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Chapter 3

The Pathogenesis of Edema and Secondary Insults after ICH

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Additional information is available at the end of the chapter

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1. Introduction

Intracerebral hemorrhage (ICH) is a type of acute stroke characterized by extravasation of blood into brain parenchyma and formation of hematoma, leading to edema and tissue damage in the brain. During ICH, rapid accumulation of blood within brain parenchyma leads to disruption of normal anatomy and increased local pressure. Depending on the dynamic of hematoma expansion (growth), the primary damage occurs within minutes to hours from the onset of bleeding and is primarily the result of mechanical damage associated with the mass effect, which compress adjacent tissues, thus destroying them. The 'mass effect' is an important factor in the pathogenic events in ICH. But it may be difficult to predict and manage this effect directly by drug therapies [1-2].

Once present, ICH causes both primary and secondary injury. The primary insult is due to disruption of adjacent tissue and mass effect. Secondary injury occurs with the development of edema, free radical formation, inflammation, and direct cellular toxicity due to the deposited hematoma and subsequent degradation byproducts [3].

2. Body

After arteriolar rupture and parenchymal hemorrhage in the brain, a combination of local compression, cytotoxic injury, inflammation, and surrounding edema ensues. Many patients with ICH deteriorate progressively with no sign of hematoma expansion, suggesting that secondary damage following ICH plays a critical role in neurological deterioration. Secondary damage is, for the most part, attributable to the presence of intraparenchymal blood and may be dependent on the initial hematoma volume, patients age, or ventricu-

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lar volume. Several lines of evidence show that secondary damage involves blood constituents such as thrombin and hemoglobin as well as its degradation products, which exert biological actions or toxic influences on brain cells. These events, primarily resulting from blood extravasation, which subsequently activates cytotoxic, oxidative and inflammatory pathways, also triggers secondary reactions in the brain parenchyma including recruitment of additional proteases, cerebral edema, and cellular apoptosis, ultimately leading to blood–brain barrier disruption and massive brain cell death. The toxic effects of extravasated blood result mainly from blood components, including red blood cells (RBCs), plasma proteins, coagulation factors, inflammatory mediators , complement components and immunoglobulins. After ICH, the extravasated blood components (primarily erythrocytes and plasma proteins) and the damage-associated molecular patterns, impose a strong cytotoxic, pro-oxidative, and proinflammatory insult toward adjacent viable brain cells and could be seen as early as minutes after onset of ICH [1-3].

In addition to the hematoma, the associated edema may also contribute to the initial neurological deficit, subsequent decline, or death. The edema related to ICH has been cited as a reason for neurological deterioration after the first 24 to 48 h from the onset of symptoms, and it has, to a lesser degree, also been implicated with deterioration as late as 3 weeks. The edema has been demonstrated to be predominately vasogenic with a cytotoxic component. The vasogenic edema is a consequence of blood brain barrier (BBB) breakdown. In the normal brain, the BBB prevents the flow of water into the brain due to hydrostatic pressure gradients. However, when the BBB is disrupted as occurs in ICH, the imbalance in hydrostatic forces result in the entry of an exudative proteinaceous fluid onto the brain parenchyma. The disruption in the BBB is likely a consequence of an inflammatory cascade with resultant expression of specific cytokines and other markers of inflammation. The presence of red blood cells and their subsequent lysis and release of oxyhemoglobin may contribute to the leakage of the BBB. The hemorrhage itself also induces the production of thrombin and the overexpression of matrix metalloproteinases. Thrombin has been demonstrated to be an important factor in the modulation of BBB breakdown. Thrombin may be a major mediator of ICH-induced tumor necrosis factor-α production and an increase of perihematomal tumor necrosis factor-α levels contributes to brain edema formation after ICH. Matrix metalloproteinases also promote BBB disruption and have been associated with increased edema volume via extracellular matrix proteolysis, basal lamina destruction, and the degradation of c-fibronectin [4].

3. Blood plasma components/products

At early stage following ICH, the toxicity of extravasated blood plasma components including blood derived coagulation factors, complement components, immunoglobulins, and other bioactive molecules are proposed to act as contributors to ICH-affected tissue damage.

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3.1. Thrombin [1-2]

Thrombin, a serine protease produced rapidly after ICH onset, plays a pivotal role in the blood coagulation cascade. In response to bleeding, a complex series of clotting-factor interactions leads to its conversion by thromboplastin to thrombin, which transforms fibrinogen in plasmainto fibrin, as well as catalyzing many other coagulation-related reactions. As part of its activity in the coagulation cascade, thrombin also promotes platelet activation and aggregation via activation of protease-activated receptors on the cell membrane of the platelet. The primary purpose of thrombin is to stop bleeding as soon as possible and prevent hematoma expansion. Besides its physiological role, substantial lines of evidence indicate that thrombin participates in various pathological conditions in the brain, which contributes to edema formation and blood–brain barrier damage in early brain injury, and activates the cytotoxic, excitotoxic and inflammatory pathways that are involved in secondary injury following ICH.

