after SAH in animal models[13] and within the first week in SAH patients.[14] Importantly, radiographic signs of global edema based on computerized tomography during hospitalization is an independent predictor of death, severe disability, and poor cognitive outcome at 3-month follow-up.[12,15]

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Delayed Cerebral Ischemia

Despite appropriate treatment of the ruptured aneurysm, as many as half of the SAH patients develop reduced levels of consciousness and/or focal neurologic deficits 5 to 14 days after the initial bleeding, so-called delayed cerebral ischemia (DCI).[1] The symptoms are poorly localized and develop gradually over hours, suggesting a progressing, global disease process.[16] The development of ischemic damage often coincides with the emergence of vasospasm—widespread constrictions throughout the cerebrovasculature.[17]

Causes of Vasospasm: Vessel Responses to Subarachnoid Blood and Blood Breakdown Products

After the release of blood into the subarachnoid space in humans, immune cells infiltrate the meninges within hours, and erythrocytes are gradually removed by phagocytosis and hemolytic breakdown.[18] Powerful vasoconstrictors such as thromboxane, endothelin-1, serotonin, platelet-activating factor, and 20-hydoxyeicosatetraenoid acid are hence found in increased levels in the cerebrospinal fluid (CSF) after the hemorrhage.[19,20] Endothelin-1 has received special attention in that the combination of oxyhemoglobin (HgbO) and endothelin-1 can elicit ischemia and spreading depolarizations (SDs),[21] an important feature of human DCI.[22] Spreading depolarizations are self-propagating tissue depolarizations associated with cessation of synaptic activity, surges of extracellular potassium, opening of the BBB with edema formation,[23] tissue hypoxia,[24] and inversion of the normal CBF responses to neuroglial activity.[21]

Hemolysis peaks after approximately 1 week,[18] and the period of the most intense angiographic vasospasms thus coincides with peaking levels of HgbO in the subarachnoid space in both humans[18] and primates.[25] Studies have shown that hemoglobin breakdown products can affect vessel tone in several ways—see refs. 18 and 26 for detailed reviews. First, the spontaneous autoxidation of HgbO to methemoglobin, and the iron released from hemoglobin, cause the release of highly reactive superoxide radicals.[18,26] Superoxides are thought to cause vasoconstriction by depleting vascular nitric oxide (NO) levels[22,27] and to cause lipid peroxidation, which in turn causes vasoconstriction and structural damage to the cerebral arteries, including the endothelial cell layer.[28] Second, the breakdown of heme into bilirubin under such oxidative conditions results in the formation of bilirubin oxidation products that change the contractility, signaling, and metabolism in large vessels—see ref. 29 for a review. Bilirubin is produced during the time period associated with DCI, and CSF levels of bilirubin oxidation products are higher in patients who develop DCI than in those who do not.[30] Third, HgbO has very high affinity for the NO and acts as a sink for this vasodilator.[18] Finally, NO production is reduced after SAH, first as neuronal nitric oxide synthetase disappears from nerve fibers in the arterial adventitia,[31] and later, when elevated levels of asymmetric dimethyl arginine inhibit the activity of endothelial NOS.[32] These reductions in NOS availability and activity have been shown in relation to the development of vasospasm in animal models[31] and patients,[33] respectively.

The Relation between Vasospasm and Delayed Cerebral Ischemia

Delayed cerebral ischemia and SD may develop in the absence of angiographic vasospasm, just as angiographic vasospasms may resolve without causing ischemic lesions.[17,34,35] Disappointingly, treatment with clazosentan, an endothelin receptor antagonist that effectively resolves angiographic vasospasms, has failed to reduce mortality, DCI-related morbidity, or functional outcome in relation to SAH.[36–38] It is now believed that vasospasms, rather than causing ischemic damage in their own right, render brain tissue vulnerable to the development of SD which, in turn, cause ischemic lesions—the so-called Double Hit Model of DCI.[39]

According to this model, the initial bleeding causes generalized macro- and microvascular vasospasm (first hit), whereas the ensuing spreading depressions and further vasoconstrictions cause a critical energy depletion that result in irreversible ischemic damage (second hit).[40] The presence of SD indeed correlates with the development of DCI in animal models of SAH and in SAH patients,[40,41] and the inversion of normal arteriolar responses (to elicit vasoconstriction rather than vasodilation) during SD was recently attributed to increased Ca^{++} oscillations in astrocytes, caused by the presence of blood degradation products.[40,42] The combined increase in perivascular K^+ concentration in relation to hemolysis,[39] elevated K^+ efflux from astrocytic endfeet, and further K^+ efflux during SD, are thought to elevate K^+ concentrations above a critical threshold at which vascular responses are inverted.[37]

Long-Term Brain Atrophy

The origin of the long-term cognitive symptoms after SAH remains poorly understood.[4] Studies that have compared cognitive outcome scores with lesion location after SAH, suggest that the loss of some aspects of executive function can be ascribed to ischemic lesions in specific brain regions.[43] Meanwhile, studies performed 1 year after SAH find ventricular dilation and sulcal enlargement that suggest general atrophy.[44] Importantly, outcome and neuropsychological scores after SAH appear to correlate with total atrophy,[44,45] cortical atrophy,[46] and hippocampal atrophy.[47] The long-term structural and neuropsychological effects of SAH therefore resemble those of neurodegenerative and neuropsychiatric disorders such as Alzheimer's disease, mild cognitive impairment, posttraumatic stress disorder, and depression.[47]

THE METABOLIC ROLE OF CHANGE IN CAPILLARY MORPHOLOGY AND PERICAPILLARY EDEMA AFTER SUBARACHNOID HEMORRHAGE

The complex pathophysiology of SAH raises several questions, which we attempt to address from the perspective of changes in the capillary circulation below: Why did clazosentan, a drug that not only restores vessel diameters in angiographic vasospasm, but also restores cerebral blood flow (CBF) to values above the ischemic thresholds,[48] fail to improve patient outcome? What are the roles of BBB damage and tissue edema in the development of DCI—if any? Can changes in the morphology and function of capillaries reviewed below contribute to the development of DCI, and to the risk of long-term, diffuse atrophy and poor neuropsychological outcome?

