Emerging experimental therapies for intracerebral hemorrhage: targeting mechanisms of secondary brain injury

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Intracerebral hemorrhage (ICH) is associated with a higher degree of morbidity and mortality than other stroke subtypes. Despite this burden, currently approved treatments have demonstrated limited efficacy. To date, therapeutic strategies have principally targeted hematoma expansion and resultant mass effect. However, secondary mechanisms of brain injury are believed to be critical effectors of cell death and neurological outcome following ICH. This article reviews the pathophysiology of secondary brain injury relevant to ICH, examines pertinent experimental models, and highlights emerging therapeutic strategies. Treatment paradigms discussed include thrombin inhibitors, deferoxamine, minocycline, statins, granulocyte-colony stimulating factors, and therapeutic hypothermia. Despite promising experimental and preliminary human data, further studies are warranted prior to effective clinical translation. (<http://thejns.org/doi/abs/10.3171/2013.2.FOCUS1317>)

KEY WORDS • intracerebral hemorrhage • secondary brain injury •
pathophysiology • therapeutic intervention • animal model • neuroprotection

INTRACEREBRAL hemorrhage accounts for 10%–15% of all strokes, but is associated with disproportionately high morbidity and mortality rates.⁶³ Nearly 50% of patients with ICH die within 1 month of presentation, and only 20% of survivors achieve functional independence at 6 months.¹⁴ Standard management is principally supportive, including airway protection, maintenance of hemodynamic stability, and control of intracranial pressure.⁴

Extravasation of blood products into brain parenchyma initiates hematoma formation, edema, and cell death.³⁴ Hematoma development and resultant mass effect account for the initial neurological deficits associated with ICH.^{8,37} Early administration of hemostatic agents^{46,47} and meticulous blood pressure control^{1,2,59,60} have been used to limit hematoma expansion. Large studies have examined the impact of reducing clot burden and mass effect through early surgical evacuation and catheter hematoma aspiration.^{24,48,79} These studies have yielded mixed results.⁶³

Recently, in translational ICH studies the attention of

investigators has shifted toward secondary mechanisms of injury following hematoma expansion. The pathophysiological basis of cerebral edema and cell death has been examined in the laboratory. Putative agents targeting mechanisms of secondary brain injury have been assessed in animal ICH models and clinical trials. Our paper reviews these treatments. We discuss the pathophysiological mechanisms underlying secondary brain injury in ICH, review the virtues and limitations of the major animal models, and survey emerging therapeutic strategies targeting secondary mechanisms of injury in the setting of ICH.

Mechanisms of Secondary Brain Injury

In the hours following ICH, mass effect generated by the hematoma mechanically deforms and disrupts neuronal and glial cell membranes, resulting in calcium influx and excitotoxic neurotransmitter release.⁸⁹ This results in cell necrosis and cytotoxic edema.³⁹ Homeostasis is altered at the cellular level and further injury occurs. Secondary brain injury following ICH involves direct cellular toxicity, BBB disruption, vasogenic edema, and up-regulation of inflammatory mediators.³¹ These processes function in a positive feedback cycle.

Abbreviations used in this paper: BBB = blood-brain barrier; G-CSF = granulocyte colony-stimulating factor; ICH = intracerebral hemorrhage; MMP = matrix metalloproteinase; TNF = tumor necrosis factor.

Activation of the coagulation cascade results in thrombin formation.³¹ Thrombin initially limits hematoma expansion and at low concentrations induces neuroprotective heat shock proteins and iron scavengers. However, at the high concentrations evident in ICH, thrombin initiates multiple destructive pathways.²⁸

Thrombin stimulates glial cells to produce inflammatory cytokines. Upregulation of TNF- α results in disruption of BBB integrity, apoptosis, and recruitment of proinflammatory mediators.^{5,29} Upregulation of various MMPs⁶¹ results in degradation of the extracellular matrix.¹⁸ The BBB breakdown then leads to vasogenic edema and infiltration by inflammatory cells.³¹