In the case of ICH, a large amount of blood-derived thrombin invades the brain tissue and exerts biological actions through its proteolytic activity. The substrates for thrombin include proteinase-activated receptors that transduce intracellular signals via trimeric G proteins.The role of thrombin in ICH pathogenesis was first suggested by its possible involvement in edema formation. That is, injection of whole blood into the striatum of rats induced edema, which was prevented by addition of hirudin, a thrombin inhibitor. Edema induced by autologous blood injection into the striatum was attenuated also by argatroban, another inhibitor of thrombin, even when the drug was systemically administered from 6 h after blood injection. ICH-associated edema results from disruption of the blood–brain barrier and death of brain parenchymal cells, both of which may be induced by thrombin.Application of thrombin to rat corticostriatal cultures induced delayed neuron death in the cortical region and shrinkage of the striatal region. Various pharmacological examinations revealed distinct properties of the mechanisms of injury between the cerebral cortex and the striatum. For example, extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), three major members of mitogen-activated protein kinase (MAPK) family, all contributed to thrombin-induced injury of the striatum, whereas ERK, but not p38 or JNK, was involved in cortical injury. In addition, depletion of microglia from slice cultures rescued striatal tissue, but not cortical cells, from thrombin-induced injury, suggesting that microglia participate only in striatal tissue injury. Involvement of MAPKs and activated microglia in striatal tissue injury was confirmed by an in vivo study where thrombin was directly injected into the striatum of adult rats. On the other hand, plasminogen was found to cooperate with thrombin in inducing cortical injury but not striatal injury.Thrombin-mediated cellular injury may also be mediated by activation of matrix metalloproteinase (MMP)-9. That is, concurrent application of MMP-9 exacerbates cytotoxicity of thrombin in neurons in primary culture, and thrombin cytotoxicity is partially attenuated by MMP inhibitors. Moreover, brain damage induced by autologous blood injection is synergistically attenuated by deletion of the gene encoding MMP-9 and administration of the thrombin inhibitor hirudin.

So, Thrombin can be a double-edged sword, It should be protective during the early stage in ICH to rapidly stop bleeding and prevent hematoma enlargement , whereas its augment in the late stage may result in edema formation and be toxic.

3.2. Haptoglobin (Hp) and Hemopexin(Hx) [1-2]

Haptoglobin and Hemopexin Acts to Combat Hb/Heme Toxicity After ICH.

Haptoglobin (Hp), an acute phase protein, is an abundant blood plasma component that is normally synthesized and released into blood circulation primarily by hepatocytes. The primary function of Hp in blood is to bind and neutralize nephrotoxic free Hb in case of intravascular hemolysis.

Under normal circumstances, Hp represents an effective mechanism by which our body is protected from Hb toxicity. However, because Hp synthesis is not increased by low Hp levels and Hp is not recycled by macrophages, it may take 5 to 7 days for the Hp level to recover if completely sequestered by Hb. Thus, massive hemolysis may lead to persistent hypohaptoglobinemia. Interestingly, Recently study demonstrated that Hp is also produced locally in rat brain after ICH and its expression is significantly increased around the hematoma within hours from the onset of ICH.

The brain-derived Hp appeared to be synthesized and released by oligodendrocytes. Because oligodendroglia are abundant in white matter and are present throughout the gray matter, local production of Hp by these cells likely represents an important endogenous mechanism protecting brain against the extravascular Hb toxicity. Indirect support for such a claim includes (1) primary oligodendrocytes protect neurons in culture from Hb toxicity via Hp release; (2) animals made hypohaptoglobinemic with repetitive Hb administration, before ICH, experience more extensive brain damage; (3) mice genetically engineered to overexpress Hp are less susceptible to ICH injury; and (4) Hp-deficient mice are more vulnerable to ICH injury. In the context of therapeutic relevance, there have been determined that pharmacological intervention with sulforaphane, a naturally occurring agent that acts as NF-E2–related factor-2 (Nrf2) transcription factor activator, increases Hp in blood plasma and brain, and notably reduces brain damage in animal models of ICH. The relevance of Hp genotype as a factor modifying the outcome after ICH has not been studied to date. It could also be relevant for this review to indicate that in addition to the Hp-Hb/CD163 scavenging system, an independent system exists to help remove Hb breakdown products, heme, and iron.

Hemopexin (Hx) is a blood plasma glycoprotein synthesized primarily by hepatocytes. Hx has been shown to bind to heme with a high affinity and forms stable Hx-heme complexes. The heme–Hx complexes are readily endocytosed by macrophages expressing CD91 macroglobulin receptor, (also known as low-density lipoprotein receptor-related protein-1). Although under physiological conditions CD91 plays a role in recycling iron in response to extravascular hemolysis in hematoma-affected tissue, the Hx-heme/CD91 system may facilitate removal of prooxidative heme by icroglia/macrophages.

Recent studies and ongoing research in this laboratory support this notion and suggest that Hx-deficient mice experience augmented ICH injury. More studies of the role of Hx in ICH are warranted.