We recently showed that the availability of oxygen in brain tissue depends not only on the CBF, but also on the microscopic distribution of the blood, the so-called capillary transit time heterogeneity (CTH).[49] By a model that determines the availability of oxygen in tissue for a given CBF, CTH, and tissue oxygen tension, we have analyzed the metabolic effects of gradual increases in CTH, which we expect to parallel changes in capillary morphology in ageing and disease.[50,51] We found that as CTH increases, oxygenated blood is increasingly shunted through the capillary bed. To maintain sufficient oxygen availability to support neuronal function and survival, we showed that the vasculature must attenuate CBF responses (and ultimately resting CBF) to improve blood–tissue oxygen concentration gradients and blood–tissue oxygen exchange times. The resulting vascular oxidative stress and tissue hypoxia, however, comes at the expense of increased thrombogenicity, tissue inflammation, and neurodegenerative changes, which we have proposed may have a role in the etiopathogenesis of Alzheimer's disease[51] and ischemia–reperfusion injury.[50]

Below, we review the changes in the capillary morphology and function that are known or likely to occur in relation to SAH, and
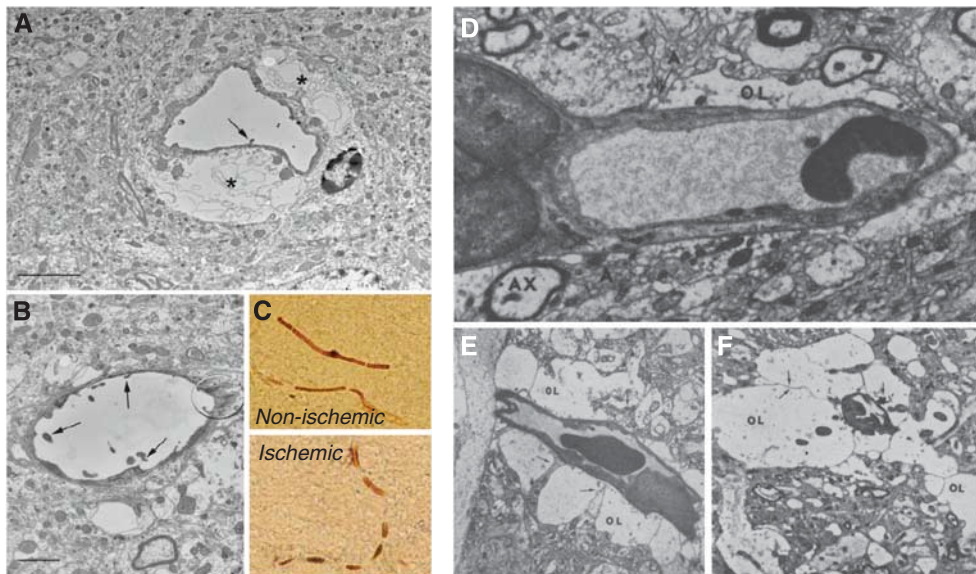
Figure 1. Changes in the capillary morphology after subarachnoid hemorrhage (SAH), ischemia, and hypotonic hyponatremia. Panels **A** and **B** show swelling of astrocytic endfeet (*) and endothelial protrusions (arrows) after SAH. Scale bars indicate 5 μm in panel **A** and 2 μm in panel **B**, respectively. Reproduced from ref. 52 with permission from the publisher. Panel **C** shows segmental narrowing of capillaries due to capillary constrictions after ischemia and reperfusion (bottom) compared with slender, thread-like, horseradish peroxidase-filled capillaries in the normal hemisphere (top). Reproduced from ref. 53 with permission from the publisher. Panels **D** and **E** show tangential sections through capillaries in sham-operated brain (**D**) and in a hypotonic hyponatremic edema model (**E**) in rabbit. The distended astrocytic endfeet (OL), the membranes of which are marked by arrows, clearly compress the capillary lumen, as shown in the transverse section (**F**). Other astrocytic membranes are labeled 'A', and axons 'AX'. The magnifications were $\times 6500$ (**D** and **E**) and $\times 5000$ (**F**), respectively. Reproduced from ref. 59 with permission from the publisher.

how any accompanying changes in CTH may interfere with oxygen availability and tissue microenvironment in the various phases of disease progression after SAH.

Changes in Capillary Morphology and Blood–Brain Barrier Function after Subarachnoid Hemorrhage

Sehba and Friedrich[52] recently reviewed the molecular and morphologic changes that occur in the capillary wall in relation to SAH. These changes involve the development of luminal endothelial protrusions that are thought to restrict capillary flows, and swelling of astrocytic endfeet that cause compression of the capillary lumen—see Figures 1A and 1B. In addition, cerebral ischemia has been shown to result in the constriction of cerebral pericytes, seemingly due to the increased levels of oxidative and nitrosative stress[53,54]—see Figure 1C. Experimental studies have shown profound damage to both capillary basement membrane and endothelial cells, followed by disruptions of the blood–brain barrier (BBB) and development of vasogenic edema in both experimental ischemia and SAH.[55,56] These changes are paralleled by openings of tight junctions between capillary endothelial cells in models of SAH,[9,52] and likely to be exacerbated by SDs, during which swelling of astrocytic endfeet[24] and BBB breakdown[23] occur.

Up to 57% of SAH patients develop hypovolemic hyponatremia in the week after the initial bleeding.[57] The effects of hyponatremia on cerebral and pericapillary swelling in humans remain unclear, but animal models of osmotic brain edema induced by hyposmotic hyponatremia (intraperitoneal or central venous injection of distilled water) show both brain swelling[58] and capillary compression owing to profound swelling of astrocytic endfeet[59]—see Figures 1D–1F.

Exposure of Capillaries to Vasoactive Substances after Subarachnoid Hemorrhage

In the absence of lymphatic vessels, interstitial fluid and solutes from brain parenchyma are removed along the basement membranes of arteries and capillaries to the cervical lymph nodes.[60] Recent studies of the clearance of solutes from the CSF in mice show that after intracisternal injection, molecules in the size range 3 to 2,000 kDa distribute rapidly along penetrating arteries into brain tissue, along the basement membranes of arterioles and capillaries, after which they drain into the cervical lymph nodes.[61] The molecular weight of the hemoglobin tetramer falls within this size range (64 kDa), and reports on the distribution of horseradish peroxidase[62] (44 kDA) and albumin (66 kDa)[63] confirm that hemoglobin and its breakdown products are likely to be cleared from the subarachnoid space via perivascular transport to the cervical lymph nodes. The clearance of CSF and interstitial fluid may be disturbed after SAH, but animal studies in which the cervical lymph drainage has been blocked suggest that the perivascular pathway is active and crucial for fluid drainage after SAH.[64,65] It is therefore likely that not only smooth muscle cells, but also pericytes, are exposed to high concentrations (similar to or above those found in CSF) of hemoglobin and other vasoactive substances as these are transported through the narrow basement membranes in which these contractile cells are embedded.[61]

