Molecular mechanisms of early brain injury after subarachnoid hemorrhage

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Objectives: Increasing body of experimental and clinical data indicates that early brain injury after initial bleeding largely contributes to unfavorable outcome after subarachnoid hemorrhage (SAH). This review presents molecular mechanisms underlying brain injury at its early stages after SAH.

Methods: PubMed was searched using term ‘subarachnoid hemorrhage’ and key words referring to molecular and cellular pathomechanisms of SAH-induced early brain injury.

Results: The authors reviewed intracranial phenomena and molecular agents that contribute to the early development of pathological sequelae of SAH in cerebral and vascular tissues, including cerebral ischemia and its interactions with injurious blood components, blood–brain barrier disruption, brain edema and apoptosis.

Discussion: It is believed that detailed knowledge of molecular signaling pathways after SAH will serve to improve therapeutic interventions. The most promising approach is the protection of neurovascular unit including anti-apoptosis therapy. [Neurol Res 2006; 28: 399–414]

Keywords: Subarachnoid hemorrhage; brain injury; signaling pathways; early vasospasm

INTRODUCTION

General aspects

Spontaneous subarachnoid hemorrhage (SAH) represents 5–7% of all strokes and ~30,000 Americans will suffer SAH each year.1,2 Following aneurismal SAH, 30% of patients died within the first few days of initial bleeding, 10% died of rebleeding3 and 10% died or less in major cities3 in the following weeks owing to cerebral vasospasm4,5. Autopsy studies reveal that ~2% of the population have an unruptured intracranial aneurysm, which means that six million Americans have cerebral aneurysms6.

No effective treatment is available for early brain injury after the initial bleeding.7 Therapy to limit the impact of the initial bleeding includes reducing intracranial pressure (ICP) and relieving hydrocephalus. Most deaths from the initial bleeding occur in the first 48 hours and result from increased ICP.1

Pathophysiology

When a saccular aneurysm ruptures, the ICP rises to diastolic blood pressure in 1 to 2 minutes8,9. ICP will fall and reach a steady level in ~10 minutes, unless a hematoma is formed. Although reduced, a regional cerebral blood flow (rCBF) may be relatively high as compared with the largely reduced cerebral metabolic rate of oxygen resulting in so-called luxury perfusion owing to an uncoupling between flow and metabolism10,11. The arteriovenous oxygen difference is always reduced. Cerebral autoregulation is disturbed even in mild cases. On the other hand, the reactivity of the cerebral vasculature to changes in arterial pCO2 is often preserved, although it will be reduced12. Both cerebral autoregulation and reactivity to carbon dioxide (so-called total vasoparalysis) will be damaged only in the presence of severe tissue acidosis13. Reactivity to carbon dioxide may be lost after SAH in a graded fashion or completely in patients who go on to die14.

Factors responsible for the impact of the initial bleeding in SAH include raised ICP, decreases in CBF and cerebral perfusion pressure (CPP), blood–brain barrier (BBB) disruption, brain swelling, brain edema, acute vasospasm and dysfunction of autoregulation. The change of these factors within the first 48 hours after SAH constitutes the early brain injury period. Figure 1 demonstrates in a schematic the cascade of pathophysiologic factors in the early brain injury after SAH (Figure 1). Plenty of complex molecular changes occur in subarachnoid cerebrospinal fluid (CSF) space, intracranial vessels and brain tissue, with the importance in early brain injury after SAH. It involves multiple interactions between proteases, inflammatory mediators and apoptotic cascades. Studies of these alterations are summarized in Tables 1 and 2 and discussed in the text (Tables 1 and 2).

Animal models for early brain injury

Endovascular filament perforation51 and blood injection through the cisterna magna52 are the most popular models to study early brain injury after the initial bleeding in rats. Most early studies used a single blood
injection through the cisterna magna and produced limited impact on the brain with no or low mortality and transient cerebral vasospasm. A modified version of this model was adapted in which two blood injections were given through the cisterna magna and an extension of the time course of cerebral vasospasm was obtained. Because aneurysmal rupture is the usual cause of SAH in humans, the endovascular perforation model appears to more closely mimic the human situation. In this model, a nylon monofilament is inserted into the internal carotid artery (ICA) through the external carotid artery and advanced rostrally to perforate the MCA in the proximity of ICA bifurcation. These three models (single-, double-injections and endovascular puncture) were compared and it was found that even though double blood injections via cisterna magna produced prolonged cerebral vasospasm, the endovascular filament perforation model was superior for the study of early brain injury after the initial bleeding. The endovascular perforation model caused increased ICP, reduced CBF and CPP, enhanced BBB permeability, deterioration in neurological function and led to 40–50% mortality, which resembles closely SAH in humans.