Vasogenic edema resulting from BBB permeability may contribute to functional deterioration over the course of weeks.⁹⁴ The mass effect of perihematomal edema can cause regional hypoperfusion by mechanically compressing blood vessels.⁷⁷ This leads to dysfunctional adenosine triphosphate generation, failure of ion and neurotransmitter regulation, and free radical generation.⁶⁹ Whether this process generates true ischemia is controversial.⁴

Thrombin-initiated activation of the complement cascade further enhances inflammatory cell migration through anaphylatoxins and direct cellular destruction via the membrane attack complex. Lysis of endothelial cells by the membrane attack complex in turn causes additional BBB disruption.³⁰ Iron released from red blood cell lysis and free radicals produced by inflammatory cells generate oxidative stress, leading to further cell death and BBB breakdown.^{4,31} It should be noted, however, that although many of these inflammatory pathways appear to be harmful in the short term, they are critical for hematoma clearance and long-term recovery.³¹

The mechanisms of secondary brain injury outlined above suggest potential avenues for therapeutic intervention in the setting of ICH. Treatment modalities have been tailored according to these concepts and are currently progressing toward clinical testing and application. However, translational progress remains slow. One potential reason is the inability of current animal models to provide a sufficient recapitulation of the clinical disease.⁴⁰ The following sections will describe relevant animal ICH models and review the treatments (in both experimental paradigms and clinical trials) applied toward secondary brain injury following ICH.

Experimental Animal Models of ICH

There are 2 widely used experimental techniques to simulate ICH in animal models: an autologous whole-blood protocol and a collagenase protocol. In the autologous whole-blood model, blood is harvested from a superficial vessel and stereotactically injected into the striatum.⁴⁰ This method was originally hindered by backflow along the needle tract, resulting in undesired intraventricular and subarachnoid hemorrhaging.⁹¹ Thus, it was modified into a double injection. Initially, a small volume of blood is slowly infused into the striatum to allow clotting along the needle tract. The remaining blood is then injected to generate the hematoma. Double injection allows for reproducible hematoma volumes with substan-

tially lower incidences of ventricular and subarachnoid infiltration.¹³ However, because the hematoma arises from a single bolus injection of blood, it fails to reproduce the temporal expansion evident in clinical ICH. Furthermore, this model does not achieve the rebleeding phenomenon that is typical of clinical ICH.⁴³ Finally, injection of whole blood produces a less severe neurological deficit than seen in the clinical setting⁴⁵ and can even allow for spontaneous recovery.^{21,33,70}

In the collagenase model, bacterial collagenase is injected into the caudate nucleus to erode the basal lamina of blood vessels and induce in situ hemorrhage. Bleeding is typically seen 10 minutes after injection, but hematoma expansion continues up to 24 hours. This more accurately simulates clinically relevant ICH hematoma expansion.⁶² Furthermore, because of the diffuse activity of collagenase, rebleeding is often achieved.⁸¹ Neurological deficits seen in this model are typically more severe than in whole-blood injection,⁴⁵ and animals do not frequently exhibit spontaneous recovery.^{6,42,80} The major drawback of the collagenase model is that levels of inflammation and cellular toxicity tend to be significantly higher than those evident in spontaneous human ICH.⁴⁵

Both ICH models are performed in rodents, which are relatively inexpensive to house, genetically modifiable, and have well-established parameters for functional assessment.⁴⁰ However, mice and rats are disadvantaged by a relative lack of white matter when compared with humans.¹⁷ The techniques have also been adapted in pigs, dogs, and rabbits. Species-specific differences in brain matter composition and structure are less extensive in these animals than in mice and rats, but criteria for neurobehavioral testing are not as well defined. The inability of any one model truly to simulate human ICH renders assessment in multiple paradigms useful prior to clinical testing.⁴⁰ Furthermore, translation is impacted by experimental parameters. Age is an important predictor of functional outcome in clinical ICH.¹² Also, ICH is most prevalent in older patients with substantial medical comorbidities.⁷⁸ Evidence suggests that aged rats demonstrate more profound inflammation and neurological deficits than their younger counterparts.¹⁹ However, the majority of ICH experiments are performed in young, healthy animals.⁴⁰ These disparities have tempered the process of translating positive preclinical results to the clinical arena.⁴⁰

Therapies Under Investigation

Literature Review

Table 1 lists published reports for each kind of therapy discussed.