The control of pericyte tone remains much less studied than that of arterioles.[66] The vasoactive substances released after SAH are likely to interfere with both arteriolar and pericyte tone. In vitro experiments suggest that NO acts as a pericyte dilator[67,68] whereas the oxidative and nitrosative stress that result from the release of superoxide radicals have been shown to cause pericyte constrictions in vitro[53] and in vivo.[53] Furthermore, brain pericytes express endothelin-1 receptors,[69] and in vitro studies suggest that pericytes and capillaries constrict upon endothelin-1 exposure.[70,71] The period of maximum vasospasm in cerebral resistance vessels is therefore likely to coincide with a period of poor vascular control, or even constrictions, throughout the capillary bed. In the section below, we describe how any resulting changes in capillary flow patterns can affect tissue oxygenation, independent of any vasoconstrictions at either the arteriolar or arterial level.
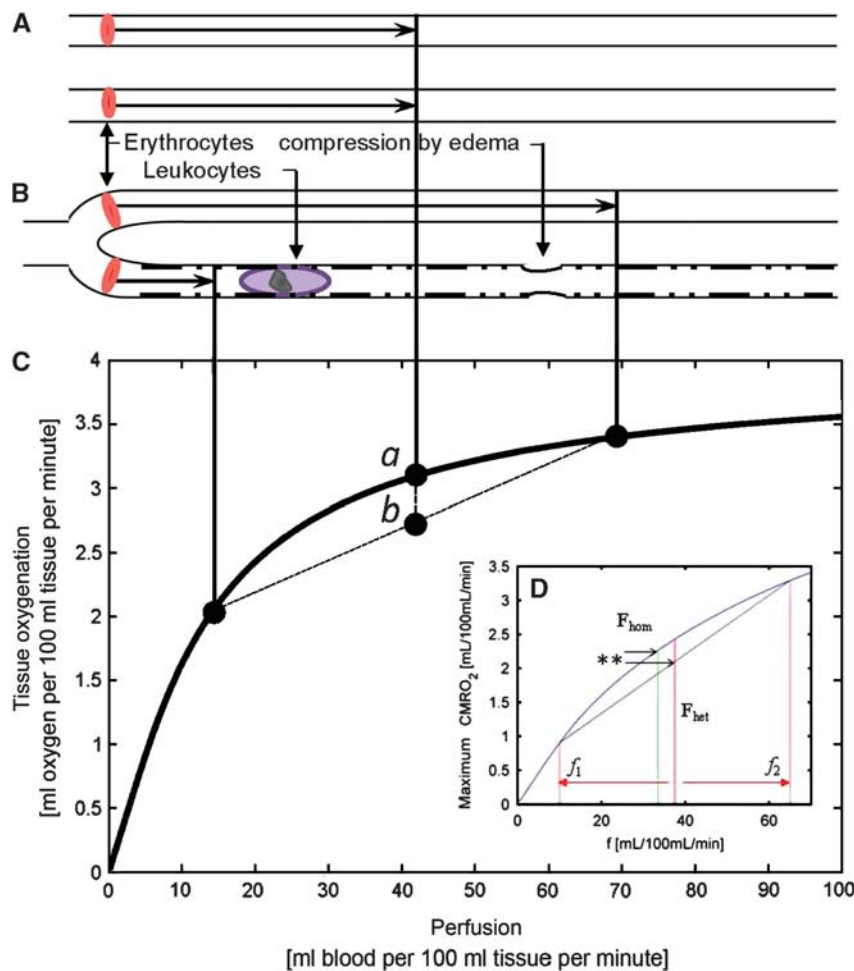
Figure 2. The relation between blood flow and net oxygen extraction in single capillaries. The curve in plot **C** shows the so-called flow–diffusion equation[72] for oxygen, that is, shows the maximum amount of oxygen that can diffuse from a single capillary into tissue, for a given perfusion rate. The curve shape predicts three important properties of parallel-coupled capillaries: First, the curve slope decreases towards high-perfusion values, making vasodilation increasingly inefficient as a means of improving tissue oxygenation towards high-perfusion rates. Second, if erythrocyte flows differ among capillary paths (case **B**) instead of being equal (case **A**), then net tissue oxygen availability declines. This can be observed by using the curve to determine the net tissue oxygen availability resulting from the individual flows in case **B**. The resulting net tissue oxygen availability is the weighted average of the oxygen availabilities for the two flows, labeled b in the plot. Note that the resulting tissue oxygen availability will always be less than that of the homogenous case, labeled a. Conversely, homogenization of capillary flows during hyperemia has the opposite effect, and serves to compensate for the first property. By a similar argument, insert **D** disproves the traditional assumption that increased perfusion always results in improved tissue oxygenation:[49] By increasing tissue perfusion from F_{hom} to F_{het}, and again subdividing capillary flows in the latter case into f_1 and f_2, tissue oxygen availability in fact decreases in response to a flow increase, as indicated by the double asterisk. Note that, if erythrocyte flows are hindered (rather than continuously redistributed) along single capillary paths (as indicated by slow-passing white blood cell (WBC) and/or rugged capillary walls), upstream vasodilation is likely to amplify the redistribution losses, as erythrocytes are forced through other branches at very high speeds, with negligible net oxygenation gains. Reproduced from refs. 49 and 50.

THE RELATION BETWEEN ERYTHROCYTE VELOCITIES AND OXYGEN EXTRACTION IN CAPILLARIES

Figure 2 illustrates how the heterogeneity of erythrocyte velocities, as it occurs either naturally or because of the disturbances in the capillary wall or in blood cell morphology, reduces the efficacy of oxygen extraction from blood. As illustrated by the figure, both white blood cell and erythrocyte dimensions exceed the average capillary diameter, and these cells must therefore undergo deformation to enter and pass the capillaries. Changes in the adhesion of blood cells to endothelium have been shown in SAH,[73] and such changes are known to disturb capillary flow patterns and lead to 'shunting' of erythrocytes through the capillary bed.[74] Studies by direct microscopy in animals,[75,76] and by perfusion MRI in human

stroke,[77–79] confirm that capillary flow patterns undergo profound changes in cerebral ischemia.

THE COMBINED EFFECT OF CEREBRAL BLOOD FLOW, CAPILLARY TRANSIT TIME HETEROGENEITY, AND TISSUE OXYGEN TENSION ON BRAIN OXYGENATION