Experimental SAH in rats can also be produced by transclival puncture of the basilar artery, which affects ICP, CBF and CPP to a similar level as the above mentioned model, but requires significant surgical skills. In addition, blood injections into the pre-chiasmatic cistern have been tested and their influences on the pathologic and pathophysiologic processes were compared. The mortality rates were 44% after endovascular perforation, 25% after pre-chiasmatic blood injection and 0% after blood injection via the cisterna magna. CBF recovered within 15 minutes in the cisterna magna group, but had recovered to normal at minute 90 in only 60 and 89% of the perforation and pre-chiasmatic groups, respectively. Therefore, the endovascular perforation model offers a unique opportunity to evaluate the pathophysiology in SAH as well as the therapeutic effect of drug treatment which remains a significant problem for neurosurgeons.

CEREBRAL ISCHEMIA IN EARLY BRAIN INJURY

ICP, CBF, acute vasospasm and autoregulation

Elevation of ICP and acute vasoconstriction, especially at the microcirculation level, are the two major factors that reduce CBF and CPP after initial bleeding in SAH and lead to a global ischemia. After the initial bleeding, there is a loss of autoregulation and the reduction in CPP produces cerebral ischemia. This fall in CPP may be due to elevated ICP or reduced mean arterial blood pressure. Experimental evidence has indicated that at the time of bleeding, there is a profound and extensive cerebral ischemic insult. The severity of the ischemia depends upon the nature, size and rapidity of onset of hemorrhage. It is believed that if the patient does not lose consciousness with the SAH then possibly ICP did not increase over the diastolic blood pressure; however, vasoconstrictor elements in blood produce spasm which may further reduce CBF, sometimes even remotely from the lesion.

In an endovascular perforation SAH rat model, a ‘lethal’ SAH was induced when CBF was reduced to <40% of baseline for 60 minutes after SAH. In addition, extracellular glutamate concentration increased to 600% of baseline after lethal SAH in both hippocampus and cortex and was inversely correlated with CBF. Acute vasocostriction observed after SAH occurs independently of changes in ICP and CPP and is associated with decreased CBF, larger hemorrhage size, persistent elevations of extracellular glutamate and poor outcome. Lower levels of energy substrates and higher levels of lactate and neuronal injury markers were observed in patients with severe and complete ischemia when compared with patients without symptoms of ischemia. It should be emphasized that SAH produces ischemic insult that is very different from global ischemia caused by occlusion of cerebral arteries or cardiac arrest. In the latter, there is an absolute cessation of blood supply to the brain and the duration of ischemic insult is the most important factor determining the outcome. The acute cerebral ischemia after SAH interacts with increased ICP and extravasated blood components that contribute to sustained hypoperfusion, enhanced inflammation, oxidative injury and ultimately cell death in cerebral tissues.
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that with except for minor leaks, the initial bleeding itself has a global ischemic impact.

**Mechanisms of cerebral ischemia**

Multiple pathogenic factors have been described for the reduction of CBF after the initial bleeding. First, total blood released in the subarachnoid space after SAH is the main factor leading to CBF reduction. The components of blood can cause acute CBF reduction including aggregating platelets through mechanically blocking arteries and releasing vasoactive compounds.

Hemoglobin released into subarachnoid space is involved in the pathogenesis of cortical spreading ischemia, characterized by cortical blood flow below ischemic threshold and propagating together with neuronal and astroglial depolarization waves. Hemoglobin has an inhibitory effect on the Na⁺/K⁺ ATPase activity, which plays an important role in the mechanism of depolarization. Second, ET-1 released from cerebral arteries reduces Na⁺/K⁺ ATPase activity and works in concert with hemoglobin in the induction of cortical spreading ischemia. Third, activation of 5-HT1B receptors induces HETE which produces potent vasoconstriction and reduction in CBF. Fourth, immediate reduction of NO availability after the initial bleeding is linked to the ‘sink-effect’ of hemoglobin, which scavenges NO. Replacing oxyhemoglobin with the NOS inhibitor Nomega-nitro-L-arginine mimicked these effects, implicating NO scavenging functions of oxyhemoglobin. Fifth, elevations of ICP and global hypoxia/ischemia immediately after the initial bleeding impair autoregulation, decrease CBF and may contribute to brain swelling. Sixth, the impact of the initial bleeding enhances the production of superoxide anion catalyzed by vascular NADPH oxidase. CuZn-SOD gene transfer after the initial bleeding restored blunted vasodilation of pial arteries in response to calcitonin gene-related peptide and K⁺ channel opener levocromakalim. Seventh, early brain injury activated tyrosine kinase, which inhibits K⁺ channels resulting in vasoconstriction. In experimental settings the presence of K⁺ and hemoglobin in the subarachnoid space is sufficient to induce cortical ischemia and necrosis. Early literature on K⁺ in the CSF shows, however, that K⁺ does not increase enough to contribute to vasoconstriction after SAH and vasodilatory effect may be more prominent owing to activity of inwardly rectifying K⁺ channels. Finally, it should be emphasized that contribution of the ‘no-reflow’ phenomenon may be considered in CBF reductions after SAH. It might contribute to the reduction of CBF observed within 24 hours from the initial bleeding even after ICP elevation is resolved.

