Thrombin and hemin as central factors in the mechanisms of intracerebral hemorrhage–induced secondary brain injury and as potential targets for intervention

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Intracerebral hemorrhage (ICH) is a subtype of stoke that may cause significant morbidity and mortality. Brain injury due to ICH initially occurs within the first few hours as a result of mass effect due to hematoma formation. However, there is increasing interest in the mechanisms of secondary brain injury as many patients continue to deteriorate clinically despite no signs of rehemorrhage or hematoma expansion. This continued insult after primary hemorrhage is believed to be mediated by the cytotoxic, excitotoxic, oxidative, and inflammatory effects of intraparenchymal blood. The main factors responsible for this injury are thrombin and erythrocyte contents such as hemoglobin. Therapies including thrombin inhibitors, N-methyl-D-aspartate antagonists, chelators to bind free iron, and antiinflammatory drugs are currently under investigation for reducing this secondary brain injury. This review will discuss the molecular mechanisms of brain injury as a result of intraparenchymal blood, potential targets for therapeutic intervention, and treatment strategies currently in development.

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Stroke affects 15 million people worldwide and accounts for approximately 10% of all deaths.40 Strokes are classified as either ischemic or hemorrhagic, and occur due to blood vessel occlusion or blood vessel rupture, respectively. Approximately 13% of strokes are of the hemorrhagic subtype and include ICH and SAH.40 Intradecerebral hemorrhage is the most common cause of hemorrhagic stroke and causes extravasation of blood into the parenchyma and subsequent hematoma formation, resulting in brain damage.40 Intracerebral hemorrhage frequently causes significant morbidity and death, with as many as 50% of patients dying within 1 month of presentation, and only 20% of survivors able to function independently at 6 months.39 Also, with a worldwide incidence of 10–20 cases per 100,000 people, ICH is a global public health problem.39,118

Spontaneous ICH is mainly caused by hypertension, which causes microaneurysms at the bifurcation of intracerebral arterioles that can immediately rupture.29,123 These microaneurysms may be different from the berry aneurysms at the Circle of Willis branch points that cause SAHs. Intracerebral hemorrhage may also be due to cerebral amyloid angiopathy, anticoagulant use, hematological disorders, arteriovenous malformations, arteriovenous fistulas, cavernous angiomas, and brain tumors. Intracerebral hemorrhage can be further distinguished from SAH as it is more commonly found near gray-white junctions in cerebral lobes, subcortical structures such as the basal ganglia, the brainstem, and deep cerebellar nuclei.99,101 Current management for ICH immediately after onset involves airway management, monitoring of hemodynamic parameters, control of intracranial pressure, and hematoma evacuation.

Brain injury from ICH can be described by primary and secondary mechanisms (Fig. 1). The majority of the brain injury due to ICH typically occurs within the first few hours as a result of mass effect due to hematoma formation.39 This primary injury results in increased pressure and disruption of the surrounding neural structures,
resulting in early neurological deterioration. Although randomized trials have not consistently shown a clear benefit of surgical management compared with medical therapy, there may be a role for ICH evacuation in an attempt to reduce intracranial pressure and reduce mass effect to try and improve outcomes in select cases. Lack of Class I data supporting evacuation may be due to the added morbidity of the surgical procedure in eloquent areas (such as the basal ganglia), inappropriate timing of clot evacuation, variability of ICH and techniques used, and insufficient sample sizes in clinical trials.

Because the optimal therapy for treating the primary injury associated with ICH has not yet been identified, prevention and treatment of secondary injury is imperative. As many patients continue to deteriorate clinically despite no signs of rehemorrhage or hematoma expansion, there is increasing interest in the mechanisms of secondary brain injury following ICH. Vasogenic and cytotoxic edema due to the breakdown of the BBB and cellular injury have been implicated in this process. Additional mechanisms for this secondary injury are believed to be due to the intraparenchymal accumulation of various blood components following ICH, activating cytotoxic, excitotoxic, oxidative, and inflammatory pathways. As a result of increased awareness of this secondary injury, specific therapeutic targets have been identified in hopes of preventing further brain damage following ICH. In this review, we will discuss the various molecular mechanisms of secondary brain injury as a result of intraparenchymal blood, potential therapeutic targets, and the various treatment strategies currently under investigation.