Figure 3 shows how blood mean capillary transit time (MTT), CTH, and tissue oxygen tension (along the three axes) in combination can secure sufficient oxygen to support normal brain function—see also ref. 50. Mean capillary transit time (MTT) is given by the central volume theorem[81] as the ratio between capillary blood volume and CBF, which is shown in the secondary x-axis for convenience. The green surface show all combinations
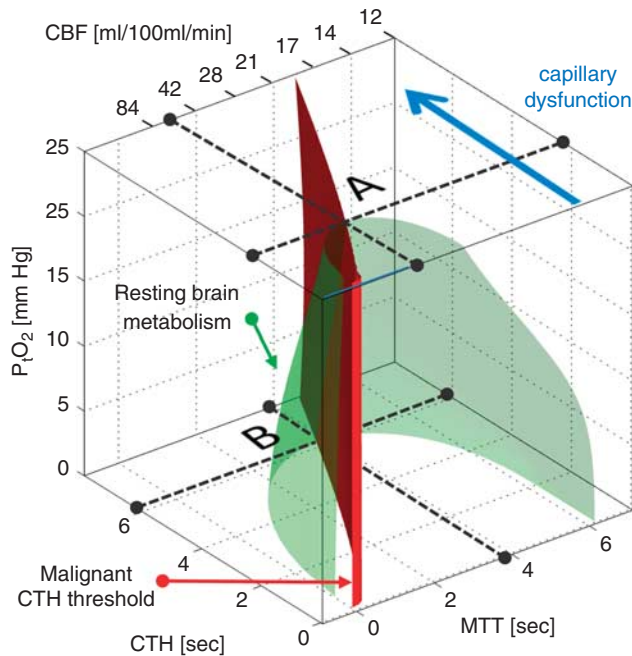
Figure 3. Metabolic thresholds. The green iso-contour surface corresponds to the metabolic rate of contralateral tissue in patients with focal ischemia.[80] The red plane marks the boundary, left of which vasodilation fails to increase tissue oxygen availability (malignant capillary transit time heterogeneity (CTH)). The maximum value that CTH can attain at a tissue oxygen tension (P_tO_2) of 25 mm Hg, if oxygen availability is to remain above that of resting tissue, is indicated by the label **A**. As CTH increases further, a critical limit is reached as P_tO_2 approaches 0—label **B**. At this stage, the metabolic needs of tissue cannot be supported unless mean transit time (MTT) is prolonged to a threshold of approximately 4 seconds, corresponding to cerebral blood flow (CBF) = 21 mL/100 mL/minute. Modified from ref. 50.

of MTT (or CBF), CTH, and tissue oxygen tension that make 2.5 mL oxygen available to each 100 mL of tissue per minute. This rate of oxygen delivery matches the metabolic need of resting brain tissue,[80] and the interior of the green half-cone therefore represents hemodynamic conditions that can support normal brain function.

The figure illustrates why, theoretically, reductions in tissue oxygen tension *and* resting CBF are the only alternatives to immediate tissue damage if CTH increases uncontrollably. Label **A** in Figure 3 shows the theoretical maximum for the increase in CTH (indicated by a broken line parallel to the MTT and CBF axes) that brain tissue can sustain at the tissue oxygen tension of normal brain tissue (25 mm Hg)[82] before neurologic symptoms ensue. As CTH approaches this limit, oxygen availability gradually approaches the tissue consumption, causing tissue oxygen tension to decrease.[50] At lower tissue oxygen tensions, the green cone is wider, and tissue can therefore accommodate increases in CTH until oxygen tension cannot be reduced further (label **B**). Note that as CTH increases, CBF must be attenuated (MTT prolonged) to meet the metabolic needs of the tissue. As CTH reaches this maximum and tissue oxygen tension becomes negligible, CBF is predicted to approach 21 mL/100 mL/minute. So, if CBF is indeed adjusted to optimize brain oxygenation as capillary flow patterns become increasingly disturbed, CBF values would therefore be predicted to be close to the classic ischemic threshold of 20 mL/100 mL/minute[83] when symptoms arise. Accordingly, this threshold may not only be characteristic of vascular narrowing/occlusion, but also of CBF

adaptations to secure sufficient oxygenation under conditions of critically elevated CTH, caused by changes in the capillary flow patterns.[50]

THE DYNAMICS OF CEREBRAL BLOOD FLOW, CEREBRAL BLOOD FLOW RESPONSES, AND OXYGEN EXTRACTION FRACTION AS CAPILLARY TRANSIT TIME HETEROGENEITY INCREASES: THE THREE STAGES

Figure 4A illustrates the dynamics of capillary flow patterns during rest and during increased metabolic needs in the normal brain. The flux of erythrocytes through the cortical brain capillaries is highly inhomogeneous during rest,[84–86] and the limited increase in net extraction of oxygen as flow in individual capillaries is increased (cf. Figure 2D) therefore contribute to the modest (30%) net oxygen extraction fraction (OEF) in the resting brain.[49] A range of CBF-modifying stimuli cause parallel homogenizations of capillary flow patterns, including functional hyperemia,[49] hypocapnia,[87] hypercapnia,[88] and hypoxemia.[89] We recently showed that the combined reductions in MTT and CTH during functional hyperemia seemingly ensure close coupling of oxygen availability to the metabolic needs of the tissue by this additional, neurocapillary coupling.[49]

Mild Capillary Transit Time Heterogeneity Increase: The Hyperemic Stage

Figure 4B illustrates the metabolic consequence of *capillary dysfunction*: disturbances that elevate flow heterogeneity, and prevent the normal flow homogenization during hyperemia. Elevated CTH reduces the OEF that can be attained for a given tissue oxygen tension,[49] and the metabolic needs of tissue can therefore be met by slight increases in CBF, both during rest and during hyperemia (e.g. functional activation or hypercapnia). We therefore refer to states of mild CTH increases as hyperemic.

Increases in the blood flow velocity in the intracranial vessel are indeed observed in the days after SAH in both animal models[90] and patients[91] by transcranial Doppler sonography (TCD). Increased flow velocities may, in principle, be caused by either or both relative vasoconstriction and increased CBF.[92] Parallel recordings of arteriovenous oxygen differences and TCD flow velocities in the first days after SAH, however, show *decreased* OEF during the gradual increase in flow velocity, consistent with such relative hyperemia.[93] Similarly, direct measurements of CBF show occasional hyperemia and early reductions in OEF in patients after SAH.[94] The molecular underpinnings of the early vasodilation after SAH have been explored in animal models: within the first few days of SAH, the production of NO indeed appears to be upregulated in the walls of pial arterioles (in contrast to the subsequent downregulation reviewed above), as evidenced by increased expression of endothelial endothelial NOS and increased levels of NO breakdown products.[95]

Moderate Capillary Transit Time Heterogeneity Increase: The Flow Suppression Stage