**HIF-1α**

HIF-1α is a transcription factor specifically activated by hypoxia. Even though accumulation of HIF-1α in the ischemic or hypoxic tissues might promote adaptive mechanisms for cell survival, high levels of HIF-1α might bind with tumor suppressor p53 to activate apoptotic pathways in brain tissues after cerebral ischemia and traumatic brain injuries. HIF-1α, which has been found at elevated levels in brain tissues after the initial bleeding, may participate in the pathogenesis of early brain injury.

Amongst HIF-1 target genes BNIP3 mediates cell death upon hypoxia. BNIP3 is a proapoptotic member of Bcl2 family. BNIP3 positive immunostained cells and increased protein content were found in vulnerable sector CA1 of the hippocampus in perforation model of SAH accompanied by significant HIF-1α up-regulation and a profound cell injury. BNIP3 induces cell death through heterodimerization with Bcl-2/Bcl-X (L), cell death repressors (Figure 2).

**Ischemic secondary injury**

Other aspects of cerebral ischemia including membrane depolarization in the penumbra, Ca²⁺ accumulation in the nerve cells, and glutamate receptor activation are present after initial bleeding. Glutamate levels were elevated six-fold in hippocampus and cortex and Ca²⁺ accumulation and membrane depolarization were detected in cerebral arteries after SAH. Elevation of Ca²⁺ promotes the formation of NO and reactive oxygen species, dysfunction of mitochondria, activation of proteases and inflammation.

Multiple interactions between acute ischemia and injurious agents, including the extravasated vasoactive compounds, affect the outcome of SAH over and above ischemia alone. Acute global ischemia may trigger inflammation in brain regions remotely from blood clot (e.g. hippocampus and small intraparenchymal arteries). However, both parenchymal and vascular inflammation 48 hours after SAH was aggravated in the proximity of extravasated blood that induces intercellular adhesion molecule-1 (ICAM-1), IL-1β, IL-6 and TNF-α in cerebral tissue. Additionally, inflammation overlapped with apoptotic cell death which further strengthens the significance of interactions between distinct mediators of the brain injury. It is unlikely that therapeutic efficacy will be developed without knowledge of these interactions.

**BRAIN EDEMA IN EARLY BRAIN INJURY**

**BBB and brain edema**

Brain (global) edema after SAH presents on admission CT scans in 8% and develops secondarily in 12% of patients. Brain edema developing after the initial bleeding could be classified as having both a primary vasogenic and secondary cytotoxic component. Brain edema occurred in gray and white matter as well as in cortex and deep nuclei, particularly in the parasagittal watershed areas.

Early dysfunction of BBB contributes to brain edema which expands brain volume and prolongs elevated ICP values after SAH. The combination of initially induced global cerebral ischemia and the subsequent recovery of cerebral circulation aggravates brain edema. Studies report that SAH causes increases...
in BBB permeability in tight junctions (paracellular flux) and transcytotic vesicles in brain microvascular endothelial cells (transcellular flux)\textsuperscript{93,98}. Two studies of SAH separately emphasize the importance of transcellular flux in the acute stage\textsuperscript{99} and the importance of an opening in the inter-endothelial space in the chronic stage\textsuperscript{100}. Disruption in the BBB following SAH may be the underlying mechanism for the toxicity of blood breakdown products or the production of free radicals, ET-1, arachidonic acid and TXA\textsubscript{2}\textsuperscript{15,101} resulting in raised ICP\textsuperscript{93} and hypertension\textsuperscript{93}.

