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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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Key Words • intracerebral hemorrhage • thrombin • hemin • brain injury • neuroprotective drug • protease-activated receptor

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.[107] Intracerebral 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.[33] Also, with a worldwide incidence of 10–20 cases per 100,000 people, ICH is a global public health problem.[99,118]

Abbreviations used in this paper: ATP = adenosine triphosphate; BBB = blood-brain barrier; ICH = intracerebral hemorrhage; IL = interleukin; MMP = matrix metalloproteinase; NF = nuclear factor; NMDA = *N*-methyl-D-aspartate; PAR = protease-activated receptor; RBC = red blood cell; rCBF = regional cerebral blood flow; SAH = subarachnoid hemorrhage; TNF = tumor necrosis factor.

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.[98] This primary injury results in increased pressure and disruption of the surrounding neural structures,

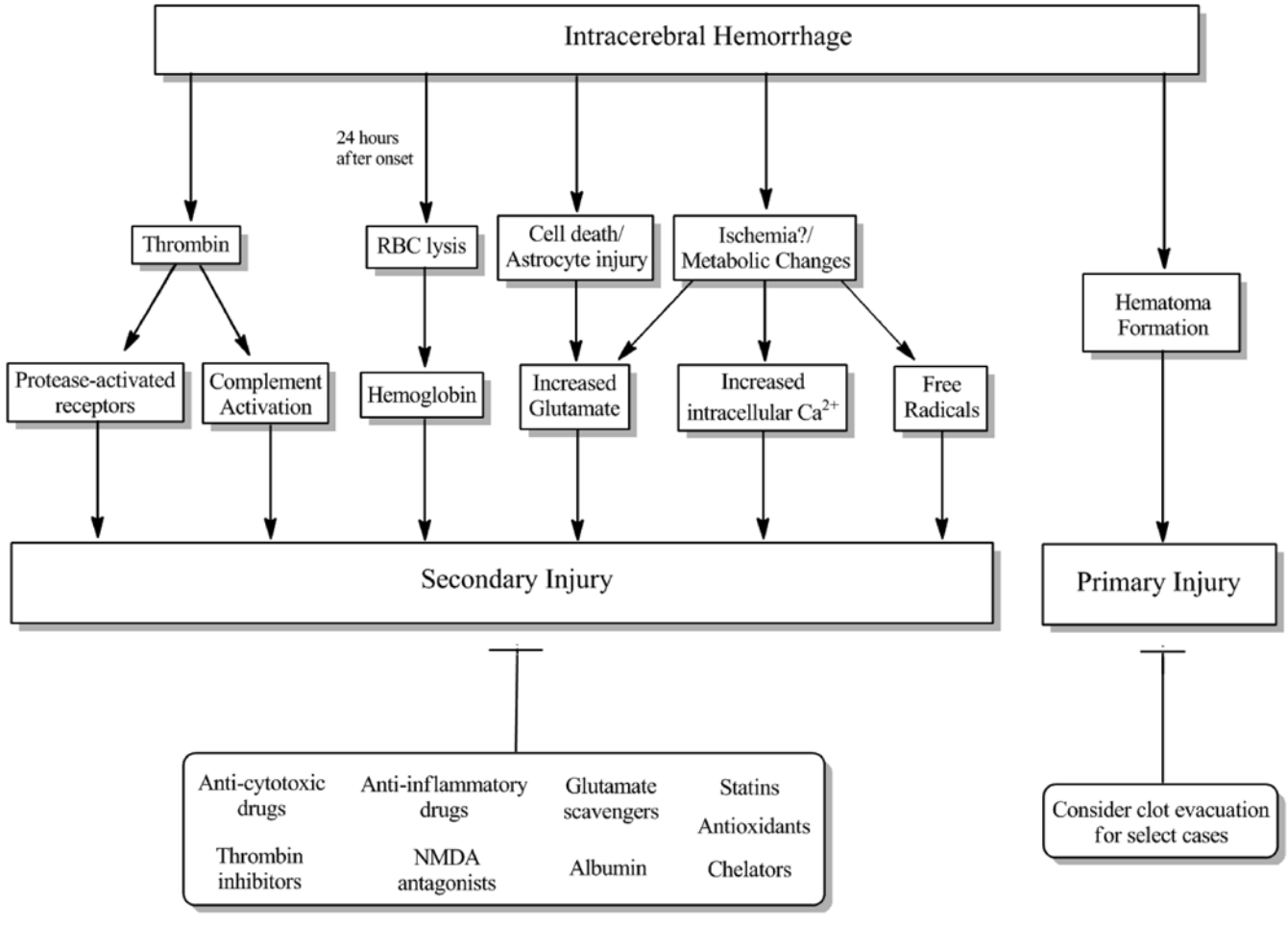

Fig. 1. Mechanisms and potential treatments for primary and secondary brain injury following ICH.

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.[30] Vasogenic and cytotoxic edema due to the breakdown of the BBB and cellular injury have been implicated in this process.[146] 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.[61] 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.[137,148] Entry of blood into the brain parenchyma activates this process, releasing large amounts of thrombin that is known to cause perihematomal edema formation after ICH due to endothelial cell damage.[75,146,148] Studies have also shown continuous release of thrombin from intracerebral hematomas for 2 weeks after clot formation due to fibrinolysis.[121]

Thrombin-induced injury may be a central mechanism for secondary injury in ICH, as many pathways are