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4. Red blood cells degradation products [1-2, 5-6]

Aside from proteases and plasma products , important blood constituents contributing to ICH pathogenesis are erythrocyte and its degradation products. Following ICH, a large number of red blood cells penetrate into brain parenchyma. Hemolysis does not occur promptly after ICH, but rather, proceeds slowly, taking several days to weeks. Packed RBCs do not cause acute edema development after infusion into the basal ganglia or frontal white matter. However, they do cause delayed edema that appears to be related to release of hemoglobin. Usually, most RBCs start to lyse several days after ICH, but RBC lysis can occur very early.A cascade of events triggered by erythrocyte lysis is critical for the delayed development of edema and the secondary brain damage after ICH.

4.1. Hemoglobin (Hb) and Heme and heme oxygenase-1 (HO-1) [1-2, 5-6]

Red blood cell lysis lead to release of cytotoxic hemoglobin (Hb) with further deterioration of the pathological status quo . Hb and its degradation products, heme and iron, directly compromise the well-being of neighboring brain cells. Hb and heme are potent cytotoxic chemicals capable of causing death to many brain cells.

Hemoglobin(Hb) is a major component of blood and a potent mediator of oxidative stress after ICH. After a cerebral hemorrhage, large numbers of hemoglobin-containing red blood cells are released into the brain's parenchyma and/or subarachnoid space. Hemoglobin is released from lysed red blood cells, heme is liberated from hemoglobin, and hemoglobin as well as heme is taken up by brain parenchymal cells such as microglia and neurons. Prominently, the mechanism of hemoglobin toxicity is via generating free radicals (mainly through Fenton-type mechanism) and massive oxidative damage to proteins, nucleic acids, carbohydrates, and lipids. Heme itself or its degradation products may contribute to formation of brain edema and secondary brain injury after hemorrhagic insults. Hemin, the oxidative form of heme, plays a critical role in Hb-induced brain injury following ICH. Hemin exerts its neurotoxic effects via release of excessive iron, depletion of glutathione and production of free radicals.

Breakdown of the heme moieties of hemoglobin is catalyzed by heme oxygenase-1 (HO-1) into iron, carbon monoxide, and biliverdin, the latter two of which are thought to mediate the anti-inflammatory and antioxidant actions. HO-1, the rate-limiting enzyme for heme catabolism and iron production, after induction of ICH by collagenase or injection of autologous blood, robust expression of HO-1 is induced predominantly in non-neural cells such as microglia/macrophages and endothelial cells. The role of HO-1 following ICH is controversial.

Under normal conditions, HO-1 is expressed at a very low level, but it is rapidly induced by hemoglobin, heme, and various oxidants. HO-1 has a convincing role in the regulation of intracellular iron. HO-1increased heme catabolism, so it plays a protective role against oxidative injuries in the vascular system.HO-1 induction protects astrocytes from the oxidative toxicity of hemoglobin and heme. HO-1 has a protective role as an intrinsic factor against oxidative stress and the protection depends on the degree of oxidative stress by generating antioxidant bilirubin and vasodilating carbon monoxide. Deficiency of HO-1 in humans and

in an HO-1 knockout mouse model leads to vulnerability to oxidant stress and inflammation. In the present study, intravenous administration of nicaraven (a marked synergistic induction of HO-1 protein, for 2 days after subarachnoid hemorrhage) ameliorated delayed cerebral vasospasm in rat subarachnoid hemorrhage models. These results suggest that the enhanced HO-1 expression through a combination of pathological state and pharmacological agent could be an effective strategy to improve the prognosis of heme- and oxidative stress-induced diseases. However, contradictory results have been reported in other researches. Overexpression of HO-1 may be cytotoxic when excessive free iron exceeds the antioxidant properties of heme derived biliverdin. Earlier studies on ICH models in pigs and rabbits demonstrated that tin-mesoporphyrin, an isoform-nonselective HO inhibitor, attenuated edema formation and neuron loss. HO-1 and peroxidized lipid content were significantly higher in CSF from SAH patients with vasospasm, compared with nonvasospasm SAH CSF and correlated with occurrence of vasospasm. It is suggested that bilirubin and HO-1 was induced after hemorrhagic stroke, reflecting the intensity of oxidative stress. One potential explanation for such discrepancy is that HO-1 deficiency could reduce the excessive liberation of free iron from erythrocytes in hematoma (feature that is unique to ICH) and consequently limit the iron-mediated oxidative stress.After some controversy over the beneficial or toxic roles of HO-1, a better understanding of the pivotal HO-1 has evolved. We recently proposed a novel role of HO-1 in ICH pathogenesis. We concluded that upregulation of HO-1 after ICH may be a double-edged sword. The early mild upregulation possibly fit with the events and its overexpression in the late stage may result in its dysfunction and be toxic. It should be prudent to intervene ICH with the inhibitor or activator of HO-1 and think over its potential dual effects.