Hemoglobin and ET-1 both have inhibitory effects on the Na\textsuperscript{+}/K\textsuperscript{+} ATPase activity\textsuperscript{10,69} and may induce cytotoxic brain edema after SAH. Another cause of cytotoxic edema is water intoxication, which follows the acute systemic hypo-osmolarity that is caused by the influx of water and associated with acute Na\textsuperscript{+} depletion or inappropriate secretion of antidiuretic hormone. Brain natriuretic peptide (BNP) is a potent natriuretic factor responsible for hyponatremia observed in patients with SAH. Through its systemic effects (reduction of blood volume and blood pressure) BNP may augment CBF reduction and SAH-induced ischemia\textsuperscript{102}. Both plasma BNP and ANP (atrial natriuretic peptide) increase after SAH and often result in a negative fluid balance\textsuperscript{103}.

**Brain water and edema**

Badaut and colleagues found marked increase in water channels-AQP 1 and 4 protein expression 24 hours after SAH in human neocortex\textsuperscript{27}. In the normal conditions AQP4 protein was localized on astrocytic foot ends around vascular tissues; however, following SAH a marked swelling of astrocytes was accompanied by diffuse AQP4 labeling of astrocytic processes. The authors suggested that AQPs may contribute to the dynamics of edema formation after...
SAH. To address this issue, studies with AQP4 knockout animals are needed.

BBB, brain edema and VEGF

SAH promotes the accumulation of HIF-1α in which in turn activates a gene of VEGF involved in the dysfunction of BBB. The levels of VEGF in the bloody CSF and the expression of VEGF in the brain tissues are both increased after SAH, which might enhance BBB permeability. Src tyrosine kinase is downstream of VEGF activation and regulates VEGF-mediated BBB permeability alteration. In an endovascular perforation model of SAH, within 24 hours after the initial bleeding, VEGF and MAPK activities increased remarkably in cerebral arteries and to a lesser degree in cerebral cortex beside the basal cistern. These molecular changes contributed to the altered permeability of BBB, enhanced brain edema and elevated ICP and these pathological events led to neurological deficit and eventually death in rats. Inhibition of Src-family tyrosine kinase abolished these molecular changes, reduced BBB disruption, brain edema and ICP increases and decreased mortality. An interesting observation was that PP1, the Src-family tyrosine kinase inhibitor, also reduced the expression of VEGF in cerebral cortex and arteries. This new observation indicates that Src not only is downstream of VEGF receptor activation and leads to MAPK signaling cascades, but also is upstream of VEGF and may be involved in the signaling pathways that lead to the activation of VEGF. MAPK p38 is involved in HIF-1α-dependent or independent activation of VEGF. (Figure 3). MAPK ERK1/2 activates VEGF promoter at proximal region under normal conditions and stabilizes HIF-1α under hypoxic conditions to enhance VEGF activation. Even though further evidence is needed, it is possible that SAH activates Src-MAPK, which enhances HIF-1α-VEGF, which activates Src-MAPK to contribute to early brain injury following SAH. In recent study neurovascular protection of cerebral endothelial cells induced by pan-caspase inhibitor reduced early brain injury after SAH.

APOTOPSIS IN EARLY BRAIN INJURY

One of the key factors for BBB disruption and brain edema is apoptotic cell death occurring in neurons and cerebral endothelial cells. A large family of cysteine aspartyl proteases known as caspases mediates apoptosis. The caspase family has two major subfamilies: initiators (caspase 2, 8, 9 and 10) and effectors (caspase 3, 6 and 7). Among caspases, caspase-3 seems to play an essential role in the cell death machinery. In recent study, protection of cerebral endothelial cells against apoptosis reduced early brain injury after SAH.

Apoptosis in neuronal tissues

Apoptosis appears to be the predominant mechanism of death in dentate granule cells, whereas neuronal death in the hippocampus generally is of necrotic morphology. After the initial bleeding, apoptosis in brain tissues may be caused by elevated ICP, toxicity of blood components, ischemia and reperfusion, as well as by acute vasospasm. In series of studies, Matz et al. injected lyed blood into the subarachnoid space over the dorsal aspect of the cortex beneath the coronal suture and observed TUNEL positive cells in the neocortex closest to the site of hemolysate injection. In an endovascular perforation model, apoptotic changes were noted in most brain regions, especially in the basal cortex and hippocampus. The occurrence of apoptosis in the hippocampus is probably due to the use of endovascular filament model, which results in a drastic rise of ICP and reduction of CBF, which causes global ischemia. Therefore, caspase-3 and TUNEL positive cells were present not only in the basal cerebral cortex, which is exposed to hematoma caused by endovascular puncture, but were evident also in dentate gyrus and CA1 region of the hippocampus, which is known to be the most vulnerable to ischemic injury. Additionally, in certain brain regions, TUNEL positive staining in astrocytes and oligodendroglia occurs earlier than in neurons suggesting that staging post-SAHP injury in different cell compartments may be useful for the development of antiapoptotic therapies.