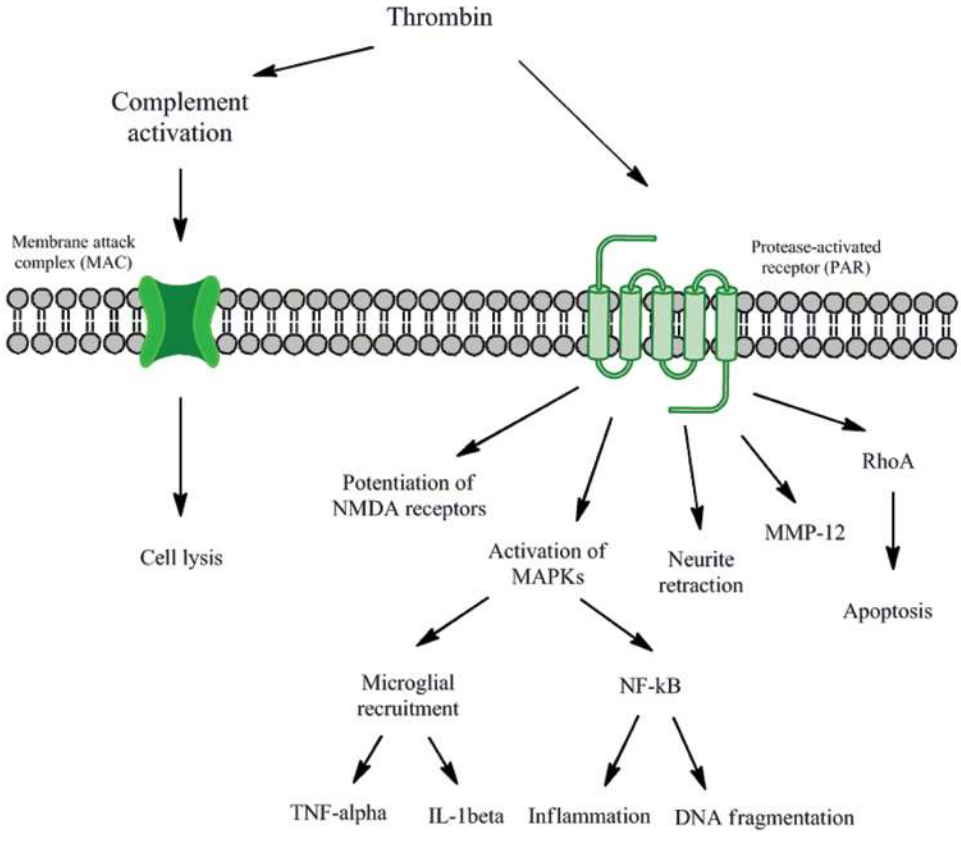

Fig. 2. Once released after ICH, thrombin is able to activate the complement pathway and PARs. This leads to a variety of cytotoxic, excitotoxic, and inflammatory effects that all lead to secondary brain injury. MAPKs = mitogen-activated protein kinases.

implicated. 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.[24] Of these receptors, PAR-1, PAR-3, and PAR-4 have been shown to be activated by thrombin.[24–26] 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.[49,78,132] 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.[37,119,128,129] It has been shown that mice lacking PAR-1 have a reduction in infarct volume following focal ischemia, indicating its importance in brain injury.[64,149] Additionally, studies have shown continued PAR-1 activation following ICH, with PAR-1 levels peaking at 3 days after onset.[162] 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.[162] 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.[91]

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.[87,133,139] 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.[55] 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.[138] 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.[53,133,140,145] Infusion of lysed RBCs also causes disruption of the BBB, DNA injury, and expression of heat shock proteins, indicating cell stress.[80,140,143,145] Once released from RBCs, hemoglobin is degraded into heme and iron, causing injury to surrounding cells.[95,133,139,157]

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.[38,50,55] 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.[13,55] This activity leads to the formation of a trans-

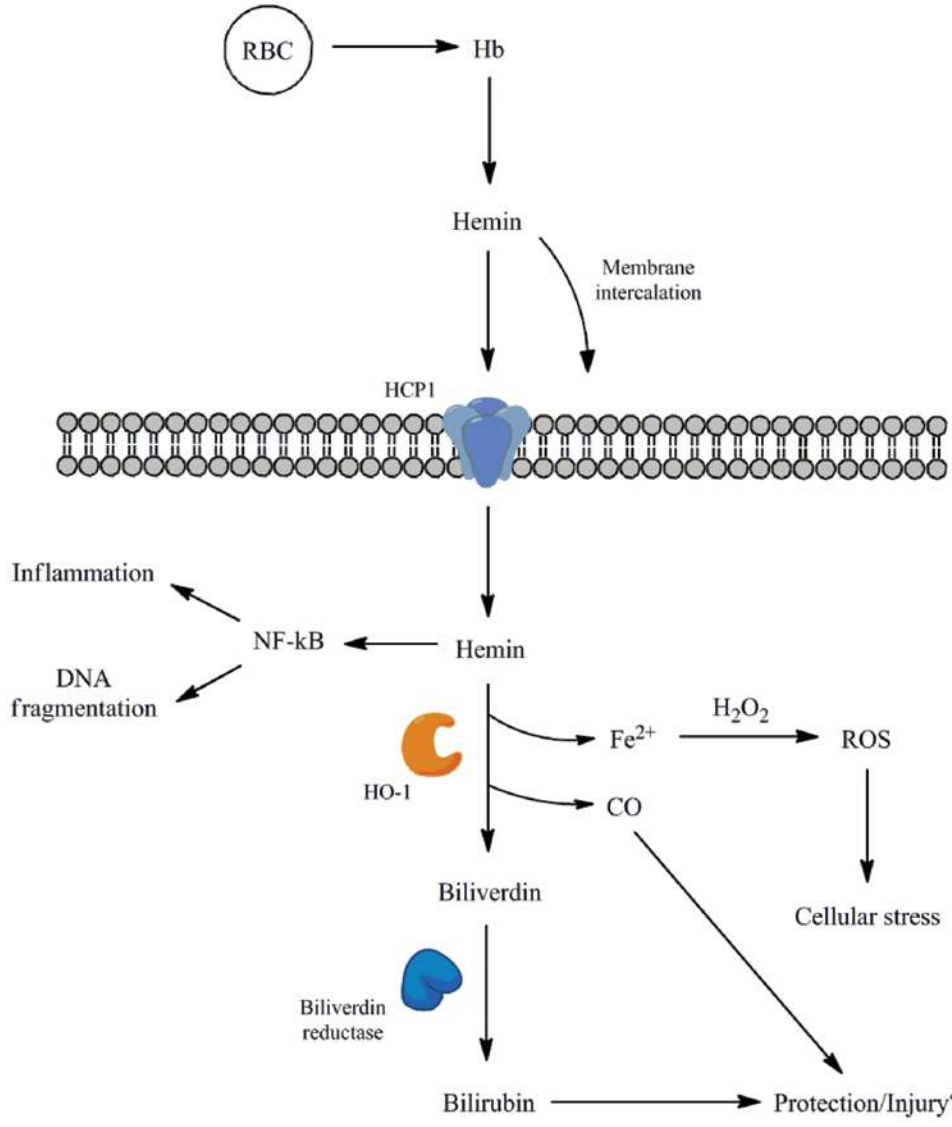

Fig. 3. Hemolysis leads to the release of hemin into the extracellular space. Hemin may then intercalate into cell membranes or enter cells via the heme carrier protein 1 (HCP1). Intracellularly hemin may activate cytotoxic and inflammatory pathways. It is then degraded by heme oxygenases, producing prooxidative iron, carbon monoxide (CO), and bilirubin. Thus far, the role of CO and bilirubin in ICH-mediated injury is unclear. Hb = hemoglobin; HO-1 = heme oxygenase-1; ROS = reactive oxygen species.

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.[55] Additionally, lysis of erythrocytes may result in further damage through hemoglobin-mediated edema formation.[145]

Thrombin is also able to induce apoptosis in neurons and astrocytes by activation of various intracellular pathways.[27,90] This occurs via RhoA, a small guanosine triphosphate-binding protein part of the Ras superfamily.[27] 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.[128]

Excitotoxicity

Potentiation of NMDA receptors by PAR-1 may cause neuronal death following ICH due to glutamate-induced excitotoxicity.[37,43] This notion is supported by studies showing that PAR-1 knockout mice had reduced thrombin-mediated NMDA receptor potentiation.[37,43] 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.[43] 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.[113] This activity is confirmed by increased expression of Src kinases following ICH.[113]