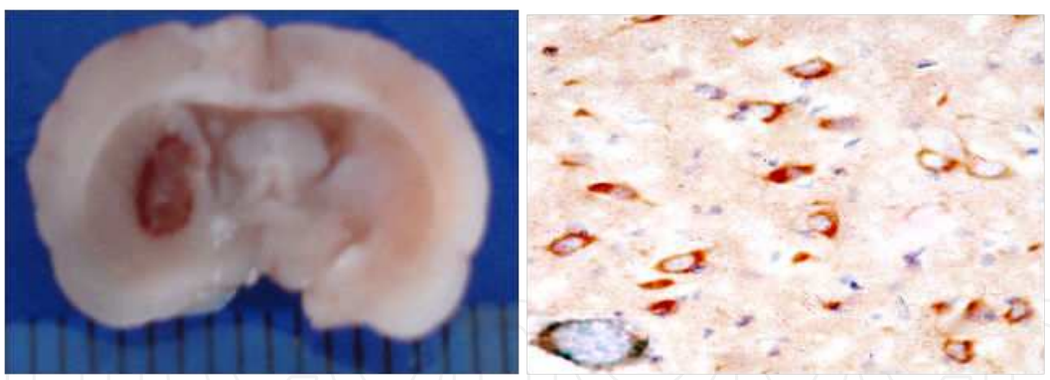


Figure 1. Cerebral slice with autologus blood HO-1 positive staining in ICH rat in ICH rat

4.2. Iron deposits and AQP4 [1-2, 5-6]

As indicated, toxicity of free iron originated from extravascular hemolysis, and HO-mediated catabolism is well-documented. Iron derived from heme degradation may also play a key role in ICH pathogenesis, presumably via acceleration of oxidative stress. After autologous blood injection in rats, induction of HO-1 is followed by a gradual increase in tissue levels of non-heme iron. Iron concentrations in the brain can reach very high levels following RBC lysis, possibly contributing to acute brain edema formation (1st week) and delayed brain atrophy (1

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month later). Lysis of RBCs and iron overload contribute to delayed edema formation after ICH. Iron accumulation in brain tissue is toxic and may result in brain damage after ICH. A recent clinical study showed that high serum levels of ferritin, a soluble protein for iron storage, are associated with poor outcome in ICH patients, suggesting that iron is involved in brain damage by ICH. Consistent with this idea, several studies on animal models of ICH showed that the iron chelator deferoxamine attenuated tissue damage and neurological deficits. For instance, intraperitoneal administration of deferoxamine starting from 2 or 6 h after autologous blood injection lessened brain edema, oxidative stress, and motor function deficits in rats. Another study on aged rats showed that deferoxamine was effective in reducing brain edema and motor dysfunction even when administration of the drug was delayed and 48 h, respectively, after autologous blood injection. However, the therapeutic efficacy of deferoxamine might depend on the type of ICH model. Reports mentioned above demonstrating beneficial effects of deferoxamine are based on the model made by autologous blood injection, whereas deferoxamine did not improve the outcome of the collagenase- induced ICH model in rat. On the other hand, a study on clioquinol, another kind of ferrous iron chelator, has demonstrated that oral administration of the drug, starting 6 h after induction of hemorrhage near the internal capsule by collagenase, alleviated motor dysfunction of rats . It recently has been demonstrated that estrogen reduces ferrous iron toxicity in vivo and in vitro, indicating that gender difference in susceptibility to ICH may, in part, be associated with differences in handling ferrous iron toxicity. Iron (II), by reacting with H2O2 generates hydroxyl radicals and clioquinol, by forming stable complexes with ferrous iron prevents its engagement in oxidative reactions. So , all these data suggest that iron particluarly ferrous iron (II) plays an important part in brain injury after ICH.

Iron has the potential to mediate a number of deleterious reactions both in vitro and in vivo. Iron accumulation in tissues, particularly if the labile iron pool is increased, is associated with tissue damage.Iron overload in the brain can cause free-radical formation and oxidative damage such as lipid peroxidation after ICH. Brain cells, including neurons, astrocytes, and microglia, show a decreased ability to respond to oxidative stress, particularly with respect to their levels of glutathione and lutathione peroxidase, such that alteration in their iron status may predispose them to iron-induced oxidative stress.

Among the aquaporins(AQP) family, a major water-channel in the central nervous system(CNS)is AQP4, which is a key molecule for maintaining water balance, and its dysfunction or structural damage may cause brain edema.Aquaporin 4, a major water channel protein that is expressed in the brain, plays a key role in the maintenance of brain water homeostasis. It has been proposed that AQP4 may play an important role in the formation of cerebral edema. Because of restricted space within the cranium, salt and water fux in the CNS must be strictly regulated to maintain neuronal functions of the brain. In the CNS, most of the AQP4 is expressed in perimicrovessel astrocyte foot processes, and alterations in AQP4 expression are associated with perturbations of brain water homeostasis. The pattern of AQP4 expression was correlated with blood-brain barrier permeability, which was assessed using contrast enhanced Computed Tomography scanning. Our results showed that AQP4 was mainly located around blood vessels. The current study provided more direct evidence that AQP4 in perivascular

astroglial end feet plays a key role in exchange of water between brain, blood, and cerebrospinal fuid. Upregulation of AQP4 induced by iron overload may cause an increased permeability to water in astrocytic membranes. The faint positive immunoreactivity of AQP4 possibly prevent the astrocytes from swelling. So, AQP4 in the brain may be viewed as a final common pathways of cerebral edema.