Mechanisms of Secondary Brain Injury

Thrombin-Induced Injury

Thrombin, a serine protease found in the brain after ICH, has been shown to induce brain injury (Fig. 2). This enzyme is produced on the plasma membranes of platelets, neutrophils, monocytes, and lymphocytes as a result of cleavage of prothrombin following activation of the intrinsic and/or extrinsic coagulation cascades. Entry of blood into the brain parenchyma activates this process, releasing large amounts of thrombin that is known to cause perihematoma edema formation after ICH due to endothelial cell damage. Studies have also shown continuous release of thrombin from intracerebral hematomas for 2 weeks after clot formation due to fibrinolysis.

Thrombin-induced injury may be a central mechanism for secondary injury in ICH, as many pathways are
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Secondary injury due to thrombin primarily occurs through PARs, a family of G protein–coupled proteins found on the surface of various cells including platelets, neurons, and endothelial cells. Of these receptors, PAR-1, PAR-3, and PAR-4 have been shown to be activated by thrombin. This activation occurs by cleavage of the exodomains of PARs, forming a new amino terminus that acts as a tethered ligand for receptor activation, resulting in the activation of various signaling pathways. Protease-activated receptor-1 has been shown to be upregulated in ischemia models and is implicated in potentiation of NMDA receptors, neurite retraction, and cell death. It has been shown that mice lacking PAR-1 have a reduction in infarct volume following focal ischemia, indicating its importance in brain injury. Additionally, studies have shown continued PAR-1 activation following ICH, with PAR-1 levels peaking at 3 days after onset. This effect may last for up to 14 days, implicating this process in cerebral edema initiation as it often peaks approximately 3 days after ICH. Protease-activated receptors also activate various intracellular enzymes such as mitogen-activated protein kinases, which play a role in the recruitment of microglia and neuronal injury.

Red Blood Cell Lysis

The presence of extravasated RBCs in the brain following ICH also stimulates a variety of cytotoxic, oxidative, and inflammatory processes (Fig. 3). Red blood cell lysis begins to occur approximately 24 hours following ICH and occurs for several days after onset. This primarily occurs due to intracellular energy depletion, loss of structural integrity, and the formation of the membrane attack complex due to activation of the complement system. The release of the intracellular contents of these cells induces brain edema, as studies have shown increases in edema volume following reductions in hematoma size due to clot lysis. Studies in animals have shown delayed brain injury with intracerebral infusion of packed RBCs and dramatic edema formation within 24 hours following infusion of lysed RBCs. Infusion of lysed RBCs also causes disruption of the BBB, DNA injury, and expression of heat shock proteins, indicating cell stress. Once released from RBCs, hemoglobin is degraded into heme and iron, causing injury to surrounding cells.

Cytotoxicity

Thrombin has been shown to induce various components of the complement system, an enzymatic cascade of blood and cell surface proteins. Thrombin primarily activates complement C3d and C9. The presence of C3d following ICH indicates activation of the complement cascade, while deposition of C9 on the neuronal cell membranes indicates membrane attack complex formation. This activity leads to the formation of a trans-
membrane pore and subsequent cell lysis, which may be one of the mechanisms of neuronal death and disruption of the BBB as a result of endothelial cell damage following ICH. Additionally, lysis of erythrocytes may result in further damage through hemoglobin-mediated edema formation.

Thrombin is also able to induce apoptosis in neurons and astrocytes by activation of various intracellular pathways. This occurs via RhoA, a small guanosine triphosphate-binding protein part of the Ras superfamily. RhoA inhibitors have been noted to attenuate thrombin-mediated cell death, implicating this mechanism as a major cause of neuronal loss following ICH. However, the exact mechanism by which RhoA induces apoptosis is currently unknown. This process may involve caspase activation, as inhibitors to these enzymes have been shown to prevent thrombin-induced cell death.

