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Preclinical and clinical research on inflammation after intracerebral hemorrhage

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Abstract

Intracerebral hemorrhage (ICH) is one of the most lethal stroke subtypes. Despite the high morbidity and mortality associated with ICH, its pathophysiology has not been investigated as well as that of ischemic stroke. Available evidence from preclinical and clinical studies suggests that inflammatory mechanisms are involved in the progression of ICH-induced secondary brain injury. For example, in preclinical ICH models, microglial activation has been shown to occur within 1 h, much earlier than neutrophil infiltration. Recent advances in our understanding of neuroinflammatory pathways have revealed several new molecular targets, and related therapeutic strategies have been tested in preclinical ICH models. This review summarizes recent progress made in preclinical models of ICH, surveys preclinical and clinical studies of inflammatory cells (leukocytes, macrophages, microglia, and astrocytes) and inflammatory mediators (matrix metalloproteinases, nuclear factor erythroid 2-related factor 2, heme oxygenase, and iron), and highlights the emerging areas of therapeutic promise.

Keywords

Heme oxygenase; Hemorrhagic stroke; Leukocytes; Matrix Metalloproteinase; Microglia; NF-E2-related factor 2; Iron

1. Introduction

Intracerebral hemorrhage (ICH) results when a weakened blood vessel ruptures and bleeds into the surrounding brain. Spontaneous ICH accounts for 15–20% of all strokes and affects more than 2 million people worldwide each year (Qureshi et al., 2009; Ribo and Grotta, 2006). The prevalence of ICH is higher in certain populations, including blacks and Asians (Qureshi et al., 2009). Parts of the brain that are particularly vulnerable to ICH include the basal ganglia, cerebellum, brainstem, and cortex. Most cases of ICH are caused by primary hypertensive arteriolosclerosis and amyloid angiopathy (reviewed in Mayer and Rincon, 2005; Sutherland and Auer, 2006). Secondary ICH accounts for 15–20% of patients and usually results from vascular malformation, neoplasia, coagulopathy, and the use of thrombolysis in ischemic stroke (reviewed in Mayer and Rincon, 2005; Sutherland and Auer, 2006; Wang and Tsirka, 2005a). No matter the cause, the extravasated blood compresses the surrounding brain tissue, increasing the intracranial pressure. The prevalence of ICH is expected to increase slightly as improvements in blood pressure management are

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counteracted by the trends that favor ICH incidence, such as population aging, increasing use of thrombolytics and anticoagulants, and lack of effective prevention for cerebral amyloid angiopathy in the elderly.

The incidence of fatality is much higher among individuals who suffer ICH than among those who experience ischemic stroke. Those who do survive usually experience long-term physical and mental disability, although some patients can recover most neurologic function. Treatment for ICH is primarily support and control of general medical risk factors. The prognosis of ICH depends on the location, amount of bleeding, extent of subsequent brain swelling, the level of consciousness at admission, concomitant diseases, and the age of the patient. Interestingly, the data from a recent clinical ICH study indicate that the degree of perihematomal edema and subsequent edema expansion are positively correlated with the underlying hematoma size but are not major independent determinants in the outcome (Arima et al., 2009).

Although ICH research has received far less attention than has ischemic stroke (Donnan et al., 2010; NINDS ICH Workshop Participants, 2005), during the past few years, progress has been made toward identifying the roles of inflammatory signaling molecules, cells, and proteins in initiation and progression of post-ICH inflammation. We and others have reviewed the roles of cytokines, proteases, and reactive oxygen species (ROS) in ICH-induced brain injury (Aronowski and Hall, 2005; Wang and Doré, 2007b; Wang and Tsirka, 2005a; Xi et al., 2006). A recent review has highlighted the important functions of complement activation in ICH (Ducruet et al., 2009). The focus of this review will be primarily on recent progress made in the use of preclinical ICH models, understanding the changes in cellular components (leukocytes, microglia/macrophages, and astrocytes) and inflammatory mediators [matrix metalloproteinases (MMPs), nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase (HO), and iron toxicity], and emerging opportunities for novel therapeutic strategies such as stem cell therapy.