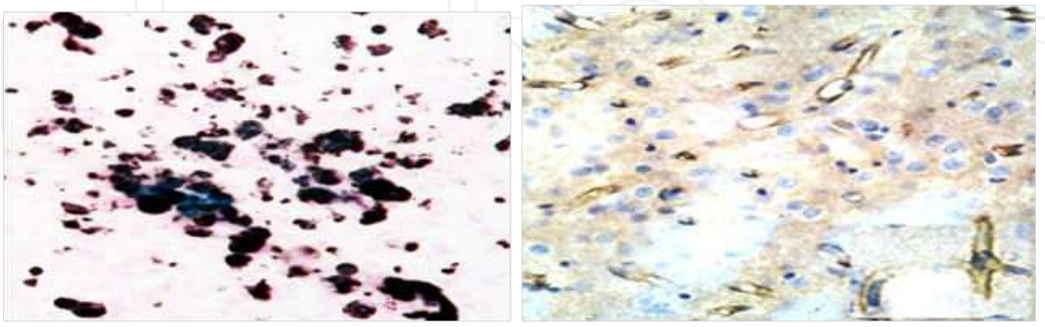


Figure 2. Iron staining after ICH (Perl's staining) AQP4 positive staining in ICH rat

5. Inflammatory mediators [1-4]

Whereas inflammatory mediators generated locally in response to brain injury have the capacity to augment damage caused by ICH (secondary injury), the involvement of inflammatory cells, eg, microglia/macrophages, is vital for removal or cleanup of cellular debris from hematoma, the source of ongoing inflammation. The timely removal of damaged tissue is essential for reducing the length of deleterious pathological process and thereby allowing for faster and more efficient recovery.

Several lines of evidence showed that activation of innate immunity and inflammatory responses contributes to the pathogenesis of secondary injury after ICH. An inflammatory response occurs after ICH, which aggravates ICH-induced brain injury, leading to further tissue damage, blood–brain barrier disruption and edema. The inflammatory mechanisms involved in progression of ICH-induced brain injury include activation of microglial cells, infiltration of inflammatory cells and production of cytokines and chemokines.

5.1. Microglias activation [1-4, 7]

The brain-resident phagocytes, microglia, are highly abundant (10%–15% of total glial cells) in brain and become readily activated within minutes after ICH. Microglia are a type of glial cell that are the resident macrophages of the brain and spinal cord, and thus act as the first and main form of active immune defense in the central nervous system(CNS). Microglial cells fulfill a variety of different tasks within the CNS mainly related to both immune response and maintaining homeostasis.

Microglia constitute 10-15% of the total glial cell population within the brain. Microglia (and astrocytes) are distributed in large non-overlapping regions throughout the brain and spinal

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cord. Microglia are constantly scavenging the CNS for plaques, damaged neurons and infectious agents. The brain and spinal cord are considered "immune privileged" organs in that they are separated from the rest of the body by a series of endothelial cells known as the blood–brain barrier, which prevents most infections from reaching the vulnerable nervous tissue. In the case where infectious agents are directly introduced to the brain or cross the blood–brain barrier, microglial cells must react quickly to decrease inflammation and destroy the infectious agents before they damage the sensitive neural tissue. Due to the unavailability of antibodies from the rest of the body (few antibodies are small enough to cross the blood brain barrier), microglia must be able to recognize foreign bodies, swallow them, and act as antigen-presenting cells activating T-cells. Since this process must be done quickly to prevent potentially fatal damage, microglia are extremely sensitive to even small pathological changes in the CNS.

Microglia can be activated by a variety of factors including: glutamate receptor agonists, pro-inflammatory cytokines, cell necrosis factors, lipopolysaccharide, and changes in extracellular potassium (indicative of ruptured cells). Once activated the cells undergo several key morphological changes including the thickening and retraction of branches, uptake of major histocompatibility complex (MHC) class I/II proteins, expression of immunomolecules, secretion of cytotoxic factors, secretion of recruitment molecules, and secretion of pro-inflammatory signaling molecules (resulting in a pro-inflammation signal cascade). Activated non-phagocytic microglia generally appear as "bushy, " "rods, " or small ameboids depending on how far along the ramified to full phagocytic transformation continuum they are. In addition, the microglia also undergo rapid proliferation in order to increase their numbers. From a strictly morphological perspective, the variation in microglial form along the continuum is associated with changing morphological complexity and can be quantitated using the methods of fractal analysis, which have proven sensitive to even subtle, visually undetectable changes associated with different morphologies in different pathological states.

Microglial cells are activated within minutes after the onset of ICH. The activated microglia release proinflammatory cytokines and chemotactic factors, which help to recruit hemotogeneous inflammatory cells to the ICH injury sites. Activated microglial cells undergo morphological and functional changes that include enlargement and thickening of processes, upregulation of proinflammatory proteins, and behavioral changes, including proliferation, migration and phagocytosis. Timely clearance of the extravasated RBCs by activated microglia/macrophages can provide protection from local damage resulting from RBC lysis. The primary neuroprotective role of activated microglia is to clear the hematoma and damaged cell debris through phagocytosis, providing a nurturing environment for tissue recovery. This is characterized first by the transient (18 hours–4 days) infiltration of neutrophils and then a long-term (1 day–months) presence of hematogenous macrophages. However, accumulating evidence has shown that microglial activation contributes to ICH-induced secondary brain injury by releasing a variety of cytokines, chemokines, free radicals, nitric oxide and other potentially toxic chemicals. In addition, several studies have shown that inhibition of microglial activation reduces brain damages in animal models of ICH. Microglial inhibitors, such as minocycline and microglia/macrophage inhibitory factors (tuftsin fragment 1–3), reduce ICH-induced brain injury and improve neurological function in rodents. Clearly, microglial activation mediates ICH-mediated brain injury. Successful removal of injured cells can reduce

secondary damage by preventing discharge of injurious proinflammatory cell contents. Resolution of hematoma and inhibition of inflammation are considered potential targets for ICH treatment.