Apoptosis in cerebral endothelial cells

In vitro studies provided evidence that apoptosis occurred when endothelial cells were exposed to specific inducers, which are the degradation products of blood such as oxymemoglobin, inflammatory mediators or vasooactive substances occurring in patients with SAH such as TNF-α or TXA2 and natriuretic peptides. It was also reported that a lesser number of selective inhibitors have been identified which inhibit
the function of caspase 2, 3, 8 and 9 and effectively protected endothelial cells from oxyhemoglobin-induced apoptosis\(^\text{125}\) (Figure 4).

Erythrocyte lysate causes important events such as intracellular Ca\(^{2+}\) elevation\(^\text{126}\) and the generation of reactive oxygen species (ROS) in endothelial cells\(^\text{127}\). ROS-induced lipid peroxidation of cell membranes causes increases in eicosanoid TXA\(_2\)\(^\text{128}\). Stimulation of the endothelial cells with TXA\(_2\) mimetic IBOP induced apoptosis by inhibiting Akt phosphorylation, an intracellular mediator required for cell survival\(^\text{45}\). In addition, natriuretic peptides that are elevated in the plasma or CSF of patients with SAH\(^\text{102}\) are capable of inducing endothelial apoptosis via the cGMP-dependent mechanism\(^\text{43}\).

Several authors have noted ultrastructural changes in the endothelium of cerebral arteries after SAH, including cellular distortion, formation of intracellular vacuoles, disruption of tight junctions and widening of interendothelial spaces. This damage may lead to detachment of endothelial cells\(^\text{129,130}\). The denuded arterial state allows for direct contact of smooth muscle cells with vasoactive agents in the blood stream, such as ATP, which can cause constriction\(^\text{131}\). Furthermore, endothelial apoptosis and the resultant endothelial detachment expose the collagen of the internal lamina which in turn promotes platelet adherence and thrombus formation\(^\text{132,133}\). Initiated thrombogenesis causes embolic infarction and worsens the ischemic symptoms of SAH. Therefore, direct damage to the endothelial cells is considered to be responsible for cerebral ischemia after SAH\(^\text{132,134,135}\).

Some recent observations demonstrated the presence of apoptosis in brain microvessel endothelial cells overlapping with the disruption of BBB after SAH\(^\text{136}\). Endothelial apoptosis after SAH may be caused by mechanical stimulation such as elevated ICP\(^\text{51}\), ischemia and reperfusion injuries\(^\text{56}\), toxic components of blood clots\(^\text{46,125}\), activation of MMP-9\(^\text{137,138}\) and acute

Figure 4: The inflammatory mediators and products of blood degradation together with an excess of glutamate, NO and free radicals trigger apoptotic cascades in endothelial cells. Compromised function of endothelial cells underlies acute vasospasm, BBB rupture and brain edema, leading to a secondary increase in ICP and CBF reduction.
vasospasm\(^{19}\). Because a general caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp (OMe) fluoromethylketone (Z-VAD-FMK) has been shown to attenuate BBB permeability, decrease brain edema and reduce mortality, it was postulated that Z-VAD-FMK produced anti-apoptotic effect primarily in cerebral endothelial cells but not in neuronal tissues\(^{34}\). Interestingly, apoptotic endothelial death is accompanied by the activation of MMP-9, which plays a role in the disruption of BBB especially during hemorrhagic transformation\(^{137,138}\) (Figure 5). Apoptosis and activation of MMP-9 contribute to the enhanced permeability of BBB and result in a mixture of vasogenic and cytotoxic brain edema after SAH\(^{139,140}\).

**BLOOD COMPONENTS IN EARLY BRAIN INJURY**

**Sink-effect and depolarization**

The presence of large amounts of hemoglobin is in direct relationship with the availability of NO in the brain after the initial bleeding because hemoglobin has ‘sink-effect’, which scavenges NO\(^{21,70}\). Furthermore, hemoglobin has inhibitory effect on the Na\(^+\)/K\(^+\) ATPase activity, which plays an important role in the mechanism of depolarization\(^{69}\). Depolarization of smooth muscle cell membranes causes the opening of voltage-dependent Ca\(^{2+}\) channels and the contraction of cerebral arteries\(^{141}\).