2. Preclinical models of ICH

Preclinical studies of ICH have been carried out in many species, but rodents are most commonly used. Rodent models of ICH are fundamentally different from the human condition, and the paucity of white matter, lower glia-to-neuron ratio, and differences in homeostasis limit their clinical relevance. Two main animal models are used to reproduce the clinical condition of ICH, the whole-blood model and the collagenase model. The whole-blood model, in which an animal's own blood or donor blood is injected directly into the striatum, has been used in various animals (Gu et al., 2009; Koeppen et al., 2004; Okauchi et al., 2009; Qureshi et al., 2001). Recently this model has been adapted for use in mice (Rynkowski et al., 2008; Tejima et al., 2007; Wang et al., 2008; Xue et al., 2006; Zhao et al., 2007b), thereby enabling the use of transgenic or knockout ($^{-/-}$) mice to study specific signaling pathways or brain injury mechanisms. The autologous whole-blood model mimics ICH better than the donor blood model because the latter induces more severe brain edema (Nakamura et al., 2004b). The advantage of the whole-blood model is that only blood is introduced into the model system. The drawbacks include: lack of underlying vascular pathology and rupture; variable lesion size caused by ventricular rupture or backflow of the injected blood along the needle track and corpus callosum; and potential effects of donor blood or anticoagulant on inflammation, complement, or the coagulation system. Given the limited volume of blood that can be infused into the mouse striatum, the technique of blood injection in mice remains challenging. Because of the shortcomings of the current blood infusion models, we established a modified double-blood infusion model in mice that does not use any anticoagulant (Wang et al., 2008). In the modified model, 10 μl of autologous whole blood is infused into the striatum at a speed of 0.2 μl/min in two phases, with a break

of 7 min in between. We keep the needle within the injection site for another 20 min after blood infusion to prevent backflow of blood along the needle track. The hematoma developed in this model is confined to the ipsilateral striatum and produces the desired brain injury and neurologic deficits (Wang et al., 2008).

In another model, the proteolytic enzyme collagenase is injected into the striatum, breaking down the blood-brain barrier (BBB) and resulting in active bleeding (Rosenberg et al., 1990; Tang et al., 2004; Wang et al., 2003). In this model, the hematoma develops gradually over 4–5 h (Wang and Doré, 2007a). The procedure itself is simple, and it mimics an acute cerebrovascular injury. Furthermore, the resultant bleed is spontaneous and reproducible in location and size (Tejima et al., 2007; Wagner, 2007; Wang and Doré, 2007b). These advantages allow investigation of the collagenase-induced bleeding response and hematoma expansion. The model is relevant to the clinical condition because continued bleeding occurs in 14–20% of all ICH patients and lasts for over 6 h in 17% of cases (Brott et al., 1997; Kazui et al., 1996). Of course the collagenase model does have some drawbacks. There is no underlying vascular pathology, and bleeding results from the rupture of many vessels, whereas in humans, rupture of a small, deep-penetrating artery is the primary cause. In addition, the introduction of bacterial collagenase into the brain could potentially enhance inflammation, although three in vitro studies, including our own, argue against the possibility by showing that collagenase alone does not activate microglia, affect prostaglandin E2 production, or induce cell death (Chu et al., 2004; Matsushita et al., 2000; Wang et al., 2003). Both models have limitations and reflect only certain clinical features of ICH, but to our knowledge, these two models are the most useful tools currently available for the study of ICH. Interestingly, a new mouse model of spontaneous ICH has been developed in which ICH is induced by acute hypertension (Iida et al., 2005; Wakisaka et al., 2010); more studies into the pathophysiology of this model are clearly needed. In translational medicine, testing in multiple related preclinical models and in different laboratories is strongly encouraged before advancing any novel medicine or therapy to a clinical trial. Early reviews of preclinical animal models of ICH are available (Andaluz et al., 2002; Strbian et al., 2008).