Activated microglia can be stained via the marker ionized calcium-binding adapter molecule 1 (IBA1), which is upregulated during activation. Microglia are the only cells in the brain to express Iba1.

One way to control neuroinflammation is to inhibit microglial activation. Studies on microglia have shown that they are activated by diverse stimuli but they are dependent on activation of mitogen-activated protein kinase (MAPK). Previous approaches to down-regulate activated microglia focused on immunosuppressants. Recently, minocycline (a tetracycline derivative) has shown down-regulation of microglial MAPK. Another promising treatment is CPI-1189, which induces cell death in a tumor necrosis factor (TNF) α-inhibiting compound that also down-regulates MAPK. Recent study shows that nicergoline (Sermion) suppresses the production of proinflammatory cytokines and superoxide anion by activated microglia. Microglial activation can be inhibited by MIF (microglia/macrophage inhibitory factor, tuftsin fragment 1–3, Thr-Lys-Pro). MIF-treated mice showed reduced brain injury and improved neurologic function in a mouse model of collagenase-induced intracerebral hemorrhage.

Albeit some inflammatory responses generated by microglia/macrophages after ICH may aggravate brain injury, microglia/macrophages-mediated phagocytosis is instrumental in conducting brain clean-up, the process that must occur to allow for tissue repair and functional recovery. A fast and efficient removal of apoptotic, dislocated (eg, extravascular erythrocytes), and damaged cells before the discharge of injurious and proinflammatory cell contents (damageassociated molecular patterns) occurs and may help to reduce secondary damage.

So, by inhibiting the activation of microglial cells, namely the inhibition of brain primary response to the timely removal of damaged tissue and self repairing systems, to reduce the amount of potential damages, the desirability of this way is still questionable .

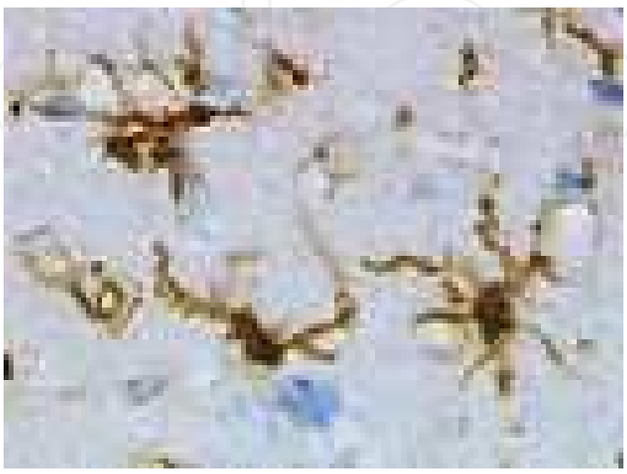


Figure 3. Microglia - ramified form from rat cortex before traumatic brain injury (lectin staining with HRP)

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Figure 4. Microglia/Macrophage - activated form from rat cortex after traumatic brain injury (lectin staining with HRP)

5.2. Infiltration of inflammatory cells [1-3]

Besides microglia, other blood-derived inflammatory cells, such as leukocytes and macrophages, are also activated after ICH and contribute to ICH-induced brain injury Neutrophil infiltration occurs less than 1day after the onset of ICH, and the infiltrating neutrophils die by apoptosis within 2days . Neutrophils are believed to contribute to brain injury after ICH. Depletion of neutrophils reduced blood–brain barrier disruption, axon injury and inflammation in a rat model of ICH and was found to prevent tissue plasminogen activator (tPA)-induced ICH in a rat model of cerebral ischemia. Neutrophils may damage brain tissues by producing reactive oxygen species (ROS) and releasing proinflammatory cytokines and matrix metalloproteinases (MMPs). Dying leukocytes can cause further brain injury by stimulating microglia/macrophages to release proinflammatory factors. Activated macrophages are indistinguishable from resident microglia in morphology and function. Similar to activated microglia, activated leukocytes and macrophages release a variety of cytokines, chemokines, free radicals and other potentially toxic chemicals.

5.3. Production of cytokines

Cytokines are well-known to be associated with inflammation and immune activation. Although cytokines are released by many cells, including microglia/macrophages, astrocytes and neurons, the major sources of cytokines are activated microglia/macrophages.