Thrombin Inhibitors. Despite concerns regarding hemorrhage exacerbation and facilitation of hematoma expansion, thrombin inhibition has been investigated in animal models of ICH. In the autologous whole-blood rat model, Sun et al.⁷⁴ demonstrated that hirudin, a direct thrombin inhibitor, was found to decrease perihematomal edema significantly through presumed downregulation of aquaporin-4 and aquaporin-9. In the rat collagenase

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TABLE 1: Literature review of studies on experimental ICH treatment modalities

Therapy; Authors & Year	ICH Study Type	Results
<i>thrombin inhibitors</i>		
Kitaoka et al., 2002	rat autologous whole-blood & collagenase models	decreased edema, no potentiation of rebleeding in collagenase model
Nagatsuna et al., 2005	rat collagenase model	decreased edema & inflammatory cell migration, no potentiation of rebleeding
Nakamura et al., 2010	rat autologous whole-blood & collagenase models	decreased edema & oxidative DNA damage, no potentiation of rebleeding in collagenase model
Sun et al., 2009	rat autologous whole-blood model	decreased edema, possibly through downregulation of aquaporin-4 & aquaporin-9
<i>deferrioxamine</i>		
Gu et al., 2009	piglet autologous whole-blood model	mitigated cell death/tissue loss
Gu et al., 2011	piglet autologous whole-blood model	decreased oxidative DNA damage
Hatakeyama et al., 2011	aged rat autologous whole-blood model	mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Nakamura et al., 2004	rat autologous whole-blood model	decreased edema & oxidative DNA damage, mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Okauchi et al., 2009 & 2010	aged rat autologous whole-blood model	decreased edema, mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Qing et al., 2009	rat autologous whole-blood model	decreased edema & oxidative DNA damage, mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Selim et al., 2011	clinical trial	Phase I: infusions found to be tolerable up to 62 mg/kg/day; Phase II: in development
Song et al., 2007	rat hippocampal hemoglobin injection model	decreased oxidative DNA damage, mitigated cell death/tissue loss
Wu et al., 2011	mouse collagenase model	decreased edema & oxidative DNA damage, mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
<i>minocycline</i>		
Shi et al., 2011	rat collagenase model	preserved BBB integrity, decreased edema & inflammatory cell migration
Wasserman & Schlichter, 2007	rat collagenase model	preserved BBB integrity, decreased edema & inflammatory cell migration
Wasserman et al., 2007	rat collagenase model	decreased expression of TNF- α & MMP-12
Wu et al., 2009	rat autologous whole-blood model	mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Wu et al., 2011	rat autologous whole-blood model	mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Zhao et al., 2011	rat autologous whole-blood model	mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
<i>statins</i>		
Cui et al., 2012	rat collagenase model	downregulation of MMP-9, decreased edema, mitigated cell death/tissue loss
Jung et al., 2004	rat collagenase model	mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Karki et al., 2009	rat autologous whole-blood model	mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Naval et al., 2008 ⁵²	retrospective clinical study on statin use prior to ICH	decreased perihematomal edema
Naval et al., 2008 ⁵³	retrospective clinical study on statin use prior to ICH	decreased mortality rate, w/ a 12-fold increase in survival (p = 0.05)
Seyfried et al., 2004	rat autologous whole-blood model	mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Tapia-Perez et al., 2009	prospective clinical cohort study	decreased mortality rate (5.6%) compared to historical controls (15.8%)
Yang et al., 2011	rat autologous whole-blood model	preserved BBB integrity, decreased edema, promoted synaptogenesis