As changes in capillary or blood morphology accumulate and CTH increases further, increases in CBF can no longer compensate for the parallel reduction in OEF[max]. The slope of the curve that depicts oxygen extraction as a function of blood flow (cf. Figure 2C) becomes more and more horizontal towards high-flow values, indicating that, as resting CBF increases, (additional) hyperemia becomes increasingly inefficient as a means of increasing oxygen availability during episodes of increased metabolic needs. As indicated by the insert in Figure 2D, CTH can become so high that increases in CBF no longer increase oxygen availability. Note that, in the absence of a mechanism that blocks further vasodilation in cases where this fails to improve
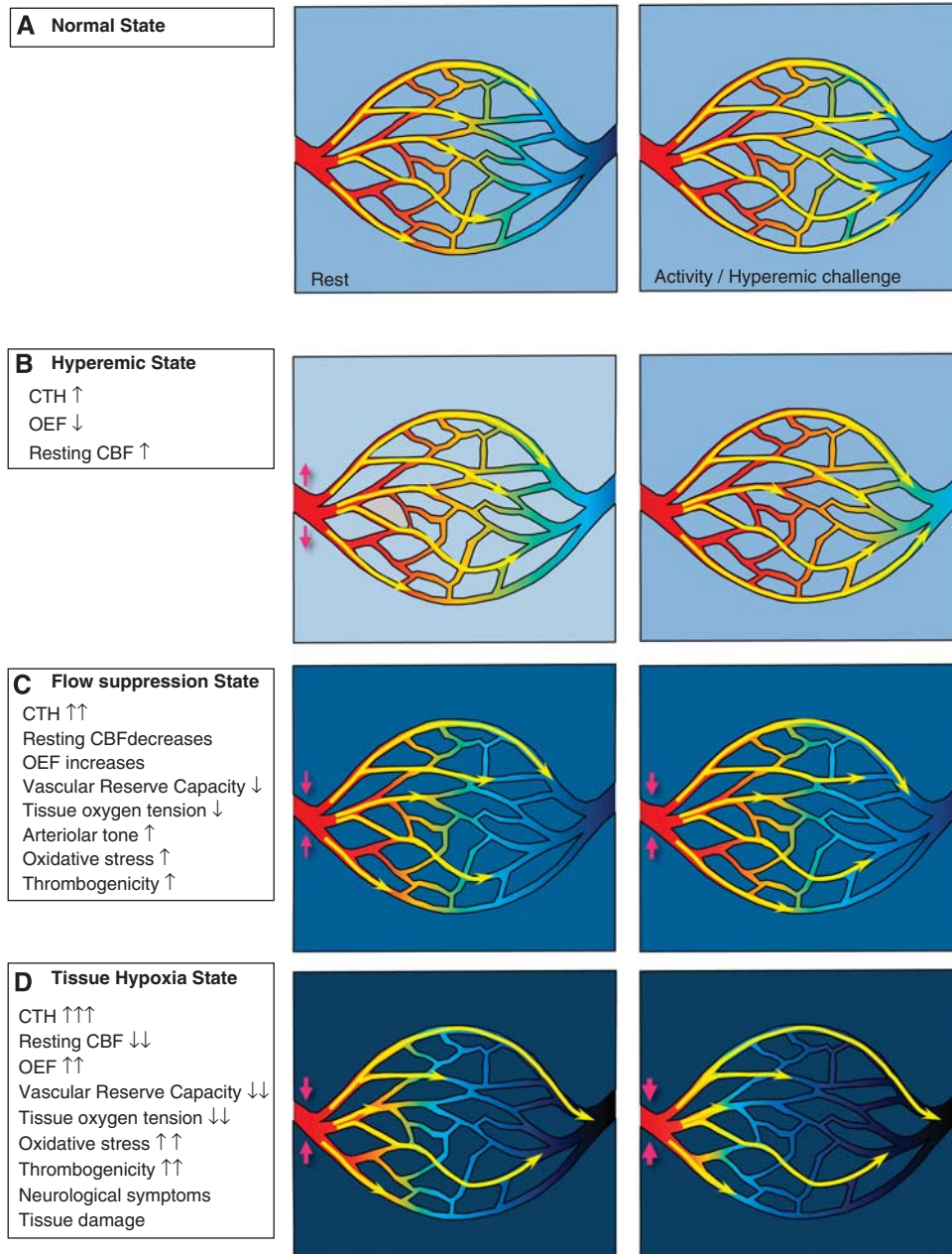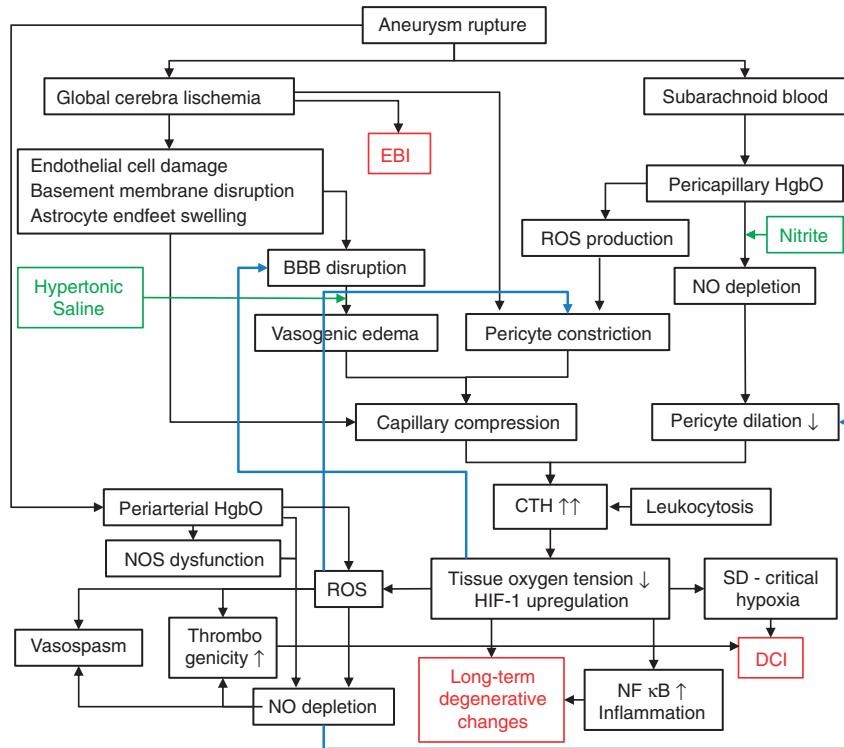
Figure 4. The subarachnoid hemorrhage (SAH) stages. The yellow arrows indicate flow with different velocities through the capillary system. The color within the vessels indicates oxygen saturation, and the background color outside the tissue oxygen tension. (**A**) The normal state, (**B**) the hyperemic state, where slight capillary transit time heterogeneity (CTH) increases lead to increased cerebral blood flow (CBF). (**C**) The flow suppression state. (**D**) Tissue hypoxia. OEF, oxygen extraction fraction.

tissue oxygenation, a state of high CTH is predicted to lead to uncontrolled hyperperfusion and hypoxic tissue damage, such as it is observed in the luxury perfusion syndrome.[96] This phenomenon, and reperfusion injury, is discussed further in refs. 49 and 50. Our analysis shows that, provided CBF is suppressed, the resulting reduction in tissue oxygen tension improves blood–tissue concentration gradients and OEF^{max} so much that oxygenation for brain function can be secured.[49] The prediction that the cerebral vasculature attempts to suppress any CBF increases is consistent with the finding that vasodilatory responses, referred to as the cerebrovascular reserve capacity, are reduced between days 3 and 13 in patients with SAH.[97]

The Hypoxic Stage: Short-Term Tissue Damage or Long-Term Neurodegeneration.