5.3.1. TNF-α and IL-1β [1-3]

Many studies have shown that two major proinflammatory cytokines, TNF-α and interleukin1β (IL-1β), exacerbate ICH-induced brain injury. After ICH, TNF-α is significantly increased both *in vivo* and *in vitro*, which may contribute to brain edema formation and brain injury in animal models of ICH. Consistent with animal studies, clinical studies support the

proposition that TNF-α contributes to ICH-induced brain injury. Plasma TNF-α has been shown to correlate with the magnitude of the perihematomal brain edema in patients with ICH. Single-nucleotide polymorphisms in the TNF-α gene promoter are associated with spontaneous deep ICH. Similarly, IL-1β has been found to be upregulated after ICH in an animal model and to produce detrimental effects, including brain edema and blood–brain barrier disruption.

5.3.2. Nuclear factor kappa-B (NF-κB) [1-3]

The apoptotic pathway in ICH may involve nuclear factor-kappa B (NF-κB), which is a ubiquitous transcription factor that, when activated, translocates to the nucleus and binds to DNA. NF-κB is associated with apoptotic cell death and has been reported in the role of cell death after experimental ICH in rats .

The inflammatory signaling involves a coordinated effort of different molecules and cell types and is largely coordinated by a ubiquitous transcription factor, NF-κB, a transcription factor involved in inflammatory responses, is a key regulator of many proinflammatory cytokines, such as TNF-α , IL-1β and MMP-9 are involved in various pathological conditions, including ICH-mediated brain injury. Activation of NF-κB occurs within minutes and lasts for at least 1 week after the onset of ICH. The activity of NF-κB correlates with perilesional cell death after ICH in rats and is positively associated with the progression of apoptotic cell death in patients with ICH. Several lines of evidence have shown that NF-κB is activated by RBCs and plasma via signaling pathways involving free radicals, cytokines and glutamate receptors. Cellular necrosis likely occurs at the core of the hemorrhage; however, apoptosis has been observed in the perihematomal region.

5.3.3. CD36 [1, 3]

Microglia and macrophages express various cell surface receptors, including scavenger receptors (eg, CD36) that assist in phagocytosis / endocytosis - mediated removal of cellular debris after tissue injury, including brain injury after ICH. One specific study evaluated CD36, a class II scavenger receptor that is transcriptionally regulated by peroxisome proliferator-activated receptors (PPARs). This study used in vitro and in vivo models and demonstrated that: (1) microglia/macrophages utilize CD36 to promote phagocytosis of red blood cell; and (2) treating animals with PPAR agonists (eg, rosiglitazone, pioglitazone, or 15D-PGJ2), which increased CD36 expression, results in faster hematoma resolution and improved functional recovery after ICH.

5.3.4. Toll-like receptors (TLRs) [3]

Toll-like receptors (TLRs) is expressed in microglia, the resident macrophages of the brain, belong to a large family of pattern recognition receptors that play a key role in innate immunity and inflammatory responses. It has been reported that TLR4 is upregulated in a rat model of ICH and that its signaling pathway contributes to poor outcome after ICH. TLR4 is activated by many endogenous ligands, such as heme and fibrinogen, which are produced in the brain

after ICH. Our recent *in vivo* study shows that activation of TLR4 by heme causes ICH-induced inflammatory injury via the MyD88/TRIF signaling pathway and that effective blockade of TLR4 by its antibody suppresses ICH-induced inflammation. Thus, the TLR4 signaling pathway could be a promising therapeutic target for ICH treatment.

5.3.5. Matrix metalloproteinases(MMPs) [1-2, 4]

The hemorrhage itself also induces the overexpression of matrix metalloproteinases(MMPs). Matrix metalloproteinases also promote BBB disruption and have been associated with increased edema volume via extracellular matrix proteolysis, basal lamina destruction, and the degradation of c-fibronectin.

Matrix metalloproteinases (MMPs) are a large family of calcium-dependent zinc-containing endopeptidases, which are responsible for the tissue remodeling and degradation of the extracellular matrix (ECM), including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycan. Matrix metalloproteinases are excreted by a variety of connective tissue and pro-inflammatory cells including fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes. These enzymes are expressed as zymogens, which are subsequently processed by other proteolytic enzymes (such as serine proteases, furin, plasmin, and others) to generate the active forms. After hemorrhagic events in the brain, several members of the MMP family are recruited and involved in pathogenic processes. An early study on collagenase injection model reported that the MMP inhibitor BB-1101 could reduce brain edema when administered 6h after induction of ICH. In human ICH patients, expression of MMP-9 and MMP-3 increases after the incident. MMP-9 expression is induced in astrocytes and neurons in the perihematomal area, possibly by an action of hemoglobin. Increased MMP-9 is associated with the extent of perihematomal edema, whereas increased MMP-3 is associated with high mortality. Experiments using MMP-9–deficient mice demonstrated that MMP-9 derived from both blood and brain parenchyma contributes to edema formation after autologous blood injection. In addition, MMP-9–deficient mice displayed lower levels of neurodegeneration, neutrophil infiltration, and microglia/macrophage reactions than wild-type mice. In the collagenase injection model, MMP-9 expression was found mainly in neurons and vascular endothelial cells, and administration of the MMP inhibitor GM6001, beginning 2 h after induction of ICH, attenuated neutrophil infiltration, oxidative stress, brain edema, neurodegeneration, and neurological impairment. With regard to MMP-3, early induction of this enzyme may contribute to brain damage in combination with other proteases such as MMP-9 and thrombin. Other lines of evidence indicate that MMP-12 may play a key role in the pathogenesis of ICH. That is, MMP-12 was induced most prominently among MMP isozymes in the collagenase-injection model in rats and mice, and MMP-12 deficient mice showed better functional recovery after ICH as well as reduced levels of recruitment of microglia/macrophages in the perihematomal region.It should be noted that several studies reported conflicting results. For example, a study on MMP-9–deficient mice showed that collagenase injection into the striatum of the mutant mice resulted in enhanced bleeding, increased mortality, and exacerbated neurological deficits. These changes may be attributable to heightened expression