The resting membrane potentials of cerebral arteries are significantly depolarized after SAH, as early as 30 minutes post-ictus\(^{142}\). Massive cellular depolarization was recorded in cats after the initial bleeding. It was characterized by K\(^+\) efflux and Ca\(^{2+}\) influx and resulted in membrane destabilization, osmotic imbalance and a decrease in electrical conduction\(^{143-145}\). When artificial CSF containing hemoglobin and elevated K\(^+\) levels was superfused over the rat cerebral cortex, the depolarization wave triggered an ischemic event. In animals receiving nimodipine or moderate volume expansion/hemodilution with hydroxyethyl starch, the depolarization wave triggered brief initial hypoperfusion followed by brief hyperemia in the cortical area exposed to hemoglobin. These observations suggest a mechanism involving the cortical microcirculation that might underlie the therapeutic effects of nimodipine and volume expansion\(^{26}\). Ischemic depolarization and

![Figure 5: Neurovascular unit. Although extravasated blood does not penetrate to parenchymal vessels, acute ischemia, brain swelling and diffusion of blood-derived substances into brain all target neurovascular unit after SAH. MMP-2 and -9 activation underlies basal lamina disruption, endothelial cells detachment and a suppression of integrins-mediated survival signal from extracellular matrix to endothelial cells and neurons. Endothelial apoptosis underlies BBB disruption that in turn leads to brain edema](image-url)
cortical spreading depressions occurred after SAH and the neuroprotective value of Mg$^{2+}$ after SAH may, in part, be explained by a reduction in the duration of the ischemic depolarization of brain cells$^{146}$. Small human pial arteries are hyper-responsive to contractile agents and show spontaneous contractile activity within 48 hours after SAH. Such effects could result in narrowed resistance arteries and reduction in CBF$^{147}$.

Nucleotides and other vasoactive agents

Nucleotides or nucleosides regulate smooth muscle tone of cerebral arteries and potentiate the effect of hemoglobin$^{38,148}$. The corresponding nucleotide receptors are up-regulated in spastic cerebral arteries$^{149}$ and inhibition of nucleotide receptors reduces cerebral artery constriction to blood lysates$^{150}$.

Recently, propagation of vasomotor responses has been recognized as an important regulatory mechanism in microcirculation. Oxyhemoglobin inhibits the vasodilatory effect of chemical mediators such as adenosine and adenosine nucleotides at a local and/or propagated site. This indicates that vasodilatory propagation plays an important role in the regulation of brain microcirculation and its impairment by oxyhemoglobin could, in part, explain the cerebral hypoperfusion that is observed after SAH$^{151}$.

Blood clot interacts with brain tissues, CSF and cerebral vessels and generates from these interactions secondary spasmogens such as 5-HT, catecholamine, ET, PGs and TX$^{38,152–154}$. Subsequently, an interaction between 5-HT$\text{1B}$ receptors and 20-HETE contributes to the acute fall in regional CBF after SAH in rats. The released 5-HT after SAH activates 5-HT$\text{1B}$ receptors and the synthesis of 20-HETE, which enhances the vasoconstrictor response of cerebral vessels to 5-HT$^{28}$.

Blood components and MAPK mechanisms

MAPK including ERK1/2, p38 and JNK are activated in cerebral arteries and brain tissues 24 hours after SAH and the activation of MAPK is directly involved in early brain injury including BBB disruption, brain edema and elevation of ICP$^{38}$. Even though the exact mechanism of MAPK activation after SAH remains unclear, oxyhemoglobin clearly activates MAPK in rabbit basilar artery$^{47}$. Oxidation of oxyhemoglobin to methemoglobin generates free radicals that can initiate lipid peroxidation and activation of phospholipase A2, therefore releasing the products of the arachidonic acid cascade$^{153}$. Most of the eicosanoids can activate phospholipase C (PLC), leading to the formation of inositol 1,4,5-triphosphate (IP$^3$), which will subsequently release Ca$^{2+}$ from internal stores. Free radicals and elevated intracellular Ca$^{2+}$ might activate MAPK$^{154}$. The prolonged activity of MAPK for up to 2 hours after exposure to hemolysate indicates that MAPK may play an important role in the early brain injury after the initial bleeding$^{47}$.