If CTH increases even further, the reduction of tissue oxygen tension can contribute to tissue damage in several ways. First, the reduction of tissue oxygen tension is likely to increase the probability of devastating SDs.[40] Second, the reduction of tissue oxygen tension activates hypoxia-inducible transcription factors (HIFs). Increased HIFs-1 levels is a powerful stimulus for BBB opening and edema formation in SAH,[98] contributing to the vicious cycle of further CTH increase and hypoxia as indicated in Figure 5. Third, HIF-1 also upregulates nicotinamide adenine dinucleotide phosphate oxidase 2 levels,[99] the main source of

Figure 5. The figure summarizes the pathways from the acute subarachnoid hemorrhage to early brain injury (EBI), delayed cerebral ischemia (DCI), and the long-term degenerative changes that are hypothesized to be the result of irreversible increases in capillary transit time heterogeneity (CTH). Nitric oxide synthetase (NOS) dysfunction refers to the loss of neuronal nitric oxide synthetase (nNOS) from nerve fibers in the arterial adventitia, and the inhibition of endothelial NOS (eNOS) by asymmetric dimethyl arginine (ADMA)—see text. The blue arrows indicate 'vicious cycles' through which low oxygen tension exacerbates blood–brain barrier (BBB) disruption and capillary flow disturbances. The green text boxes indicate possible sites of therapeutic intervention discussed in the text. HgbO, oxyhemoglobin; NO, nitric oxide; ROS, reactive oxygen species; SD, spreading depolarizations.

reactive oxygen species in SAH.[100] Reactive oxygen species reacts with NO to form peroxynitrite.[101] Although NO depletion and peroxynitrite both cause vasoconstriction by impairing normal smooth muscle cell relaxation,[102] peroxynitrite also inactivates tissue plasminogen activator, increasing thrombogenicity, and thereby the risk of further tissue damage.[103] The latter is consistent with the recent demonstration that the extent of microthrombosis correlates with low levels of NO in models of SAH.[104] Microvascular constrictions and microthrombosis have been demonstrated within 3 hours of experimental SAH,[105] suggesting that flow suppression, and possibly relative tissue hypoxia may be present early after SAH, possibly in relation to pericyte constrictions and early BBB opening. Finally, hypoxia and peroxynitrite are also known to damage mitochondria.[102] Mitochondrial dysfunction, in turn, exacerbates the energy crisis by reducing the amount of ATP that can be obtained from the available oxygen, and amplifies the production of ROS.[102]

If CTH continues to increase, our model of oxygen availability predicts that oxygen reserves are exhausted as tissue oxygen tension become negligible and CBF approaches 21 mL/100 g/minute. This is consistent with global CBF values in patients who develop DCI, reported to range from 17 to 21 mL/100 mL/minute in some,[48,94,106] and up to 30–40 mL/100 mL/minute in others.[107–109]

If, however, (i) perivascular HgbO clearance is completed without causing critical capillary flow disturbances owing to the parallel NO depletions, (ii) capillary edema formation eventually becomes outbalanced by the normal resorption and removal of pericapillary fluid, and (iii) endothelial, basement membrane, pericyte, and astrocyte endfeet morphology and function normalize (cf. Figure 1), then the increase in CTH is predicted to halt and

potentially reverse. The additive effect of these factors, and their approximate time-scale, are illustrated by Figure 5B. According to this scenario, the reversal of hypoperfusion and angiographic vasospasm in SAH is hence in part the result of a gradual normalization of capillary flow patterns.

The extent to which the profound changes in the capillary wall morphology in relation to SAH are indeed reversible remains unclear. If not, residual CTH elevations are predicted to render tissue relatively hypoxic. Chronic hypoxia is associated with upregulation of hypoxia-inducible transcription factor-1 and nuclear factor NF-κB, a strong inflammatory signal[110] that might account for the acute and chronic inflammatory changes after SAH recently reviewed by Provencio.[73] The putative pathway from chronically elevated CTH to neurodegeneration is discussed in detail in ref. 51 The notion that the neurovascular changes in SAH survivors are permanent is supported by findings that their cerebrovascular reserve capacity in many cases remain low,[111,112] suggestive of persisting CTH elevation characteristic of the flow suppression stage described in Figure 4C.

DISCUSSION

The recent revision of the classic flow–diffusion equation to take the heterogeneity of the capillary flow patterns into account[49] implies that factors such as vasogenic edema, astrocytic endfeet swelling, and NO depletion during the pericapillary clearance of blood, may have profound implications for cerebral oxygenation after SAH. The analysis in this review suggests that the pathway from aneurysmal hemorrhage to DCI can be explained in part by adaptations to the increases in CTH that result from these

A Sources of capillary flow impairment

B Net CTH change
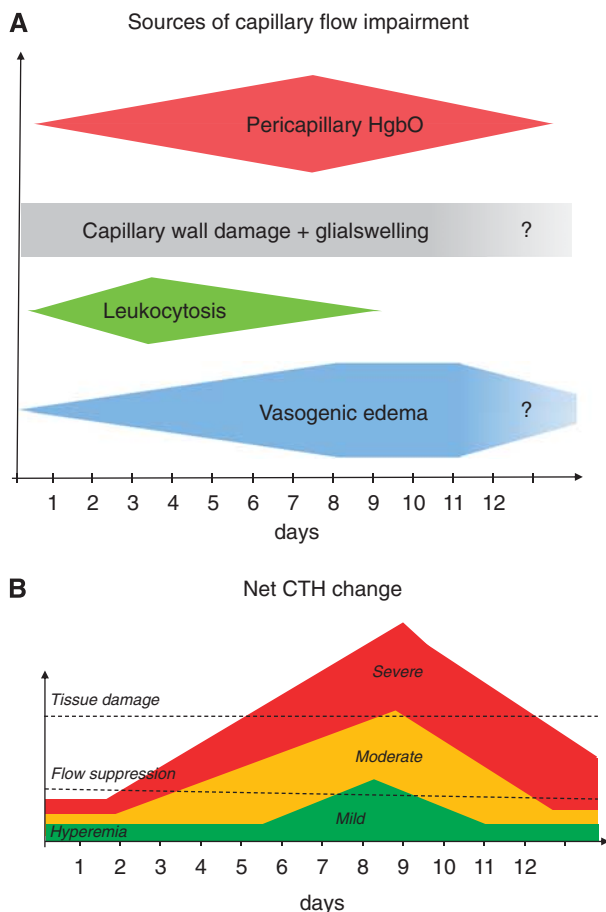
Figure 6. Panel **A** summarizes the sources of capillary flow disturbances as identified in this review, and their estimated duration. They include the oxidative stress and nitric oxide (NO) depletion caused by pericapillary oxyhemoglobin, structural damage to the capillary wall (including endothelial cells, basement membranes, pericytes, and astrocytic endfeet), leukocytosis, and compression by pericapillary edema. The extent to which the structural damage to the capillary wall is permanent remains crucial in that permanent changes in capillary transit time heterogeneity (CTH) are potential sources of long-term, neurodegenerative changes. Panel **B** indicates the added effect of the changes in panel **A** in terms of the resulting change in CTH over time. The time of the peak CTH change is likely to coincide with the peak pericapillary oxyhemoglobin HgbO concentration, whereas the height of the peak is likely to correlate with the size of the hematoma. The extent to which one observes flow increases by transcranial Doppler sonography (TCD), angiographic vasospasm, or tissue lesions by computed tomography (CT) or magnetic resonance imaging (MRI), is predicted to depend on the individual sizes of the factors in panel **A**. Note that large hematomas are likely to generate large net CTH increases owing to their larger, net capillary NO depletion, and thus to result in both angiographic vasospasm and hypoxic lesions.

factors—and that the degree of long-term neurodegenerative changes is determined by the extent to which these factors are reversible. An overview of the hypothesized chain of events is shown in Figure 5.