Excitotoxicity

Potentiation of NMDA receptors by PAR-1 may cause neuronal death following ICH due to glutamate-induced excitotoxicity. This notion is supported by studies showing that PAR-1 knockout mice had reduced thrombin-mediated NMDA receptor potentiation. Also, removal of PAR-1 and the addition of NMDA receptor antagonists reduce neuronal injury associated with the addition of NMDA and transient middle cerebral artery occlusion. The potentiation of NMDA by PAR-1 occurs through the activation of Src, a proto-oncogene tyrosine-kinase, which is known to augment NMDA activity by phosphorylation of these receptors. This activity is confirmed by increased expression of Src kinases following ICH.

Levels of extracellular amino acids such as glutamate have been shown to increase following ICH, resulting in...
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glutamate-mediated excitotoxicity. This increase in levels of extracellular amino acids may be due to the release of these molecules as a result of active ischemia, as in vivo models have shown 80-fold increases in glutamate levels after middle cerebral artery occlusion. Because neurons have high intracellular concentrations of glutamate, ICH-induced cell death may result in the release of these stores into the extracellular space. Additionally, injury of astrocytes may impair glutamate removal, resulting in extracellular accumulation.

Oxidative Injury

Hemin, the oxidative form of heme, is a potent oxidant that injures cells and is well known to cause brain injury. Its mechanism of action occurs through oxidative stress and the activation of caspases, resulting in the injury of astrocytes, neurons, and microglia. However, microglia that clear hemin have protective mechanisms that prevent cell death. Following ICH, hemogenous phagocytes, microglia, and surrounding astrocytes attempt to sequester hemin. This primarily occurs via the heme carrier protein 1. Once within the cell, hemin is degraded by heme oxygenases, producing biliverdin, carbon monoxide, and iron. Iron released due to hemin degradation can reach high levels within the brain following ICH, resulting in the formation of hydroxyl radicals and subsequent cellular stress and DNA damage via interaction with hydrogen peroxide. Iron levels after ICH may increase up to 3-fold and remain elevated for 1 month, causing continued brain injury following the initial insult. However, hemin itself can also participate in redox reactions, producing free radicals that can damage intracellular structures and cause oxidative stress. Additionally, because hemin is lipophilic, it may intercalate into lipid membranes, altering function and fluidity. The roles of biliverdin, which is converted to bilirubin by biliverdin reductase, and carbon monoxide are unclear. Small concentrations of bilirubin have been demonstrated to inhibit glutamate uptake and induce inflammation, oxidative stress, and apoptosis. However, bilirubin and carbon monoxide have also been shown to have antioxidant and antiinflammatory effects. Also unclear is the amount of bilirubin accumulation due to hemin degradation following ICH.

Inflammation

Thrombin has also been observed to increase proinflammatory cytokines such as TNF-α and IL-1β. This may occur through the activation of microglia via PARs, resulting in recruitment and proliferation of these cells at the site of injury. Tumor necrosis factor-α has been shown to increase in ICH models and is implicated in edema formation because TNF-α knockout mice have less brain edema and neurological deficits compared with wild-type mice. Plasma TNF-α has been shown to correlate with the amount of brain edema in patients. Other studies have also raised other mechanisms of TNF-α mediated injury such as enhancement of leukocyte infiltration, resulting in BBB disruption and cellular apoptosis. Thrombin also stimulates microglia to secrete IL-1β, resulting in similar damaging effects as TNF-α, such as neurotoxicity, opening of the BBB, and induction of apoptosis. The role of this mechanism in ICH-mediated injury is supported by studies showing attenuation of brain edema by the overexpression of IL-1β receptor antagonists.

Matrix metalloproteinases are zinc-containing proteases that are involved in extracellular matrix remodeling, chemotaxis, and proteolytic cleavage of various molecules. These proteins are produced by microglia, pericytes, and astrocytes, and when found in high levels in the brain, result in extracellular matrix degradation, BBB disruption, and neuronal death. The mechanism for MMP-mediated brain injury is due to activation of microglia and subsequent release of inflammatory cytokines, release of neutrophil-derived toxins from infiltrated leukocytes, and generation of toxic molecules from interaction with nitric oxide. Several MMPs including MMP-2, -3, -9, and -12 have been observed to increase following ICH and can affect clinical outcome. Additionally, studies have shown that MMP-3, -9, and -12 null mice have less brain injury as a result of ICH. As thrombin is able to increase expression of various MMPs, the effects of thrombin on microglial activation and neuronal apoptosis may be due to these mediators.