(continued)

TABLE 1: Literature review of studies on experimental ICH treatment modalities (*continued*)

Therapy; Authors & Year	ICH Study Type	Results
G-CSF		
Park et al., 2005	rat collagenase model	decreased edema & inflammatory cell migration, mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Sobrinho et al., 2009	prospective clinical cohort study	improved 3-mo clinical outcome
therapeutic hypothermia		
Fingas et al., 2007	rat autologous whole-blood model	decreased edema
Fingas et al., 2009	rat autologous whole-blood model	attenuated neurological deficits/improved functional outcome
Kawai et al., 2001	rat basal ganglia thrombin injection model	preserved BBB integrity, decreased edema
Kawanishi et al., 2008	rat autologous whole-blood model	decreased oxidative DNA damage, mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
MacLellan et al., 2004	rat collagenase model	mitigated cell death/tissue loss, attenuated neurological deficits/improved functional outcome
Rincon, 2013*	clinical trial	Phase I: in progress
Staykov et al., 2013	prospective clinical cohort study	decreased perihematomal edema, decreased mortality rate (8.3% at 3 mos & 28% at 1 yr) vs historical controls (16.7% at 3 mos & 44% at 1 yr); major adverse effect: pneumonia (adequately managed by antibiotics)

* <http://clinicaltrials.gov/ct2/show/NCT01607151>.

model, Nagatsuna et al.⁴⁹ found that systemic administration of argatroban, an inhibitor of free and clot-bound thrombin, reduced edema and neutrophil/monocyte infiltration. Delayed argatroban administration also reduced edema. Kitaoka et al.⁴¹ demonstrated improvements with argatroban delivery 3 hours (intracerebral) or 6 hours (systemically) after whole-blood or collagenase injections. No beneficial effect was seen when treatment was delayed by 24 hours. Finally, nafamostat mesilate, a serine protease inhibitor, attenuated both edema and DNA oxidation when delivered systemically to rats 6 hours after whole-blood or collagenase injections.⁵¹ Importantly, rebleeding in collagenase models was not potentiated by any of the thrombin inhibitors, because treatment and control groups had similar hematoma volumes.^{41,49,51} The success of delayed administration suggests a therapeutic window.⁵¹ Although these results are promising, they are not correlated with functional outcome. However, low concentrations of thrombin are believed to be neuroprotective in the acute phase and to be involved in neuronal plasticity after brain injury.^{27,92} Therefore, excessive thrombin inhibition could exacerbate injury and inhibit long-term recovery.⁴

Deferoxamine. The iron chelator deferoxamine has been extensively tested in animal models of ICH and has progressed further in clinical assessment than other ICH treatment modalities.⁶³ Deferoxamine rapidly crosses the BBB³⁸ and mitigates iron accumulation and oxidative stress secondary to red blood cell hemolysis.³¹ Song et al.⁷² demonstrated that deferoxamine reduces DNA damage, neuron loss, and brain atrophy caused by hippocampal hemoglobin injection in rats. In rodent whole-blood and collagenase ICH models, deferoxamine has been shown to decrease edema and oxidative DNA damage, while preventing brain atrophy and improving neurologi-

cal function.^{50,58,88} Furthermore, these results have been corroborated in aged rats and piglets.^{22,23,26,54,55} It should be noted, however, that 2 studies of deferoxamine in rat collagenase models found no improvement in neurobehavioral function.^{3,82} The multicenter Phase I study, Dose Finding and Safety Study of Deferoxamine in Patients with Brain Hemorrhage (DFO in ICH), found daily infusions of deferoxamine to be tolerable up to 62 mg/kg/day (maximum 6000 mg/day), with no serious adverse effects or deaths. High-Dose Deferoxamine in Intracerebral Hemorrhage (HI-DEF) is a Phase II trial currently in development that will evaluate the efficacy of deferoxamine in the setting of ICH.⁶⁶