The additive effect of capillary NO depletion and (cytotoxic and vasogenic) edema in terms of the CTH increase, vasospasm (micro- and/or macrovascular, angiographic), and clinical deterioration after SAH is illustrated in Figure 6. The relative importance of edema formation may be gleaned from the puzzling difference in the outcome in clot-placement and filament-puncture models of SAH: in the former, little BBB damage and edema are detected,

and little if any mortality or poor functional outcome is observed.[113] In the latter, however, extensive BBB damage and edema develops, and the rates of mortality and neurologic and long-term cognitive deficits seemingly resemble those found in patients. Brain edema thus appears to contribute to the poor long-term outcome after SAH, while NO depletion during the perivascular clearance of blood breakdown no doubt contributes to the deterioration during days 6 to 12. Further evidence in support of CTH as an indicator of metabolic derangement after SAH is offered by the finding that leukocyte counts during the first 5 days after SAH seemingly predicts clinical deterioration, the development of angiographic vasospasm,[114] and death.[115] Increased number and endothelial adhesion of leukocytes are known to disturb capillary flow patterns and lead to 'shunting' of erythrocytes through the capillary bed.[74] Leukocytosis after SAH is thought to reflect endogenous catecholamine release, and after control for the use of steroids and other known predictors of the development of angiographic vasospasm, the peak number of lymphocytes 1 to 5 days after admission has been shown to be an independent predictor of the development of angiographic vasospasm.[116]

Diagnostic Implications

The model predicts that the progression of increased flow velocity on TCD (hyperemic stage) only in the most severe cases develop into angiographic vasospasm (flow suppression stage), and that neurologic symptoms or permanent tissue damage only develop if these adaptation fails to maintain tissue metabolism during the period of pericapillary edema (hypoxic stage and beyond). See Figure 6. This is consistent with the reports that increased flow velocities by TCD is a more frequent finding than angiographic stenosis, which again is more frequent than clinical and/or radiologic signs of insufficient tissue oxygenation in SAH patients.[117] The finding that both elevated flow velocities by TCD, and angiographic vasospasm, correlate poorly with any aspects of patient outcome is in agreement with the prediction that these radiologic findings reflect early adaptations to preserve tissue oxygen availability in a condition of progressive microvascular failure. In contrast, clinical deterioration and radiologic signs of tissue infarction are predicted to reflect the exhaustion of such compensatory mechanisms. This is consistent with the findings that only radiologic signs of infarction seemingly correlate with reduced instrumental activities of daily living, cognitive impairment, and poor quality of life 3 months after the SAH.[117]

The considerations above suggest that it may be of both diagnostic and prognostic value to monitor CTH and MTT in SAH patients. Capillary transit time heterogeneity and MTT can be measured by monitoring the clearance of intravascular contrast agents as part of standard perfusion-weighted MRI, perfusion CT, or contrast-enhanced transcranial ultrasound examinations.[77,118–120]

THERAPEUTIC IMPLICATIONS

The hypothesis put forward suggests that the angiographic vasospasm that precede DCI is in part secondary to disturbances in capillary flow patterns—and predicts that normalization of vascular tone and CBF may do little to improve tissue oxygenation because of the level of capillary shunting. This is consistent with the disappointing clinical results of resolving angiographic vasospasms,[36–38] although they seemingly partly restore CBF.[48] Paradoxically, the increased free radical production and NO depletion in the walls of resistance vessels may in fact improve tissue oxygenation by attenuating CBF responses. Although the release of free radicals is a well-established source of tissue damage after SAH,[121] its hypothesized beneficial effects in the maintenance of tissue oxygenation may explain the conflicting results of anti-oxidant therapy in SAH.[121] Below, we briefly discuss

therapeutic interventions that might reverse or counteract capillary constriction and compression in the acute stages of SAH.

Management of Pericapillary Edema

Until recently, the hemodynamic management of patients with clinical signs of vasospasm aimed to achieve systemic hypertension, hypervolemia, and hemodilution—so-called triple-H therapy.[122,123] This approach is based on the assumption that augmented cardiac output and blood pressure by vasopressors and isotonic fluids help maintain CBF, cerebral microcirculation, and thereby brain oxygenation.[122,123] The extent to which each of the three components of triple-H therapy prevents DCI remains uncertain.[124] In some cases, hypervolemia and hemodilution are associated with side effects including cerebral edema, cardiac failure, and electrolyte abnormalities.[124] Experimental studies suggest that alpha-adrenoreceptor stimulation may increase BBB permeability,[125] and in patients with SAH, the use of vasopressors is independently associated with the development of global cerebral edema.[12] Although some clinicians recommend the use of triple-H therapy in some instances,[126] others have abandoned the principles of triple-H therapy,[123,127] and recent guidelines for the management of SAH therefore recommend euvolemia and induction of hypertension for patients with DCI.[124]

Given the proposed metabolic significance of pericapillary edema after SAH, means of reducing the extravasation of fluids across the BBB may prove beneficial. Contrary to triple-H therapy, the Lund-concept, originally conceived for the management of patients with severe brain trauma,[128] aims to reduce intracapillary hydrostatic pressure and maintain colloid osmotic pressure. This approach is thought to increase transcapillary fluid reabsorption and to reduce cerebral edema. In a study including 30 patients with severe brain trauma and 30 SAH patients, therapy based on the Lund-concept showed significantly lower mortality than conventional triple-H therapy.[129] Needless to say, this concept must be examined in larger SAH patient cohorts.