Nuclear factor-κB, a transcription factor involved in inflammatory processes, also contributes to brain injury following ICH. In response to various cytokines and free radicals, NF-κB translocates to the nucleus, inducing the transcription of inflammatory enzymes, chemokines, and cytokines. Activation of NF-κB occurs within minutes of ICH and can remain active for 7 days following onset. This activity results in DNA fragmentation, causing cell death. Elucidation of the mechanisms of DNA fragmentation following NF-κB may allow for the development of therapeutic interventions to inhibit this process.

Nonhematogenous Perihematomal Mechanisms of Secondary Injury

Ischemia has been believed to play a role in secondary brain injury following ICH. Several animal studies have shown reductions in rCBF and the presence of tissue ischemia around hematomas, even though blood flow is reestablished quickly. This return to normal perfusion is observed as early as 10 minutes following hemorrhage but is likely variable, depending on factors such as size of the hematoma and the presence of increased intracranial pressure. Although there may be quick recovery, ischemic damage to the cortex overlying the hematoma has been noted, consistent with histological findings of ischemia following 5 minutes of CBF cessation. In ICH, ischemia of the surrounding tissue may be due to mechanical compression of the surrounding microvasculature by the hematoma, resulting in a hypoxic environment. Hypoxia causes brain injury by a multitude of mechanisms. The inability to synthesize ATP results in Na+/K+ ATPase dysfunction, leading to neuronal membrane depolarization and ionic imbalance. This may impair the function of many enzymes such as sodium-dependent glutamate transporters, resulting in increased extracellular glutamate levels and
excitotoxicity. Low concentrations of ATP also prevent the maintenance of low calcium concentrations within cells by disrupting the Ca\textsuperscript{2+} ATPase, leading to high intracellular calcium levels that activate many DNAses and calcium-dependent proteases. Additionally, energy depletion results in the production of reactive oxygen species and the release of cytochrome c from the outer mitochondrial membrane, both of which result in apoptosis and further brain injury. Many of these mechanisms of injury overlap with the excitotoxic and oxidative pathways induced by thrombin and hemin, demonstrating the complexity of these damaging pathways and challenge of designing drugs to prevent this injury.

However, some animal and human studies have shown evidence against a significant ischemic penumbra following ICH. These studies did not show any ischemic tissue surrounding the clot, although there was evidence of hypoperfusion. Positron emission tomography has shown reductions in the oxygen extraction fraction in tissue surrounding hematomas, contrasting with what occurs during acute ischemia. Magnetic resonance imaging in patients has not shown significant changes in the apparent diffusion coefficient or mean transit time, both of which are markers of irreversible ischemia and hypoperfusion. The lack of prolonged reductions in rCBF after ICH may be due to incomplete vascular compression by the hematoma. This idea is supported by studies demonstrating rCBF within hematomas in regions of intact neural tissue. Complete compression of intracerebral vessels by the expanding hematoma may result in the disruption of the pia-microvascular interface, potentially causing alterations in BBB integrity. Because this has not been noted to occur immediately following ICH, complete vessel compression is unlikely. In addition, white matter fibers are dense structures that provide mechanical resistance against the expanding hematoma. Finally, robust collateral circulation from penetrating cortical arterioles and pial vessels from other cerebral arteries may prevent significant changes in rCBF and tissue ischemia. However, due to the relatively small sample sizes in many studies, larger human studies are needed to provide more conclusive data.

Therefore, it is unclear whether perihematomal ischemia is a significant factor in secondary brain injury following ICH. Recently there has been a paradigm shift in thinking toward a metabolic instead of an ischemic penumbra. Increases in perihematomal glucose uptake and use (hyperglycolysis) have been observed in patients following ICH, consistent with what is noted following traumatic brain injury. The mechanism of focally increased glucose uptake may be due to nonconvulsive seizure activity, which is found in many patients with acute ICH. These repetitive depolarizations may lead to secondary injury by increasing extracellular glutamate, resulting in intracellular calcium accumulation and excitotoxicity. The role of seizures as a cause of increased glucose utilization is supported by the suppression of hyperglycolysis by anticonvulsant glutamate receptor antagonists. Further studies are needed to elucidate additional metabolic changes in this perihematomal tissue and investigate potential interventions to this ongoing injury.