Levels of extracellular amino acids such as glutamate have been shown to increase following ICH, resulting in

glutamate-mediated excitotoxicity.[97] 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.[47] Because neurons have high intracellular concentrations of glutamate, ICH-induced cell death may result in the release of these stores into the extracellular space.[97] 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.[103] Its mechanism of action occurs through oxidative stress and the activation of caspases, resulting in the injury of astrocytes, neurons, and microglia.[102,135] However, microglia that clear hemin have protective mechanisms that prevent cell death.[17] Following ICH, hematogenous phagocytes, microglia, and surrounding astrocytes and neurons attempt to sequester hemin.[103,156] This primarily occurs via the heme carrier protein 1.[103] Once within the cell, hemin is degraded by heme oxygenases, producing biliverdin, carbon monoxide, and iron.[70,103] 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.[3,86,133,139] Iron levels after ICH may increase up to 3-fold and remain elevated for 1 month, causing continued brain injury following the initial insult.[139] However, hemin itself can also participate in redox reactions, producing free radicals that can damage intracellular structures and cause oxidative stress.[57] Additionally, because hemin is lipophilic, it may intercalate into lipid membranes, altering function and fluidity.[4] The roles of biliverdin, which is converted to bilirubin by biliverdin reductase, and carbon monoxide are unclear.[70] Small concentrations of bilirubin have been demonstrated to inhibit glutamate uptake and induce inflammation, oxidative stress, and apoptosis.[16,34,115] However, bilirubin and carbon monoxide have also been shown to have antioxidant and antiinflammatory effects.[103] 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.[54,142] This increase may occur through the activation of microglia via PARs, resulting in recruitment and proliferation of these cells at the site of injury.[108,120] 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.[54] Plasma TNF-α has been shown to correlate with the amount of brain edema in patients.[19] 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.[6] 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.[142] 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.[142]

Matrix metalloproteinases are zinc-containing proteases that are involved in extracellular matrix remodeling, chemotaxis, and proteolytic cleavage of various molecules.[35] 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.[149] 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.[2,96] Additionally, studies have shown that MMP-3, -9, and -12 null mice have less brain injury as a result of ICH.[136,149,150] 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.[67,150]

Nuclear factor-κB, a transcription factor involved in inflammatory processes, also contributes to brain injury following ICH.[5] 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.[161] This activity results in DNA fragmentation, causing cell death.[46] 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.[81,88,89,100,106,151] 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.[89,122] 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.[82,89] 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.[28] This may impair the function of many enzymes such as sodium-dependent glutamate transporters, resulting in increased extracellular glutamate levels and

excitotoxicity.[28] Low concentrations of ATP also prevent the maintenance of low calcium concentrations within cells by disrupting the Ca^{2+} ATPase, leading to high intracellular calcium levels that activate many DNAses and calcium-dependent proteases.[28] 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.[28,126] 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.[18,32,36,44,45,48,100,109,134,153] 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.[153] 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.[109] The lack of prolonged reductions in rCBF after ICH may be due to incomplete vascular compression by the hematoma.[100] This idea is supported by studies demonstrating rCBF within hematomas in regions of intact neural tissue.[100] Complete compression of intracerebral vessels by the expanding hematoma may result in the disruption of the pia-microvasculature interface, potentially causing alterations in BBB integrity.[100] 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.[84] Finally, robust collateral circulation from penetrating cortical arterioles and pial vessels from other cerebral arteries may prevent significant changes in rCBF and tissue ischemia.[84,100] 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.[14,154] The mechanism of focally increased glucose uptake may be due to nonconvulsive seizure activity, which is found in many patients with acute ICH.[131] These repetitive depolarizations may lead to secondary injury by increasing extracellular glutamate, resulting in intracellular calcium accumulation and excitotoxicity.[130] The role of seizures as a cause of increased glucose utilization is supported by the suppression of hyperglycolysis by anticonvulsant glutamate receptor antagonists.[20] 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 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 in vivo models, possibly by inhibiting PAR-1 expression.[68,69,74,163] 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.[72] 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.[148] This protective effect has been observed in neurons and astrocytes in 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,[79,144,147] but these protective effects are eliminated by thrombin inhibitors.[147] 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.[56,62,144,147] 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.[52] 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.[105] 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.[104,105] A

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.[7,8,10,12] In vivo studies have demonstrated improved functional outcome and BBB integrity following administration of albumin after ICH.[9,11] 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.[83] 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.[65] Outcomes of the trial were death or functional ability as determined by the Barthel Index.[42] 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.[164] 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.[39] 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.[1] 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.[1] Additionally, citicoline may decrease glutamate release from neurons and improve astrocyte uptake, decreasing extracellular glutamate levels.[58] In a randomized study of 32 patients, those receiving citicoline experienced improved muscle strength following ICH.[60] 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.[110] 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.[66]

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.[41,51] A multicenter Phase I trial showed that infusions of deferoxamine are tolerable and safe up to a daily dose of 6000 mg.[112] Preliminary data in 4 patients with hemorrhagic stroke and 3 with ischemic stroke showed decreases in serum markers of oxidative stress.[111] Currently, a Phase II trial is underway to evaluate the efficacy of deferoxamine in ICH.

Peroxisome proliferator-activated receptor γ is a transcription factor that plays a role in cellular defense mechanisms and hematoma clearance.[159] This activity occurs through the upregulation of *CD36*, the phagocytosis-facilitating gene, resulting in faster hematoma clearance.[159] In addition, it enhances expression of antioxidant molecules such as catalase and superoxide dismutase, preventing the oxidative damage of neurons and microglia.[114,160] In vivo studies have demonstrated improvements in hematoma resolution and functional outcome following treatment with peroxisome proliferator-activated receptor γ agonists in ICH models.[159] Currently the Safety of Pioglitazone for Hematoma Resolution in Intracerebral Hemorrhage (SHRINC) trial is evaluating the use of such agonists in 80 patients with 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.[157] 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.[158] 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.[125] However, hemopexin is also expressed by neurons and is present throughout the brain.[76] Mice that do not express hemopexin have greater infarct volumes and neurological deficits following middle cerebral artery occlusion.[76] Hemopexin knockout mice also had increased protein oxidation and tissue heme, and decreased cell viability and locomotor activity.[22] 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,[63,92] upregulation of nitric oxide synthase,[31,63,73] and protection from glutamate-induced excitotoxicity.[15] A prospective/retrospective nonrandomized study treated 18 patients with rosuvastatin and found improved outcomes compared with control subjects (mortality rate 5.6% vs 15.8%, respectively; NIH Stroke Scale score ≥ 15, OR 0.04).[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.[127]

Celecoxib is a nonsteroidal antiinflammatory drug that has been shown to reduce perihematomal inflammation and cell death in ICH.[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.[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.[23,59] One retrospective study analyzed the volumes of hematoma and edema in 17 patients treated with celecoxib.[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.[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 perihematomal brain edema, neuronal loss, BBB disruption, and improved functional outcome following ICH with minocycline treatment.[141,155] Minocycline also reduces brain iron accumulation and resulting toxicity by chelating iron.[21] In an open-label, blinded study, 74 patients were treated with minocycline 6–24 hours after acute ischemic stroke.[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.

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.[77,83,152] Mannitol exerts its neuroprotective effects by functioning as an osmotic diuretic, thus reducing brain edema.[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.[152] The free radical trapping agent NXY-059 prevents brain injury by quenching free radicals formed by hemoglobin degradation and ischemic tissue.[94]

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.

Author contributions to the study and manuscript preparation include the following. Conception and design: Adamson, Babu. Acquisition of data: Babu, Bagley, Di. Analysis and interpretation of data: Adamson, Babu. Drafting the article: Babu. Critically revising the article: Adamson, Friedman.