of MMP-2 and MMP-3 in response to ICH, together with lowered levels of collagen in the brain of MMP-9–deficient mice . In addition, systemic administration of BB-94, a broad spectrum MMP inhibitor, from 30 min before collagenase injection increased hemorrhagic volume and the number of cells exhibiting DNA fragmentation.

6. Oxidative injury [1-2]

Neurological deficits associated with ICH were also released from RBC lysis is a potent cytotoxic chemical that generates free radicals and oxidative damage, causing death of surrounding cells. ROS are produced after ICH and contribute to ICH pathogenesis. In addition, phagocytosis generates a large amount of ROS that can damage macrophages and neurons. Oxidative stress appears to play a prominent role in ICH pathogenesis. In addition to increased free radical generation, damage to brain tissue may result from the impairment of the endogenous antioxidative enzyme system in response to ICH. Direct evidence for the causal relationship between free radicals and ICH injury was by demonstrating the efficacy of antioxidants as therapeutic agents. Specifically, the free radical scavengers, such as dimethylthiourea, α-phenyl-N-tert-butyl nitrone, NXY-059 (a sulfonyl derivative of α-phenyl-N-tert-butyl nitrone) or deferoxamine, a drug chelating pro-oxidative iron, significantly reduced brain injury in animal models of ICH. Mice with generically deleted NADPH oxidase, a key enzyme involved in generating ROS, showed reduced damage after ICH.

It reported a significant reduction in the levels of manganese superoxide dismutase and copper/zinc superoxide dismutase, the key enzymes of the antioxidant defense system in brains, after intracerebral injection of lysed erythrocytes.

Nrf2, a key player in antioxidative homeostasis. By binding to the antioxidant response element, Nrf2 regulates the expression of many detoxification and antioxidant enzymes, including superoxide dismutase, catalase, glutathione- Stransferase, glutathione peroxidase, HO-1, NAD(P)H quinine oxidoreductase -1, peroxiredoxin, or thioredoxin. To activate Nrf2 in animals after ICH, researchers utilized a naturally occurring organosulfur compound, sulforaphane. As expected, treatment with sulforaphane effectively increased the expression of Nrf2-regulated antioxidant genes, including catalase, superoxide dismutase, and glutathione-Stransferase, in brain tissue after ICH. Notably, this expression of antioxidants corresponded to reduced oxidative damage to proteins and lipids within the ICH-affected brain and importantly, with less severe neurological deficits. Nrf2 plays critical safeguard function in defending brain against oxidative stress associated with ICH pathogenesis.

7. Glutamate [2]

Glutamate has long been recognized as the major excitatory neurotransmitter in the central nervous system (CNS). This amino acid is also well known as an important player in various

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CNS disorders, since over-activation of ionotropic glutamate receptors causes neuronal damage via processes called excitotoxicity. Several lines of evidence suggest that glutamate is involved in the pathogensis of ICH. Transient elevation of the extracellular concentration of glutamate in the perihematomal region was demonstrated in rabbits following injection of autologous blood into the gray matter of the cerebrum. Subsequently, the effect of memantine, a low-affinity blocker of the N-methyl-D-aspartate subtype of glutamate receptor–associated channels, was investigated in the collagenase-injection model in rats. Daily intraperitoneal administration of memantine, starting from 30 min after induction of ICH, reduced hemorrhage volume, apoptotic cell death, neutrophil infiltration, and the number of microglia/macrophages in the periphery of hematoma.

8. Ischemia [8, 4]

Based on the recent publications, several potential factors of secondary ischemic injury after ICH have been consistently associated with acute ischemic lesions around hematoma. Although there is restricted diffusion within the hematoma during the first 2 weeks, an effect of increased viscosity and suspectibility effects from blood breakdown products, much more attention has been paid to the potential for ischemia in the surrounding tissue.

9. Conclusion

In conclusion, blood constituents, including hemoglobin-derived products as well as proteases such as thrombin and Haptoglobin, play important roles in the pathogenic events. Inflammatory reactions involving activated microglia, neutrophils, and production of proinflammatory cytokines also constitute a critical aspect of pathology leading to neurodegeneration and tissue damage. The mechanisms of secondary cerebral injury after ICH are complex and multidisciplinary. From a protective response into the later damaged process, they are interacting and overlapping, so we have to weigh the benefits (positive effects) and risks (adverse effects) carefully when we intervene in ICH.

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