**Potential Therapeutic Targets and Current Treatments Under Investigation**

Understanding the mechanisms of secondary injury following ICH has allowed for the development of treatments aimed at preventing this damage. Some agents have been validated in vivo studies but have not yet been evaluated in clinical trials. However, several clinical trials have already been conducted to evaluate various neuroprotective drugs for the treatment of secondary injury from ICH.

**Prevention of Cytotoxicity**

One promising therapy for the prevention of secondary brain injury following ICH is the use of direct thrombin inhibitors. As thrombin plays a major role in cellular injury via a variety of pathways, inhibiting its activity would be beneficial. Inhibitors such as hirudin (a thrombin inhibitor found in leeches) and argatroban (a synthetic, direct thrombin inhibitor) have been shown to reduce brain edema following ICH in vivo models, possibly by inhibiting PAR-1 expression. Although there is concern of prolonged bleeding with the use of these anticoagulants, the use of direct thrombin inhibitors has been shown to not cause enlargement of hematoma volume, unlike with other anticoagulants such as warfarin. Clinical trials are needed to evaluate the efficacy of these drugs for the prevention of brain injury following ICH.

However, complete inhibition of thrombin may actually be deleterious as low concentrations have been shown to be neuroprotective. This protective effect has been observed in neurons and astrocytes in vitro models. Pretreatment with thrombin has been shown to prevent brain edema and damage induced by large doses of thrombin, ICH, and cerebral ischemia. But these protective effects are eliminated by thrombin inhibitors. Although the exact mechanism by which thrombin exerts its neuroprotective effects is unknown, it is believed to be due to the activation of PARs, production of heat shock proteins, and upregulation of endogenous thrombin inhibitors. Additionally, thrombin preconditioning has been shown to increase levels of hypoxia inducible factor-1α, transferrin, and transferrin receptor, increasing brain tolerance to erythrocyte- and iron-mediated injury. Further research elucidating the mechanisms of this protective effect are needed for the development of therapeutic strategies aimed to enhance this effect. The doses of thrombin inhibitors that simply reduce thrombin concentration without complete inhibition need to be clarified to augment neuroprotection. Alternatively, specific thrombin inhibitors that do not affect neuroprotective pathways should be investigated.

Due to the activation of numerous apoptotic pathways following ICH, molecules that inhibit this process have been investigated for use in ICH. One such drug is tauroursodeoxycholic acid, the taurine conjugate of the endogenous bile acid ursodeoxycholic acid. Tauroursodeoxycholic acid is able to inhibit production of reactive oxygen species, stabilize the mitochondrial membrane, activate antiapoptotic proteins such as Bcl-2, and inhibit the activity of proapoptotic proteins such as Bad.
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Phase I trial investigating the safety of this drug has been designed.

Albumin has also been investigated as a neuroprotective agent. Studies have demonstrated numerous mechanisms of this neuroprotection including reduction of brain edema, inhibition of oxidative damage, and maintenance of normal endothelial and astrocytic function. In vivo studies have demonstrated improved functional outcome and BBB integrity following administration of albumin after ICH. The Albumin for Intracerebral Hemorrhage Intervention (ACHIEVE) trial is currently evaluating the effects of albumin in 40 patients with ICH.

Inhibition of Excitotoxicity

Gavestinel, a drug that functions as an antagonist by binding to the glycine site on the NMDA receptor, has been investigated in the Glycine Antagonist in Neuroprotection (GAIN) International and Americas trials. In these trials, patients were randomized to receive the drug or placebo within 6 hours of symptom onset. This time point is considered to be crucial as the majority of hematoma enlargement occurs within this period due to continuous bleeding or rebleeding. Outcomes of the trial were death or functional ability as determined by the Barthel Index. Of the 3450 patients randomized in these trials, 571 had ICH. Analysis of these patients revealed no significant differences in mortality rates between the 2 groups (p = 0.38). There was also no difference in the distribution of Barthel Index scores at 3 months between the 2 groups, although there was a trend favoring gavestinel (p = 0.091). It may be beneficial to test this agent later during the peak of secondary brain injury from ICH.