References

1. Adibhatla RM, Hatcher JF, Dempsey RJ: Citicoline: neuroprotective mechanisms in cerebral ischemia. **J Neurochem 80:** 12–23, 2002
2. Alvarez-Sabín J, Delgado P, Abilleira S, Molina CA, Arenillas J, Ribó M, et al: Temporal profile of matrix metalloproteinases and their inhibitors after spontaneous intracerebral hemorrhage: relationship to clinical and radiological outcome. **Stroke 35:**1316–1322, 2004
3. Aronowski J, Zhao X: Molecular pathophysiology of cerebral hemorrhage: secondary brain injury. **Stroke 42:**1781–1786, 2011
4. Balla G, Jacob HS, Eaton JW, Belcher JD, Vercellotti GM: Hemin: a possible physiological mediator of low density lipoprotein oxidation and endothelial injury. **Arterioscler Thromb 11:**1700–1711, 1991
5. Barnes PJ, Karin M: Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. **N Engl J Med 336:**1066–1071, 1997

6. Barone FC, Feuerstein GZ: Inflammatory mediators and stroke: new opportunities for novel therapeutics. **J Cereb Blood Flow Metab 19:**819–834, 1999
7. Belayev L, Busto R, Zhao W, Clemens JA, Ginsberg MD: Effect of delayed albumin hemodilution on infarction volume and brain edema after transient middle cerebral artery occlusion in rats. **J Neurosurg 87:**595–601, 1997
8. Belayev L, Liu Y, Zhao W, Busto R, Ginsberg MD: Human albumin therapy of acute ischemic stroke: marked neuroprotective efficacy at moderate doses and with a broad therapeutic window. **Stroke 32:**553–560, 2001
9. Belayev L, Obenaus A, Zhao W, Saul I, Busto R, Wu C, et al: Experimental intracerebral hematoma in the rat: characterization by sequential magnetic resonance imaging, behavior, and histopathology. Effect of albumin therapy. **Brain Res 1157:**146–155, 2007
10. Belayev L, Pinard E, Nallet H, Seylaz J, Liu Y, Riyamongkol P, et al: Albumin therapy of transient focal cerebral ischemia: in vivo analysis of dynamic microvascular responses. **Stroke 33:**1077–1084, 2002
11. Belayev L, Saul I, Busto R, Danielyan K, Vigdorchik A, Khoutorova L, et al: Albumin treatment reduces neurological deficit and protects blood-brain barrier integrity after acute intracortical hematoma in the rat. **Stroke 36:**326–331, 2005
12. Belayev L, Zhao W, Pattany PM, Weaver RG, Huh PW, Lin B, et al: Diffusion-weighted magnetic resonance imaging confirms marked neuroprotective efficacy of albumin therapy in focal cerebral ischemia. **Stroke 29:**2587–2599, 1998
13. Bellander BM, von Holst H, Fredman P, Svensson M: Activation of the complement cascade and increase of clusterin in the brain following a cortical contusion in the adult rat. **J Neurosurg 85:**468–475, 1996
14. Bergsneider M, Hovda DA, Shalmon E, Kelly DF, Vespa PM, Martin NA, et al: Cerebral hyperglycolysis following severe traumatic brain injury in humans: a positron emission tomography study. **J Neurosurg 86:**241–251, 1997
15. Bösel J, Gandor F, Harms C, Synowitz M, Harms U, Djoufack PC, et al: Neuroprotective effects of atorvastatin against glutamate-induced excitotoxicity in primary cortical neurones. **J Neurochem 92:**1386–1398, 2005
16. Brito MA, Rosa AI, Falcão AS, Fernandes A, Silva RF, Butterfield DA, et al: Unconjugated bilirubin differentially affects the redox status of neuronal and astroglial cells. **Neurobiol Dis 29:**30–40, 2008
17. Cai Y, Cho GS, Ju C, Wang SL, Ryu JH, Shin CY, et al: Activated microglia are less vulnerable to hemin toxicity due to nitric oxide-dependent inhibition of JNK and p38 MAPK activation. **J Immunol 187:**1314–1321, 2011
18. Carhuapoma JR, Wang PY, Beauchamp NJ, Keyl PM, Hanley DF, Barker PB: Diffusion-weighted MRI and proton MR spectroscopic imaging in the study of secondary neuronal injury after intracerebral hemorrhage. **Stroke 31:**726–732, 2000
19. Castillo J, Dávalos A, Alvarez-Sabín J, Pumar JM, Leira R, Silva Y, et al: Molecular signatures of brain injury after intracerebral hemorrhage. **Neurology 58:**624–629, 2002
20. Chapman AG: Glutamate receptors in epilepsy. **Prog Brain Res 116:**371–383, 1998
21. Chen L, Zhang X, Chen-Roetling J, Regan RF: Increased striatal injury and behavioral deficits after intracerebral hemorrhage in hemopexin knockout mice. Laboratory investigation. **J Neurosurg 114:**1159–1167, 2011