Minocycline. Minocycline is a tetracycline-class antibiotic with documented neuroprotective effects in multiple models of CNS trauma and neurodegeneration.⁵⁷ Minocycline therapy has generated significant interest in the context of ICH, due to its ability to cross the BBB.⁸³ Multiple potential mechanisms of benefit have been proposed. Minocycline suppresses inflammation by inhibiting cyclooxygenase-2 and mitigates oxidative stress by direct scavenging of oxygen free radicals. Furthermore, it inhibits apoptosis by upregulating bcl-2 and limiting calcium influx across mitochondrial membranes.⁵⁷ Finally, minocycline blocks the activity of MMP-9 and MMP-12.^{31,34} Wasserman and colleagues^{83,85} used a rat ICH model to demonstrate that daily systemic administration of minocycline, starting 6 hours after collagenase injection, reduced TNF- α and MMP-12 expression. Minocycline also preserved BBB integrity, attenuated edema formation, and limited neutrophil infiltration.^{68,83} Other groups showed that repeated minocycline administration over several days decreased neuronal cell death, brain atrophy, and neurological deficits. However, these studies administered a first dose of minocycline within 2 hours of

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whole-blood injection.^{87,88,96} When a first dose was given at 3 or 6 hours after collagenase injection, no decrease in neuronal loss or improvement in functional outcome was noted.^{75,84} Szymanska et al.⁷⁵ propose that minocycline modulates critical inflammatory events that occur within the first 3 hours after hemorrhage, which would, ultimately, undermine its practicality in clinical use.

Statins. Statins impede cholesterol synthesis by inhibiting the hydroxymethylglutaryl-coenzyme A reductase enzyme. Statins also exhibit antiinflammatory effects and have shown neuroprotective promise in ischemic stroke.⁹⁰ These properties have prompted investigations into their utility as a treatment for ICH. Daily oral simvastatin or atorvastatin started at 24 hours after whole-blood injection and continued for 7 days reduced edema, preserved BBB integrity, and promoted synaptogenesis in rats. This enhanced neuronal plasticity could benefit long-term recovery.⁹⁰ Atorvastatin and simvastatin also inhibited neuron apoptosis and improved functional outcome in whole-blood and collagenase ICH models.^{32,33,67} Other groups suggested potential mechanisms of action through *N*-methyl-D-aspartate receptor-mediated excitotoxicity blockade⁹³ and MMP-9 downregulation.¹¹

Retrospective clinical studies suggest that statin use prior to ICH is associated with decreased perihematomal edema⁵² and a lower mortality rate. A greater than 12-fold odds of survival ($p = 0.05$) was calculated for the statin group.⁵³ Furthermore, a prospective cohort of 18 patients with ICH treated with rosuvastatin demonstrated a 5.6% mortality rate, compared with a 15.8% rate in 57 historical controls.⁷⁶ The Simvastatin for Intracerebral Hemorrhage Study, a Phase II clinical trial scheduled to begin in 2008, was terminated due to poor recruitment (<http://clinicaltrials.gov/ct2/show/NCT00718328?term=NCT00718328>). Despite this setback, statin therapy appears poised for clinical investigation.

Granulocyte Colony-Stimulating Factor. This factor has a well-known role in stimulating neutrophil differentiation and proliferation. In rat models of focal ischemia, G-CSF has also been found to reduce infarct volume⁶⁴ and decrease mortality rates through inhibition of neuronal programmed cell death.⁶⁵ Studies with collagenase-induced rat ICH models have shown that G-CSF also confers neuroprotection by facilitating hemopoietic stem cell and neural stem cell migration into the perivascular hemorrhagic region.⁹⁵ Additionally, G-CSF has demonstrated antiinflammatory effects through downregulation of TNF- α and interferon- γ .^{20,25} Using the collagenase-induced rat ICH model, recombinant human G-CSF administration resulted in less vasogenic edema and less inflammatory response as measured by fewer neutrophils and microglia. This also translated into significantly better clinical outcomes and hemispheric/cortical atrophy at 6 weeks.⁵⁶

Granulocyte colony-stimulating factor appears to be a promising therapy; studies have already shown higher levels correlating with better clinical outcome in humans with ICH. Sobrino et al.⁷¹ showed that despite confounders such as discrepancy in baseline ICH volume and clinical state, higher G-CSF serum levels were still independently associated with a better 3-month clinical outcome.