As glutamate levels have been shown to increase following ischemic injury and ICH, glutamate scavenging may provide neuroprotection. Oxaloacetate has been shown to be neuroprotective in traumatic brain injury models by reducing glutamate levels. The mechanism for this effect is due to the transformation of glutamate to 2-ketoglutarate by glutamate-oxaloacetate transaminase, an enzyme found in the blood. Human studies are needed to evaluate the efficacy of this mechanism in ICH.

Protection From Oxidative Injury

Three clinical trials have been conducted to evaluate citicoline (cytidine-5-diphosphocholine), an intermediate in the phospholipid synthetic pathway. Studies have shown its neuroprotective effects occur by maintaining the integrity of various cellular membranes, attenuating lipid peroxidation, restoring Na+/K+-ATPase activity, and enhancing the glutathione system. Additionally, citicoline may decrease glutamate release from neurons and improve astrocyte uptake, decreasing extracellular glutamate levels. In a randomized study of 32 patients, those receiving citicoline experienced improved muscle strength following ICH. Another study involving treatment of 19 patients with citicoline found that treated patients were 5-fold more likely to be functionally independent following ICH compared with those who received a placebo.

Finally, a trial of 182 patients revealed that treatment with citicoline resulted in improvement in the Barthel Index, although no effect on the modified Rankin Scale or NIH Stroke Scale was noted. Due to the neurotoxic effects of iron, there is interest in the use of iron chelators for prevention of this iron-mediated injury. In vivo studies have demonstrated that deferoxamine rapidly accumulates within brain parenchyma and reduces iron concentration, brain edema, neuronal death, and neurological deficits following ICH. A multicenter Phase I trial showed that infusions of deferoxamine are tolerable and safe up to a daily dose of 6000 mg. Preliminary data in 4 patients with hemorrhagic stroke and 3 with ischemic stroke showed decreases in serum markers of oxidative stress. Currently, a Phase II trial is underway to evaluate the efficacy of deferoxamine in ICH.

Haptoglobin is a protein found in blood plasma that has the ability to bind hemoglobin. It functions to bind extracellular hemoglobin, preventing hemoglobin-mediated oxidative damage. In the brain, haptoglobin is synthesized by oligodendrocytes, thereby protecting against extravascular hemoglobin toxicity. Animal models of ICH have demonstrated increased haptoglobin production following injury. Animals that are hypohaptoglobinemic are more susceptible to injury and have more brain damage following ICH, whereas those that overexpress haptoglobin are more protected. Haptoglobin is therefore a potential therapeutic target for the prevention of brain injury following ICH. Thus far, sulforaphane, a NF-E2–related factor–2 activator, has been shown to increase haptoglobin in the brain and reduce injury following ICH. Additional in vivo and human studies are needed to identify other agents that increase haptoglobin levels and establish their efficacy in preventing ICH-induced brain injury.

Another agent known to bind heme is hemopexin, a glycoprotein found in plasma. However, hemopexin is also expressed by neurons and is present throughout the brain. Mice that do not express hemopexin have greater infarct volumes and neurological deficits following middle cerebral artery occlusion. Hemopexin knockout mice also had increased protein oxidation and tissue heme, and decreased cell viability and locomotor activity. This protein may also be another modifiable target to decrease brain injury following ICH.

Reduction of Inflammation

Rosuvastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, has been inves-
tigated for its neuroprotective effects. Statins may exhibit their neuroprotective effects via a variety of mechanisms such as reduction of inflammation through inhibition of NF-κB, TNF-α, and chemokine expression,\textsuperscript{40,92} upregulation of nitric oxide synthase,\textsuperscript{31,63,73} and protection from glutamate-induced excitotoxicity.\textsuperscript{17} A prospective/retrospective nonrandomized study treated 18 patients with rosvastatin and found improved outcomes compared with control subjects (mortality rate 5.6% vs 15.8%; respectively; NIH Stroke Scale score ≥ 15, OR 0.04).\textsuperscript{124} Larger studies are needed to provide more conclusive evidence on the efficacy of statins for the prevention of secondary brain injury following ICH. Due to the neuroprotective effects of statins, there has also been considerable interest in using these drugs following aneurysmal SAH. A meta-analysis of double-blind randomized controlled trials showed significant reductions in delayed ischemic deficits (OR 0.41, 95% CI 0.20–0.82; p < 0.001) and mortality (OR 0.29, 95% CI 0.09–0.93; p = 0.04) following statin therapy for SAH.\textsuperscript{127}