Intracranial hypertension in patients with cerebral edema is generally treated with either mannitol or hypertonic saline (HS) according to institutional guidelines. While both agents have been proven efficacious in mixed patient populations, clinical controlled trials non-significantly favor the use of HS in terms of reducing intracranial pressure.[130,131] In addition, the use of mannitol appears to be associated with more adverse events, including hypovolemia, than HS.[132] One experimental study specifically compared the use of HS and mannitol in animal models of SAH, and demonstrated that the use of HS was associated with better intracranial pressure control and less neuronal damage.[133]

The compression of capillaries owing to hyponatremia (cf. Figure 1) might be reversed by means of restoring plasma sodium levels. Hypertonic saline is thought to create a strong transcapillary osmotic gradient that causes a shift of fluid away from brain tissue, interstitium, and endothelial cells, thereby improving the cerebral microcirculation.[134] Indeed, the infusion of HS in patients with SAH has been shown to increase both CBF and tissue oxygen tension[135]—the tell-tale signs of a reductions in CTH, cf. Figure 3. Preliminary data also show improvements in the degree of angiographic vasospasm,[136,137] in CBF,[109,138] and in modified Rankin Scale upon discharge.[139] Despite the complexity of managing hyponatremia,[57] the apparent safety of HS infusions in neurocritically ill patients[140–142] encourages further experimental and clinical studies.

Restoration of Capillary Nitric Oxide Levels

The ROS production and the NO depletion due to the pericapillary HgbO is likely to increase CTH for as long as hemolysis occurs in the subarachnoid space, unless NO levels at the capillary level can be maintained. When CTH increases and tissue oxygen tension drops, the risk of capillary NO depletion is likely to become more severe, as oxygen is the natural substrate for NO production via NO synthases.[66] NO depletion at the capillary level would therefore be expected to fuel a vicious cycle by causing further tissue hypoxia, further attenuation of upstream vessel tone, and so forth.

Endogenous nitrite is converted to NO in the tissue without the need for oxygen as a substrate,[143] and the infusion of nitrite might therefore be neuroprotective by preventing NO depletion and ROS production,[144–146] and thereby pericyte constrictions during their exposure to HgbO. By attenuating CTH increases, nitrite infusions might therefore improve oxygen delivery to tissue during this critical phase. Furthermore, nitrite reduces mitochondrial proton leakage, increasing ATP yields from oxygen and thereby tissue tolerance to hypoxia.[147] Nitrite may also attenuate thrombogenicity by inhibiting platelet aggregation.[148] Early administration of nitrite has been shown to prevent vasospasm after SAH[149,150] and to increase tissue survival in ischemia–reperfusion[151] in primate animal models. The prolonged administration of nitrite to healthy volunteers is deemed safe,[152] and a recent phase II trial confirmed that sodium nitrite salt is safe to administer to SAH patients throughout the critical phase after SAH.[153]

Stimulation of the sphenopalatine ganglion has been shown to reduce angiographic vasospasm and increase CBF in dogs[154] and monkeys[155] after experimental SAH. Fibers from the sphenopalatine ganglion release NO,[156,157] acetylcholine, and a range of vasoactive peptidergic neurotransmitters in the adventitia of cerebral vessels, causing vasodilation.[158] The effects of sphenopalatine ganglion stimulation remains to be examined in SAH patients, but it is interesting to note that NO releasing nerve fibers are present at both the arterial and capillary level.[159] Although neuronal NOS is lost in sphenopalatine nerve fibers at the arterial level after SAH,[31] our hypothesis suggests that activation of capillary NO production by this route may be of benefit after SAH.

Management of Blood Viscosity and Tissue Microcirculation

Increased numbers and endothelial adhesion of leukocytes are known to disturb capillary flow patterns,[74] and means of reducing the number of circulating leukocytes and their adhesion to capillary endothelium would therefore be predicted to reduce CTH, and thereby to reduce angiographic vasospasm and DCI after SAH. Ishikawa et al[160] indeed found increased adhesion and rolling of leukocytes, paralleled by a 60% reduction in CBF, after experimental SAH in mice, and demonstrated that these changes could be reversed by inhibiting the endothelial cell adhesion molecules. For a comprehensive review of the role of leukocyte–endothelial interactions after SAH, see ref. 161. Corticosteroid treatment is a well-known cause of leukocytosis in humans. Although corticosteroid treatment prevents hyponatremia[162] and may attenuate edema in SAH patients, we speculate that the detrimental effects of leukocytosis on tissue oxygenation may obscure the translation of such beneficial effects into better patient outcomes.[162,163]

Statin treatment of SAH patients has attracted considerable interest after reports that treatment with statins before SAH reduces the loss of vascular endothelial NOS in animal models[164] and ameliorates DCI and angiographic vasospasm in patients,[165] and that statin treatment upon admission reduces mortality after SAH.[166] Subsequent studies have yielded conflicting results, with high-dose statin treatment in animal models showing a definite neuroprotective effect, whereas studies in SAH patients remain inconclusive.[167,168] In addition to its effects of endothelial function, statins also reduce blood viscosity by lowering blood lipid levels, and would therefore be expected to facilitate the capillary passage of blood and thus reduce CTH. The lipid-lowering effect of statins becomes significant 2 to 4 days after initiation of treatment in normocholesterolemic subjects,[169] and

improved blood viscosity could therefore contribute to a protective effect in the days after SAH. The relative contribution of plasma lipids to total blood viscosity may, however, vary relative to that of leukocytes (see previous section). We speculate that studies of the effect of statins in human SAH must be controlled for the degree of leukocytosis in individual patients. The putative effects of blood viscosity may be most easily disentangled in animal models of SAH, in which concomitant leukocytosis is not a consistent finding.[160]

Although capillary dilation may be facilitated by restoring pericapillary NO levels, the extent to which capillary constriction can be inhibited remain unclear. Antihypertensives are likely to interfere with both arteriolar and pericyte tone in that pericytes constrict in response to stimulation by β_2-adrenergic,[170] Angiotensin-II type 1,[171,172] and endothelin-1[69] receptor agonists via a calcium channel-dependent mechanism. Although ET antagonist treatment is still being explored further, it is interesting to note that nimodipine, a calcium channel blocker that might be expected to prevent pericyte constrictions, has shown some efficacy in preventing DCI after SAH.[1,173]

CONCLUSION

Our review identifies a number of sources of altered capillary morphology and function, both acutely and during the first critical weeks after SAH. Our earlier re-analysis of the classic flow–diffusion equation predicts that any increase in CTH that accompany such changes tends to increase the shunting of oxygenated blood through the capillary bed. Vasospasm and inverted CBF responses may therefore, paradoxically, improve net oxygen extraction when capillary flows are disturbed by pericyte damage, nitric oxide depletion, vasogenic edema, astrocytic endfeet swelling, or leukocytosis. The role of capillary flows after SAH is supported by the results of studies that have aimed to control pericapillary edema or prevent NO depletion.

Our review further suggests that any irreversible changes in the capillary morphology or function can cause long-term degenerative changes and permanent neurocognitive symptoms after SAH.

Needless to say, the predictions and hypotheses presented in this review must be tested in experimental and clinical settings to verify the importance of the microcirculation and of BBB function after SAH. The hypothesized disturbances in CTH must be demonstrated and quantified by invasive or noninvasive approaches, and the theoretical predictions of their metabolic and functional significance assessed by independent techniques. Hopefully, better understandings of the role of capillary function in SAH can contribute to the reduce the mortality and morbidity after this devastating disease.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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