22. Chen-Roetling J, Chen L, Regan RF: Minocycline attenuates iron neurotoxicity in cortical cell cultures. **Biochem Biophys Res Commun 386:**322–326, 2009
23. Chu K, Jeong SW, Jung KH, Han SY, Lee ST, Kim M, et al: Celecoxib induces functional recovery after intracerebral hemorrhage with reduction of brain edema and perihematomal cell death. **J Cereb Blood Flow Metab 24:**926–933, 2004
24. Coughlin SR: How the protease thrombin talks to cells. **Proc Natl Acad Sci U S A 96:**11023–11027, 1999
25. Coughlin SR: Thrombin signalling and protease-activated receptors. **Nature 407:**258–264, 2000
26. Déry O, Corvera CU, Steinhoff M, Bunnett NW: Proteinase-activated receptors: novel mechanisms of signaling by serine proteases. **Am J Physiol 274:**C1429–C1452, 1998
27. Donovan FM, Pike CJ, Cotman CW, Cunningham DD: Thrombin induces apoptosis in cultured neurons and astrocytes via a pathway requiring tyrosine kinase and RhoA activities. **J Neurosci 17:**5316–5326, 1997
28. Doyle KP, Simon RP, Stenzel-Poore MP: Mechanisms of ischemic brain damage. **Neuropharmacology 55:**310–318, 2008
29. Eastern Stroke and Coronary Heart Disease Collaborative Research Group: Blood pressure, cholesterol, and stroke in eastern Asia. **Lancet 352:**1801–1807, 1998
30. Elijovich L, Patel PV, Hemphill JC III: Intracerebral hemorrhage. **Semin Neurol 28:**657–667, 2008
31. Endres M, Laufs U, Huang Z, Nakamura T, Huang P, Moskowitz MA, et al: Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. **Proc Natl Acad Sci U S A 95:** 8880–8885, 1998
32. Fainardi E, Borrelli M, Saletti A, Schivalocchi R, Azzini C, Cavallo M, et al: CT perfusion mapping of hemodynamic disturbances associated to acute spontaneous intracerebral hemorrhage. **Neuroradiology 50:**729–740, 2008
33. Fayad PB, Awad IA: Surgery for intracerebral hemorrhage. **Neurology 51 (3 Suppl 3):**S69–S73, 1998
34. Fernandes A, Falcão AS, Silva RF, Brito MA, Brites D: MAPKs are key players in mediating cytokine release and cell death induced by unconjugated bilirubin in cultured rat cortical astrocytes. **Eur J Neurosci 25:**1058–1068, 2007
35. Galis ZS, Khatri JJ: Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. **Circ Res 90:**251–262, 2002
36. Gass A: Is there a penumbra surrounding intracerebral hemorrhage? **Cerebrovasc Dis 23:**4–5, 2007
37. Gingrich MB, Junge CE, Lyuboslavsky P, Traynelis SF: Potentiation of NMDA receptor function by the serine protease thrombin. **J Neurosci 20:**4582–4595, 2000
38. Gong Y, Xi GH, Keep RF, Hoff JT, Hua Y: Complement inhibition attenuates brain edema and neurological deficits induced by thrombin. **Acta Neurochir Suppl 95:**389–392, 2005
39. Gottlieb M, Wang Y, Teichberg VI: Blood-mediated scavenging of cerebrospinal fluid glutamate. **J Neurochem 87:**119–126, 2003
40. Grysiewicz RA, Thomas K, Pandey DK: Epidemiology of ischemic and hemorrhagic stroke: incidence, prevalence, mortality, and risk factors. **Neurol Clin 26:**871–895, vii, 2008
41. Gu Y, Hua Y, Keep RF, Morgenstern LB, Xi G: Deferoxamine reduces intracerebral hematoma-induced iron accumulation and neuronal death in piglets. **Stroke 40:**2241–2243, 2009
42. Haley EC Jr, Thompson JL, Levin B, Davis S, Lees KR, Pittman JG, et al: Gavestinel does not improve outcome after acute intracerebral hemorrhage: an analysis from the GAIN International and GAIN Americas studies. **Stroke 36:**1006–1010, 2005
43. Hamill CE, Mannaioni G, Lyuboslavsky P, Sastre AA, Traynelis SF: Protease-activated receptor 1-dependent neuronal damage involves NMDA receptor function. **Exp Neurol 217:** 136–146, 2009
44. Herweh C, Jüttler E, Schellinger PD, Klotz E, Jenetzky E, Orakcioglu B, et al: Evidence against a perihemorrhagic penumbra provided by perfusion computed tomography. **Stroke 38:**2941–2947, 2007
45. Herweh C, Jüttler E, Schellinger PD, Klotz E, Schramm P: Perfusion CT in hyperacute cerebral hemorrhage within 3 hours after symptom onset: is there an early perihemorrhagic penumbra? **J Neuroimaging 20:**350–353, 2010
46. Hickenbottom SL, Grotta JC, Strong R, Denner LA, Aronowski J: Nuclear factor-kappaB and cell death after experimental intracerebral hemorrhage in rats. **Stroke 30:**2472–2478, 1999