Celecoxib is a nonsteroidal antiinflammatory drug that has been shown to reduce perihematoma inflammation and cell death in ICH.\textsuperscript{23,116} Because celecoxib selectively inhibits cyclooxygenase-2, it is a potential treatment for ICH because cyclooxygenase-2 is activated in ICH models, resulting in increased levels of prostaglandin E2.\textsuperscript{23} As prostaglandin E2 can induce free radical formation and glutamate-mediated excitotoxicity due to glutamate release from astrocytes, the neuroprotective effects of celecoxib are believed to occur through the reduction of prostaglandin E2 synthesis via cyclooxygenase-2 inhibition.\textsuperscript{23,90} One retrospective study analyzed the volumes of hematoma and edema in 17 patients treated with celecoxib.\textsuperscript{93} Treatment significantly reduced the volume of brain edema and the ratio of initial hematoma and edema volumes to follow-up volumes compared with the control group. The results of a Phase II trial investigating the efficacy of celecoxib are currently pending. Although trials have shown increased risk of serious cardiovascular events with use of celecoxib, short-term use in ICH may not increase these risks significantly.\textsuperscript{117}

Minocycline, a broad-spectrum tetracycline antibiotic, has also been investigated as a neuroprotective agent due to its antiinflammatory properties. In vivo studies have shown reduced perihematoma brain edema, neuronal loss, BBB disruption, and improved functional outcome following ICH with minocycline treatment.\textsuperscript{40,155} Minocycline also reduces brain iron accumulation and resulting toxicity by chelating iron.\textsuperscript{21} In an open-label, blinded study, 74 patients were treated with minocycline 6–24 hours after acute ischemic stroke.\textsuperscript{71} Those treated had significantly lower NIH Stroke Scale and modified Rankin Scale scores, with higher Barthel Index scores, indicating significantly better outcome. Currently, 3 trials are in progress for evaluation of the neuroprotective effects of minocycline in stroke.\textsuperscript{125}

**Other Investigated Agents**

Other studies have evaluated the use of mannitol, glycerol, and NXY-059 (disufenton sodium) for neuroprotection in patients with ICH but did not observe any improvement in mortality or functional outcome.\textsuperscript{77,83,152} Mannitol exerts its neuroprotective effects by functioning as an osmotic diuretic, thus reducing brain edema.\textsuperscript{85} It also functions as an antioxidant, protecting against free radical–mediated damage. Neuroprotection due to glycerol occurs by hemodilution, which results in increased cerebral perfusion and reduction of cerebral edema, thereby reducing intracranial pressure.\textsuperscript{152} The free radical trapping agent NXY-059 prevents brain injury by quenching free radicals formed by hemoglobin degradation and ischemic tissue.\textsuperscript{84}

**Conclusions**

The mechanisms of secondary brain injury following intracerebral hemorrhage are numerous and involve the initiation of cytotoxic, excitotoxic, oxidative, and inflammatory pathways. Optimal management of patients with ICH remains undefined. Surgical therapies have shown disappointing results in primary brain injury treatment. Medical therapies aimed at prevention of continued insult may improve mortality rates and functional outcomes. Although there is not yet an effective medical treatment, advances have been made in elucidating the mechanisms of brain injury following ICH. These advances have led to the development of neuroprotective therapies, many of which show promise in early clinical testing. However, further research is required to illuminate and better define the multitude of mechanisms involved in ICH pathogenesis in the hope of revealing targets for novel therapeutics. Additionally, large randomized trials are needed to establish the efficacy and safety of currently identified neuroprotective agents. Nonetheless, our focus must also be on finding efficient interventions to prevent ICH, decreasing the severe morbidity and mortality associated with this disease.

**Disclosure**

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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