Therapeutic Hypothermia. Hypothermia is an established neuroprotectant, which has demonstrated the capacity to prevent neuronal loss and improve functional outcome in rodent models of cerebral ischemia.^{9,10} It also has been clinically shown to reduce the mortality rate and augment neurological recovery in patients with cardiac arrest.⁷ Results have been mixed in the setting of ICH. Most studies achieve brain temperatures of 30°–35°C. Following thrombin injection into the basal ganglia, rats housed for 24 hours in a 5°C room demonstrated less edema and BBB permeability than those housed in a 25°C room.³⁵ In a rat whole-blood model, selective brain hypothermia was induced by implanting a cooling coil beneath the temporalis muscle. Hypothermia achieved at 1 hour after injection and continued for 3 days led to decreased edema. However, no improvement in tissue loss or functional outcome was evident. Delayed initiation of hypothermia (12 hours after injection) was not associated with histopathological or functional benefit.¹⁵ When treatment duration was extended to 6 days, an improvement was seen in a single test of neurological function.¹⁶

Conflicting results have been generated in the collagenase ICH model. MacLellan et al.⁴⁴ demonstrated that tissue loss was mitigated and functional outcome improved following delayed hypothermia (12 hours). One explanation is that collagenase models offer more potential for therapeutic benefit than whole-blood models, due to more severe neurological deficits and lack of spontaneous recovery in untreated animals. The authors noted no decrease in tissue loss when hypothermia was initiated less than 12 hours after collagenase injection. They postulated that early hypothermia can exacerbate hematoma expansion by causing coagulopathy and hypertension. Another group demonstrated that systemic hypothermia, initiated at 6 hours after whole-blood injection and continued for 2 days, reduced neutrophil infiltration, oxidative DNA damage, and neurological deficits. However, the long-term functional outcome was not assessed.³⁶

The most promising results, thus far, derive from clinical cohort studies. The data suggest that mild hypothermia, administered for 8–10 days, decreases perihematomal edema and mortality. Staykov et al.⁷³ induced hypothermia via endovascular catheterization in a cohort of 25 patients with ICH. The study demonstrated a mortality rate of 8.3% at 3 months, compared with 16.7% in 25 historical controls. At 1 year, the mortality rate in the treatment cohort was 28% (versus 44% in the controls). Pneumonia was the major adverse side effect, noted in 96% of treated patients versus 78% of controls. It was sufficiently managed with intravenous antibiotics. The Safety and Feasibility Study of Targeted Temperature Management After ICH (TTM-ICH) is a Phase I clinical trial in which the aim is to assess the tolerability of therapeutic hypothermia in patients with ICH. It is currently in progress (see Rincon; <http://clinicaltrials.gov/ct2/show/NCT01607151>).

Conclusions

To date, ICH treatments targeting direct mass effect and clot expansion have provided limited impact on

functional neurological outcome. Recent investigative efforts have focused on secondary mechanisms of brain injury. These processes are complicated and multifactorial. Nonetheless, we have begun to understand the underlying pathophysiological mechanisms and have identified potential treatment strategies. Various putative therapeutic agents have demonstrated efficacy in animal models. However, the current experimental systems do not ideally simulate spontaneous human ICH. Thus, generation of positive data may warrant corroboration in multiple models prior to clinical translation. Therapies targeting secondary brain injury may ultimately function in synergy with strategies designed to limit early, direct mass effect and clot expansion in the setting of ICH.

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: all authors. Drafting the article: Belur. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors.

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