47. Hillered L, Hallström A, Segersvärd S, Persson L, Ungerstedt U: Dynamics of extracellular metabolites in the striatum after middle cerebral artery occlusion in the rat monitored by intracerebral microdialysis. **J Cereb Blood Flow Metab 9:** 607–616, 1989
48. Hirano T, Read SJ, Abbott DF, Sachinidis JI, Tochon-Danguy HJ, Egan GF, et al: No evidence of hypoxic tissue on 18F-fluoromisonidazole PET after intracerebral hemorrhage. **Neurology 53:**2179–2182, 1999
49. Hollenberg MD, Compton SJ: International Union of Pharmacology. XXVIII. Proteinase-activated receptors. **Pharmacol Rev 54:**203–217, 2002
50. Hua Y, Keep RF, Hoff JT, Xi G: Brain injury after intracerebral hemorrhage: the role of thrombin and iron. **Stroke 38 (2 Suppl):**759–762, 2007
51. Hua Y, Keep RF, Hoff JT, Xi G: Deferoxamine therapy for intracerebral hemorrhage. **Acta Neurochir Suppl 105:**3–6, 2008
52. Hua Y, Keep RF, Hoff JT, Xi G: Thrombin preconditioning attenuates brain edema induced by erythrocytes and iron. **J Cereb Blood Flow Metab 23:**1448–1454, 2003
53. Hua Y, Schallert T, Keep RF, Wu J, Hoff JT, Xi G: Behavioral tests after intracerebral hemorrhage in the rat. **Stroke 33:**2478–2484, 2002
54. Hua Y, Wu J, Keep RF, Nakamura T, Hoff JT, Xi G: Tumor necrosis factor-alpha increases in the brain after intracerebral hemorrhage and thrombin stimulation. **Neurosurgery 58:** 542–550, 2006
55. Hua Y, Xi G, Keep RF, Hoff JT: Complement activation in the brain after experimental intracerebral hemorrhage. **J Neurosurg 92:**1016–1022, 2000
56. Hua Y, Xi G, Keep RF, Wu J, Jiang Y, Hoff JT: Plasminogen activator inhibitor-1 induction after experimental intracerebral hemorrhage. **J Cereb Blood Flow Metab 22:**55–61, 2002
57. Huffman LJ, Miles PR, Shi X, Bowman L: Hemoglobin potentiates the production of reactive oxygen species by alveolar macrophages. **Exp Lung Res 26:**203–217, 2000
58. Hurtado O, Moro MA, Cárdenas A, Sánchez V, Fernández-Tomé P, Leza JC, et al: Neuroprotection afforded by prior citicoline administration in experimental brain ischemia: effects on glutamate transport. **Neurobiol Dis 18:**336–345, 2005
59. Iłzecka J: Prostaglandin E2 is increased in amyotrophic lateral sclerosis patients. **Acta Neurol Scand 108:**125–129, 2003
60. Iranmanesh F, Vakilian A: Efficiency of citicoline in increasing muscular strength of patients with nontraumatic cerebral hemorrhage: a double-blind randomized clinical trial. **J Stroke Cerebrovasc Dis 17:**153–155, 2008
61. James ML, Warner DS, Laskowitz DT: Preclinical models of intracerebral hemorrhage: a translational perspective. **Neurocrit Care 9:**139–152, 2008
62. Jiang Y, Wu J, Hua Y, Keep RF, Xiang J, Hoff JT, et al: Thrombin-receptor activation and thrombin-induced brain tolerance. **J Cereb Blood Flow Metab 22:**404–410, 2002
63. Jung KH, Chu K, Jeong SW, Han SY, Lee ST, Kim JY, et al: HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. **Stroke 35:**1744–1749, 2004
64. Junge CE, Sugawara T, Mannaioni G, Alagarsamy S, Conn PJ, Brat DJ, et al: The contribution of protease-activated receptor 1 to neuronal damage caused by transient focal cerebral ischemia. **Proc Natl Acad Sci U S A 100:**13019–13024, 2003
65. Kazui S, Naritomi H, Yamamoto H, Sawada T, Yamaguchi T: Enlargement of spontaneous intracerebral hemorrhage. Incidence and time course. **Stroke 27:**1783–1787, 1996
66. Kellner CP, Connolly ES Jr: Neuroprotective strategies for intracerebral hemorrhage: trials and translation. **Stroke 41 (10 Suppl):**S99–S102, 2010
67. Kim YS, Kim SS, Cho JJ, Choi DH, Hwang O, Shin DH, et al: Matrix metalloproteinase-3: a novel signaling proteinase from apoptotic neuronal cells that activates microglia. **J Neurosci 25:**3701–3711, 2005
68. Kitaoka T, Hua Y, Xi G, Hoff JT, Keep RF: Delayed argatroban treatment reduces edema in a rat model of intracerebral hemorrhage. **Stroke 33:**3012–3018, 2002
69. Kitaoka T, Hua Y, Xi G, Nagao S, Hoff JT, Keep RF: Effect of delayed argatroban treatment on intracerebral hemorrhage-induced edema in the rat. **Acta Neurochir Suppl 86:**457–461, 2003
70. Kutty RK, Maines MD: Purification and characterization of biliverdin reductase from rat liver. **J Biol Chem 256:**3956–3962, 1981
71. Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, et al: Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. **Neurology 69:**1404–1410, 2007
72. Lauer A, Cianchetti FA, Van Cott EM, Schlunk F, Schulz E, Pfeilschifter W, et al: Anticoagulation with the oral direct thrombin inhibitor dabigatran does not enlarge hematoma volume in experimental intracerebral hemorrhage. **Circulation 124:**1654–1662, 2011
73. Laufs U, Gertz K, Huang P, Nickenig G, Böhm M, Dirnagl U, et al: Atorvastatin upregulates type III nitric oxide synthase in thrombocytes, decreases platelet activation, and protects from cerebral ischemia in normocholesterolemic mice. **Stroke 31:** 2442–2449, 2000
74. Lee KR, Colon GP, Betz AL, Keep RF, Kim S, Hoff JT: Edema from intracerebral hemorrhage: the role of thrombin. **J Neurosurg 84:**91–96, 1996
75. Lee KR, Kawai N, Kim S, Sagher O, Hoff JT: Mechanisms of edema formation after intracerebral hemorrhage: effects of thrombin on cerebral blood flow, blood-brain barrier permeability, and cell survival in a rat model. **J Neurosurg 86:**272–278, 1997
76. Li RC, Saleem S, Zhen G, Cao W, Zhuang H, Lee J, et al: Heme-hemopexin complex attenuates neuronal cell death and stroke damage. **J Cereb Blood Flow Metab 29:**953–964, 2009
77. Lyden PD, Shuaib A, Lees KR, Davalos A, Davis SM, Diener HC, et al: Safety and tolerability of NXY-059 for acute intracerebral hemorrhage: the CHANT Trial. **Stroke 38:**2262–2269, 2007
78. Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R: Proteinase-activated receptors. **Pharmacol Rev 53:**245–282, 2001
79. Masada T, Xi G, Hua Y, Keep RF: The effects of thrombin preconditioning on focal cerebral ischemia in rats. **Brain Res 867:**173–179, 2000
80. Matz PG, Weinstein PR, Sharp FR: Heme oxygenase-1 and heat shock protein 70 induction in glia and neurons throughout rat brain after experimental intracerebral hemorrhage. **Neurosurgery 40:**152–162, 1997
81. Mendelow AD: Mechanisms of ischemic brain damage with intracerebral hemorrhage. **Stroke 24 (12 Suppl):**I115–I119, 1993
82. Mendelow AD, Bullock R, Teasdale GM, Graham DI, McCulloch J: Intracranial haemorrhage induced at arterial pressure in the rat. Part 2: Short term changes in local cerebral blood flow measured by autoradiography. **Neurol Res 6:**189–193, 1984
83. Misra UK, Kalita J, Ranjan P, Mandal SK: Mannitol in intracerebral hemorrhage: a randomized controlled study. **J Neurol Sci 234:**41–45, 2005
84. Mutlu N, Berry RG, Alpers BJ: Massive cerebral hemorrhage. Clinical and pathological correlations. **Arch Neurol 8:**644–661, 1963
85. Nakajima R, Nakamura T, Miyakawa H, Kudo Y: Effects of mannitol on ischemia-induced degeneration in rat hippocampus. **J Pharmacol Sci 95:**341–348, 2004
86. Nakamura T, Keep RF, Hua Y, Hoff JT, Xi G: Oxidative DNA injury after experimental intracerebral hemorrhage. **Brain Res 1039:**30–36, 2005