Another main component in hemolysate is ATP which produces smooth muscle contraction by activation of G-protein coupled P2 receptors$^{157}$. ATP in hemolysate might activate MAPK by activating P2 receptors (a G-protein coupled receptor)$^{48}$. ET, which may be generated and released after the initial bleeding, induces contraction by activation of G-protein coupled ET receptors$^{158}$. All four G-protein subfamilies ($G_{q/11}$, $G_{i/o}$, $G_o$ and $G_{12/13}$) have been implicated in the activation of the MAPK cascade$^{159}$. Activation of these receptors leads to stimulation of PLC and formation of diacylglycerol (DAG) and IP$^3$. Ca$^{2+}$ induces smooth muscle contraction via myosin light chain phosphorylation and PKC regulates smooth muscle contraction by phosphorylating contractile proteins such as calponin. However, DAG also stimulates PKC that either activates Ca$^{2+}$-dependent proline-rich tyrosine kinase 2 (Pyk2) or directly activates Raf-1. IP$^3$ releases Ca$^{2+}$ from intracellular Ca$^{2+}$ stores and Ca$^{2+}$ may stimulate tyrosine kinases such as Pyk2. Activation of tyrosine kinases may either phosphorylate Src (or by Shc and Grb2/SOS cascades activate Ras) or activate Ras directly. Ras activates Raf-1, the cellular proto-oncoprotein counterpart of viral oncogene (v-raf), which in turn activates MAPK/ERK kinase (MEK) and MAPK$^{159}$ (Figure 6). In addition, thrombin is activated in CSF after SAH. Inhibition of thrombin activity leads to amelioration of cerebral vasospasm and suppression of MAPK diphosphorylation$^{160}$.

AMINO ACIDS, EICOSANOIDS, PEPTIDES AND CYTOKINES IN EARLY BRAIN INJURY

Excitotoxic amino acids

Increase in extracellular glutamate level is a robust marker of SAH-induced ischemia$^{161}$. Additionally, glutamate present in high concentrations in plasma and red blood cells may propagate from extravasated blood to the brain increasing local glutamate concentration$^{66,162}$. Glutamate activates N-methyl-D-aspartate, alpha-amino-3-hydroxy-5-methylisoxasole-4-propionate receptors on neurons and oligodendrocytes contributing to excitotoxic neuronal death and white matter injury after SAH.
High level of glutamate after SAH may also result from diminished uptake by glial cells. Expression of glutamate transporters excitatory amino acid transporter 4 (EAAT4) and glutamate-aspartate transporter (GLAST) was decreased in human neonatal SAH. Such glial dysfunction may lead to increased extracellular glutamate levels and contribute to excitotoxic cell death.

The significantly increased levels of excitatory amino acids observed in SAH patients with poor outcomes followed a biphasic course, with maximal concentrations on the first and second days or the seventh day after the insult. Release of these substances into the extracellular fluid of the brain might be particularly relevant for the development of secondary brain damage after SAH, e.g., infarction or brain swelling.

Eicosanoids

After SAH, SPC and CGRP are released from vascular nerve fibers. They stimulate inducible COX-2 in platelets resulting in the production of prostanoids with particular activation of PGE2 pathway. Two days after SAH, COX-2 was overexpressed in cerebral arteries. Endothelial cells subjected to TNF-α exposure also become a source of PGE2. PGE2α and PGF2α have damaging effect on BBB as suggested by in vitro study. Activated platelets produce also TXA2, which is a potent vasoconstrictor and stimulator of platelet aggregation. In addition, a leukotriene C4 (LTC4) pathway is activated in the cerebral cortex within the first hour following SAH. Infiltrating neutrophils and macrophages are major producers of this compound. LTC4 activates endothelial cells and increases their permeability, therefore contributing to a BBB breakdown after SAH.

CSF eicosanoid levels are raised following SAH but not sufficiently to be vasoactive per se within the cerebral circulation. Rebleeding and intraventricular hemorrhage are two factors associated with a worse outcome after aneurysmal SAH. Intraventricular hemorrhage increased the median levels of TXB2 (TXA2 metabolite), PG6-keto F1α (prostacyclin metabolite), PGF2α and PGE2 in ventricular CSF by 2.1–5.1-fold. In patients who rebled, the CSF median levels of all four eicosanoids were raised up to 250-fold over the normal range. These concentrations are just sufficient to have cerebrovascular and neuromodulatory effects.

ET-1

The level of ET-1 was increased in the bloody CSF after SAH. The impact of the initial bleeding and the resulting global ischemia, rather than hemoglobin, may promote the production and release of ET-1. ET-1 produces constriction, which may contribute to the acute vasospasm, reduces Na+/K+ ATPase activity to depolarize membrane potentials and works in concert with hemoglobin in the induction of cortical spreading ischemia. At least, the cerebrovascular action of ET-1 is related to MAPK activation. ET may have a profound role in the early brain injury after SAH and the therapeutic effect of endothelin receptor antagonists should be examined in future studies.