87. Nakamura T, Keep RF, Hua Y, Schallert T, Hoff JT, Xi G: Deferoxamine-induced attenuation of brain edema and neurological deficits in a rat model of intracerebral hemorrhage. **J Neurosurg 100:**672–678, 2004
88. Nath FP, Jenkins A, Mendelow AD, Graham DI, Teasdale GM: Early hemodynamic changes in experimental intracerebral hemorrhage. **J Neurosurg 65:**697–703, 1986
89. Nath FP, Kelly PT, Jenkins A, Mendelow AD, Graham DI, Teasdale GM: Effects of experimental intracerebral hemorrhage on blood flow, capillary permeability, and histochemistry. **J Neurosurg 66:**555–562, 1987
90. Noorbakhsh F, Vergnolle N, Hollenberg MD, Power C: Proteinase-activated receptors in the nervous system. **Nat Rev Neurosci 4:**981–990, 2003
91. Ohnishi M, Katsuki H, Fujimoto S, Takagi M, Kume T, Akaike A: Involvement of thrombin and mitogen-activated protein kinase pathways in hemorrhagic brain injury. **Exp Neurol 206:** 43–52, 2007
92. Ortego M, Bustos C, Hernández-Presa MA, Tuñón J, Díaz C, Hernández G, et al: Atorvastatin reduces NF-kappaB activation and chemokine expression in vascular smooth muscle cells and mononuclear cells. **Atherosclerosis 147:**253–261, 1999
93. Park HK, Lee SH, Chu K, Roh JK: Effects of celecoxib on volumes of hematoma and edema in patients with primary intracerebral hemorrhage. **J Neurol Sci 279:**43–46, 2009
94. Peeling J, Del Bigio MR, Corbett D, Green AR, Jackson DM: Efficacy of disodium 4-[(tert-butylimino)methyl]benzene-1,3-disulfonate N-oxide (NXY-059), a free radical trapping agent, in a rat model of hemorrhagic stroke. **Neuropharmacology 40:** 433–439, 2001
95. Peeling J, Yan HJ, Chen SG, Campbell M, Del Bigio MR: Protective effects of free radical inhibitors in intracerebral hemorrhage in rat. **Brain Res 795:**63–70, 1998
96. Power C, Henry S, Del Bigio MR, Larsen PH, Corbett D, Imai Y, et al: Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. **Ann Neurol 53:**731–742, 2003
97. Qureshi AI, Ali Z, Suri MF, Shuaib A, Baker G, Todd K, et al: Extracellular glutamate and other amino acids in experimental intracerebral hemorrhage: an in vivo microdialysis study. **Crit Care Med 31:**1482–1489, 2003
98. Qureshi AI, Mendelow AD, Hanley DF: Intracerebral haemorrhage. **Lancet 373:**1632–1644, 2009
99. Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF: Spontaneous intracerebral hemorrhage. **N Engl J Med 344:**1450–1460, 2001
100. Qureshi AI, Wilson DA, Hanley DF, Traystman RJ: No evidence for an ischemic penumbra in massive experimental intracerebral hemorrhage. **Neurology 52:**266–272, 1999
101. Rasool AH, Rahman AR, Choudhury SR, Singh RB: Blood pressure in acute intracerebral haemorrhage. **J Hum Hypertens 18:**187–192, 2004
102. Regan RF, Wang Y, Ma X, Chong A, Guo Y: Activation of extracellular signal-regulated kinases potentiates hemin toxicity in astrocyte cultures. **J Neurochem 79:**545–555, 2001
103. Robinson SR, Dang TN, Dringen R, Bishop GM: Hemin toxicity: a preventable source of brain damage following hemorrhagic stroke. **Redox Rep 14:**228–235, 2009
104. Rodrigues CM, Solá S, Brito MA, Brondino CD, Brites D, Moura JJ: Amyloid beta-peptide disrupts mitochondrial membrane lipid and protein structure: protective role of tauroursodeoxycholate. **Biochem Biophys Res Commun 281:**468–474, 2001
105. Rodrigues CM, Sola S, Nan Z, Castro RE, Ribeiro PS, Low WC, et al: Tauroursodeoxycholic acid reduces apoptosis and protects against neurological injury after acute hemorrhagic stroke in rats. **Proc Natl Acad Sci U S A 100:**6087–6092, 2003
106. Ropper AH, Zervas NT: Cerebral blood flow after experimental basal ganglia hemorrhage. **Ann Neurol 11:**266–271, 1982
107. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, et al: Heart disease and stroke statistics—2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. **Circulation 117:**e25–e146, 2008 (Erratum in **Circulation 122:**e10, 2010)
108. Ryu J, Min KJ, Rhim TY, Kim TH, Pyo H, Jin B, et al: Prothrombin kringle-2 activates cultured rat brain microglia. **J Immunol 168:**5805–5810, 2002
109. Schellinger PD, Fiebach JB, Hoffmann K, Becker K, Orakcioglu B, Kollmar R, et al: Stroke MRI in intracerebral hemorrhage: is there a perihemorrhagic penumbra? **Stroke 34:**1674–1679, 2003
110. Secades JJ, Alvarez-Sabín J, Rubio F, Lozano R, Dávalos A, Castillo J: Citicoline in intracerebral haemorrhage: a double-blind, randomized, placebo-controlled, multi-centre pilot study. **Cerebrovasc Dis 21:**380–385, 2006
111. Selim M: Deferoxamine mesylate: a new hope for intracerebral hemorrhage: from bench to clinical trials. **Stroke 40 (3 Suppl):**S90–S91, 2009
112. Selim M, Yeatts S, Goldstein JN, Gomes J, Greenberg S, Morgenstern LB, et al: Safety and tolerability of deferoxamine mesylate in patients with acute intracerebral hemorrhage. **Stroke 42:**3067–3074, 2011
113. Sharp F, Liu DZ, Zhan X, Ander BP: Intracerebral hemorrhage injury mechanisms: glutamate neurotoxicity, thrombin, and Src. **Acta Neurochir Suppl 105:**43–46, 2008
114. Shimazu T, Inoue I, Araki N, Asano Y, Sawada M, Furuya D, et al: A peroxisome proliferator-activated receptor-gamma agonist reduces infarct size in transient but not in permanent ischemia. **Stroke 36:**353–359, 2005
115. Silva RF, Rodrigues CM, Brites D: Rat cultured neuronal and glial cells respond differently to toxicity of unconjugated bilirubin. **Pediatr Res 51:**535–541, 2002
116. Sinn DI, Lee ST, Chu K, Jung KH, Song EC, Kim JM, et al: Combined neuroprotective effects of celecoxib and memantine in experimental intracerebral hemorrhage. **Neurosci Lett 411:**238–242, 2007
117. Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, et al: Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. **N Engl J Med 352:**1071–1080, 2005
118. Steiner T, Rosand J, Diringer M: Intracerebral hemorrhage associated with oral anticoagulant therapy: current practices and unresolved questions. **Stroke 37:**256–262, 2006
119. Suidan HS, Stone SR, Hemmings BA, Monard D: Thrombin causes neurite retraction in neuronal cells through activation of cell surface receptors. **Neuron 8:**363–375, 1992
120. Suo Z, Wu M, Ameenuddin S, Anderson HE, Zoloty JE, Citron BA, et al: Participation of protease-activated receptor-1 in thrombin-induced microglial activation. **J Neurochem 80:**655–666, 2002
121. Suzuki M, Ogawa A, Sakurai Y, Nishino A, Venohara K, Mizoi K, et al: Thrombin activity in cerebrospinal fluid after subarachnoid hemorrhage. **Stroke 23:**1181–1182, 1992
122. Suzuki R, Yamaguchi T, Kirino T, Orzi F, Klatzo I: The effects of 5-minute ischemia in Mongolian gerbils: I. Blood-brain barrier, cerebral blood flow, and local cerebral glucose utilization changes. **Acta Neuropathol 60:**207–216, 1983
123. Tanaka H, Ueda Y, Hayashi M, Date C, Baba T, Yamashita H, et al: Risk factors for cerebral hemorrhage and cerebral infarction in a Japanese rural community. **Stroke 13:**62–73, 1982
124. Tapia-Perez H, Sanchez-Aguilar M, Torres-Corzo JG, Rodriguez-Leyva I, Gonzalez-Aguirre D, Gordillo-Moscoso A, et al: Use of statins for the treatment of spontaneous intracerebral hemorrhage: results of a pilot study. **Cen Eur Neurosurg 70:** 15–20, 2009
125. Tolosano E, Altruda F: Hemopexin: structure, function, and regulation. **DNA Cell Biol 21:**297–306, 2002
126. Traystman RJ, Kirsch JR, Koehler RC: Oxygen radical mechanisms of brain injury following ischemia and reperfusion. **J Appl Physiol 71:**1185–1195, 1991
127. Tseng MY: Summary of evidence on immediate statins therapy

following aneurysmal subarachnoid hemorrhage. **Neurocrit Care 15:**298–301, 2011
128. Turgeon VL, Lloyd ED, Wang S, Festoff BW, Houenou LJ: Thrombin perturbs neurite outgrowth and induces apoptotic cell death in enriched chick spinal motoneuron cultures through caspase activation. **J Neurosci 18:**6882–6891, 1998
129. Turgeon VL, Milligan CE, Houenou LJ: Activation of the protease-activated thrombin receptor (PAR)-1 induces motoneuron degeneration in the developing avian embryo. **J Neuropathol Exp Neurol 58:**499–504, 1999
130. Vespa P, Prins M, Ronne-Engstrom E, Caron M, Shalmon E, Hovda DA, et al: Increase in extracellular glutamate caused by reduced cerebral perfusion pressure and seizures after human traumatic brain injury: a microdialysis study. **J Neurosurg 89:** 971–982, 1998
131. Vespa PM, O'Phelan K, Shah M, Mirabelli J, Starkman S, Kidwell C, et al: Acute seizures after intracerebral hemorrhage: a factor in progressive midline shift and outcome. **Neurology 60:**1441–1446, 2003
132. Vu TK, Hung DT, Wheaton VI, Coughlin SR: Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. **Cell 64:**1057–1068, 1991
133. Wagner KR, Sharp FR, Ardizzone TD, Lu A, Clark JF: Heme and iron metabolism: role in cerebral hemorrhage. **J Cereb Blood Flow Metab 23:**629–652, 2003
134. Wagner KR, Xi G, Hua Y, Kleinholz M, de Courten-Myers GM, Myers RE, et al: Lobar intracerebral hemorrhage model in pigs: rapid edema development in perihematomal white matter. **Stroke 27:**490–497, 1996
135. Wang X, Mori T, Sumii T, Lo EH: Hemoglobin-induced cytotoxicity in rat cerebral cortical neurons: caspase activation and oxidative stress. **Stroke 33:**1882–1888, 2002
136. Wells JE, Biernaskie J, Szymanska A, Larsen PH, Yong VW, Corbett D: Matrix metalloproteinase (MMP)-12 expression has a negative impact on sensorimotor function following intracerebral haemorrhage in mice. **Eur J Neurosci 21:**187–196, 2005
137. West KL, Adamson C, Hoffman M: Prophylactic correction of the international normalized ratio in neurosurgery: a brief review of a brief literature. A review. **J Neurosurg 114:**9–18, 2011
138. Wu G, Xi G, Huang F: Spontaneous intracerebral hemorrhage in humans: hematoma enlargement, clot lysis, and brain edema. **Acta Neurochir Suppl 96:**78–80, 2006
139. Wu J, Hua Y, Keep RF, Nakamura T, Hoff JT, Xi G: Iron and iron-handling proteins in the brain after intracerebral hemorrhage. **Stroke 34:**2964–2969, 2003
140. Wu J, Hua Y, Keep RF, Schallert T, Hoff JT, Xi G: Oxidative brain injury from extravasated erythrocytes after intracerebral hemorrhage. **Brain Res 953:**45–52, 2002
141. Wu J, Yang S, Xi G, Fu G, Keep RF, Hua Y: Minocycline reduces intracerebral hemorrhage-induced brain injury. **Neurol Res 31:**183–188, 2009
142. Wu J, Yang S, Xi G, Song S, Fu G, Keep RF, et al: Microglial activation and brain injury after intracerebral hemorrhage. **Acta Neurochir Suppl 105:**59–65, 2008
143. Xi G, Hua Y, Bhasin RR, Ennis SR, Keep RF, Hoff JT: Mechanisms of edema formation after intracerebral hemorrhage: effects of extravasated red blood cells on blood flow and blood-brain barrier integrity. **Stroke 32:**2932–2938, 2001
144. Xi G, Hua Y, Keep RF, Hoff JT: Induction of colligin may attenuate brain edema following intracerebral hemorrhage. **Acta Neurochir Suppl 76:**501–505, 2000
145. Xi G, Keep RF, Hoff JT: Erythrocytes and delayed brain edema formation following intracerebral hemorrhage in rats. **J Neurosurg 89:**991–996, 1998
146. Xi G, Keep RF, Hoff JT: Mechanisms of brain injury after intracerebral haemorrhage. **Lancet Neurol 5:**53–63, 2006
147. Xi G, Keep RF, Hua Y, Xiang J, Hoff JT: Attenuation of thrombin-induced brain edema by cerebral thrombin preconditioning. **Stroke 30:**1247–1255, 1999
148. Xi G, Reiser G, Keep RF: The role of thrombin and thrombin receptors in ischemic, hemorrhagic and traumatic brain injury: deleterious or protective? **J Neurochem 84:**3–9, 2003
149. Xue M, Hollenberg MD, Demchuk A, Yong VW: Relative importance of proteinase-activated receptor-1 versus matrix metalloproteinases in intracerebral hemorrhage-mediated neurotoxicity in mice. **Stroke 40:**2199–2204, 2009
150. Xue M, Hollenberg MD, Yong VW: Combination of thrombin and matrix metalloproteinase-9 exacerbates neurotoxicity in cell culture and intracerebral hemorrhage in mice. **J Neurosci 26:** 10281–10291, 2006
151. Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT: Experimental intracerebral hemorrhage: relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. **J Neurosurg 81:**93–102, 1994
152. Yu YL, Kumana CR, Lauder IJ, Cheung YK, Chan FL, Kou M, et al: Treatment of acute cerebral hemorrhage with intravenous glycerol. A double-blind, placebo-controlled, randomized trial. **Stroke 23:**967–971, 1992
153. Zazulia AR, Diringer MN, Videen TO, Adams RE, Yundt K, Aiyagari V, et al: Hypoperfusion without ischemia surrounding acute intracerebral hemorrhage. **J Cereb Blood Flow Metab 21:**804–810, 2001
154. Zazulia AR, Videen TO, Powers WJ: Transient focal increase in perihematomal glucose metabolism after acute human intracerebral hemorrhage. **Stroke 40:**1638–1643, 2009
155. Zhao F, Hua Y, He Y, Keep RF, Xi G: Minocycline-induced attenuation of iron overload and brain injury after experimental intracerebral hemorrhage. **Stroke 42:**3587–3593, 2011
156. Zhao X, Grotta J, Gonzales N, Aronowski J: Hematoma resolution as a therapeutic target: the role of microglia/macrophages. **Stroke 40 (3 Suppl):**S92–S94, 2009
157. Zhao X, Song S, Sun G, Strong R, Zhang J, Grotta JC, et al: Neuroprotective role of haptoglobin after intracerebral hemorrhage. **J Neurosci 29:**15819–15827, 2009
158. Zhao X, Sun G, Zhang J, Strong R, Dash PK, Kan YW, et al: Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. **Stroke 38:**3280–3286, 2007
159. Zhao X, Sun G, Zhang J, Strong R, Song W, Gonzales N, et al: Hematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor gamma in microglia/macrophages. **Ann Neurol 61:**352–362, 2007
160. Zhao X, Zhang Y, Strong R, Grotta JC, Aronowski J: 15d-Prostaglandin J2 activates peroxisome proliferator-activated receptor-gamma, promotes expression of catalase, and reduces inflammation, behavioral dysfunction, and neuronal loss after intracerebral hemorrhage in rats. **J Cereb Blood Flow Metab 26:**811–820, 2006
161. Zhao X, Zhang Y, Strong R, Zhang J, Grotta JC, Aronowski J: Distinct patterns of intracerebral hemorrhage-induced alterations in NF-kappaB subunit, iNOS, and COX-2 expression. **J Neurochem 101:**652–663, 2007
162. Zheng GQ, Wang XT, Wang XM, Gao RR, Zeng XL, Fu XL, et al: Long-time course of protease-activated receptor-1 expression after intracerebral hemorrhage in rats. **Neurosci Lett 459:**62–65, 2009
163. Zhou ZH, Qu F, Zhang CD: Systemic administration of argatroban inhibits protease-activated receptor-1 expression in perihematomal tissue in rats with intracerebral hemorrhage. **Brain Res Bull 86:**235–238, 2011
164. Zlotnik A, Gurevich B, Tkachov S, Maoz I, Shapira Y, Teichberg VI: Brain neuroprotection by scavenging blood glutamate. **Exp Neurol 203:**213–220, 2007

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