Inflammatory cytokines

The inflammatory cytokines released after SAH may be in contact with microvessels and enhance vascular permeability through BBB disruption. IL-6, IL-1β, IL-1 receptor antagonist and TNF-α levels all increased in bloody CSF after SAH. In addition, brain-derived cytokines may enter the systemic circulation in the presence of post-SAH BBB disruption to activate inflammatory cascades systemically and therefore contribute to the development of post-SAH systemic inflammatory response syndrome and extracerebral organ system failures.

OXIDATIVE STRESS

Free radicals, triggered by clot-derived hemoglobin, include superoxide anion, hydrogen peroxide and hydroxyl radical. Superoxide (O2−) anion is produced by hemoglobin auto-oxidation and consequent dismutation of two O2− forms hydrogen peroxide. The latter is the source of highly reactive hydroxyl radical in the reaction catalyzed by ferric ion. Activated platelets produce also TXA2, which is a potent vasoconstrictor and stimulator of platelet aggregation. In addition, a leukotriene C4 (LTC4) pathway is activated in the cerebral cortex within the first hour following SAH. Infiltrating neutrophils and macrophages are major producers of this compound. LTC4 activates endothelial cells and increases their permeability, therefore contributing to a BBB breakdown after SAH.

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NO PATHWAYS

After SAH, cerebral extracellular concentrations of NO metabolites decrease over time. Because NO-dependent vasodilatory mechanisms are still intact in this setting, acute vasoconstriction may be the result of limited NO availability after SAH. Blood released during SAH leads to vasoconstriction by scavenging NO2. CSF concentration of NO metabolites may correlate with the amount of bleeding, inasmuch as the values in patients with Fisher Grade 3 were higher than those in patients with Fisher Grade 2. The concentration of nitrate was higher than that of nitrite, suggesting that NO in the subarachnoid space is mainly absorbed by hemoglobin and degraded to nitrate.

During the acute stage the lower limit of CBF autoregulation significantly shifted to the higher arterial blood pressure in association with suppressed vasodilation in response to acute hypotension, which was accompanied by significantly increased expression of...
endothelial nitric oxide synthase mRNA and increased production of superoxide anion in cerebral vessels. Endogenously produced NO is implicated in the preservation of CBF autoregulation during the acute stage after SAH via its capability to scavenge superoxide anion. Arterioles from the SAH animals demonstrated attenuated dilation to the endothelium-dependent dilator ADP and accentuated constriction to ET-1. Therefore, the endothelial dysfunction in cortical arterioles may be the basis for microvascular spasm.

**GENOMIC CHANGES IN EARLY BRAIN INJURY**

Genes activated in vascular tissues after SAH include immediate early genes, genes encoding stress-induced and inflammatory response molecules as well as genes related to metal ion metabolism, ion channels, membrane receptors, extracellular matrix, signaling molecules, hormones and neurotransmitters metabolism.

Immediately early genes are involved in the control of transcription and their activation may occur within minutes following brain injury. In the presence of hemolysate, c-fos, jun B and c-jun mRNAs were upregulated in cultured vascular smooth muscle cells starting from 15 minutes after exposure and reaching peak expression between 30 and 60 minutes later. Microarray investigations by Onda et al. revealed vascular activation of genes related to inflammatory process including monocoye chemotactic protein-1, cystatin B inhibitor of the cysteine protease, inter-z-trypsin inhibitor family heavy chain-related protein–member of the family of serine protease inhibitors, serum amyloid A protein (acute phase protein) and gp130, constituent of interleukin-6 receptor complex. In cerebral tissues, polymerase chain reaction (PCR) studies revealed early activation of heme oxygenase-1 and iNOS after SAH; however, genomic investigations are still awaited.

**PERSPECTIVES**

It is believed that knowledge of molecular signaling pathways in the acute stage of SAH will allow researchers to invent novel therapies against brain injury. The fact that there are few specific and potent drugs targeting pathological signal transduction provides a great challenge for investigators. An alternate approach would be to develop double stranded RNA-based gene-silencing therapeutics to target genes mediating brain injury and apply cytoprotective gene therapy. Molecular pathways of SAH-induced early brain injury show high level of signal redundancy. Therefore, implementing and testing novel molecular therapies in SAH will require proteomic and genomic studies.

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