3. Inflammation and the cellular response to ICH

ICH can cause primary and secondary brain injury. The immediate effects of ICH, such as hematoma expansion and the consequent increase in intracranial pressure, lead to primary injury, whereas subsequent effects, such as inflammation, contribute to secondary injury. Inflammation is characterized by the accumulation and activation of inflammatory cells and mediators within the hemorrhagic brain. ICH allows the immediate infiltration of blood components, including red blood cells, leukocytes, macrophages, plasma proteins, etc, into the injury site. The inflammatory response that follows this infiltration involves inflammatory mediator release, protease activation, microglia and astrocyte activation, brain tissue breakdown, and repair (Wang and Doré, 2007b; Wang and Tsirka, 2005a). Inflammatory cells include blood-derived leukocytes and macrophages, resident microglia, astrocytes, and mast cells. Microglia are believed to be the first non-neuronal cells to react to brain injury; they act as guardians of neuronal survival and function under various pathologic conditions in the brain (van Rossum and Hanisch, 2004). When fully activated, phagocytic microglia are impossible to differentiate from infiltrating macrophages. Increasing evidence suggests that leukocytes/macrophages, activated microglia, and astrocytes are major cellular mediators of secondary brain damage after ICH based on their local release of cytokines, chemokines, prostaglandins, proteases, ferrous iron, and other immunoactive molecules (Aronowski and Hall, 2005; Wang and Doré, 2007b; Zhang et al., 2009). Interestingly, cerebral mast cells have gained attention lately, as blocking them has been reported to reduce brain edema and hematoma volume and to improve ICH outcomes

(Lindsberg et al., 2010; Strbian et al., 2007). A new study suggests that Toll-like receptor 4-mediated nuclear factor kappa-B signaling mediates the activation and regulation of inflammatory responses in the hemorrhagic brain and could be a therapeutic target for ICH (Teng et al., 2009).

Recent preclinical and clinical studies have enabled us to better understand the impact of post-ICH inflammation. Preclinical studies of ICH rely heavily on animal models, whereas clinical studies are usually restricted to blood or cerebrospinal fluid sampling. A recent human ICH study that used microarray analysis demonstrated significant up-regulation of both pro- and anti-inflammatory genes in the peri-ICH brain tissue (Carmichael et al., 2008), a finding consistent with the results of a previous animal study (Lu et al., 2006). Little data regarding ICH pathogenesis have been obtained from postmortem human brain, and more studies are critically needed. Anti-inflammatory therapies have been tested in preclinical and clinical studies (Table 1), but as yet, no direct anti-inflammatory treatments have been approved for clinical use. Therapeutic hypothermia has been extensively studied as a means to reduce ischemic or hemorrhagic brain injury through its anti-inflammatory effect. Although therapeutic hypothermia provides considerable protection after ischemic stroke, evidence for a beneficial effect in ICH is insufficient, and potential complications can arise, such as infection and increased blood pressure. Thus, its clinical usefulness for ICH is in question (MacLellan et al., 2009).

3.1. Leukocytes

3.1.1. Preclinical studies—Infiltrating leukocytes are believed to play a role in ICH-induced secondary brain injury. Preclinical studies of ICH provide substantial histopathologic evidence for the presence of infiltrating leukocytes in and around the hematoma (Wang and Doré, 2007a,b; Zhao et al., 2006c). Neutrophils are the earliest leukocyte subtype to infiltrate into the hemorrhagic brain, and these may damage brain tissue directly by producing ROS, releasing proinflammatory proteases (Nguyen et al., 2007), and modulating BBB permeability (Joice et al., 2009). Isolated neutrophils have been shown to aggravate neuronal cell death induced by excitotoxicity or oxygen–glucose deprivation (Dinkel et al., 2004). Once they have entered the hemorrhagic brain, leukocytes will die by apoptosis within two days (Savill and Haslett, 2001). The contents of dying leukocytes can further damage brain tissue by stimulating neighboring microglia/macrophages to secrete pro-inflammatory toxic factors. Under a microscope, neutrophils can be identified easily and specifically by using immunohistochemistry with anti-myeloperoxidase antibody (Wang and Tsirka, 2005b). We observed in the collagenase-induced ICH model that infiltrating neutrophils appeared in and around the hematoma of mice at 4 h (Fig. 1A) (Wang and Doré, 2007a) and that the number of neutrophils peaked at 3 days (Wang and Tsirka, 2005b). This temporal pattern of neutrophil infiltration was reported to be similar in both ICH models in rat (Peeling et al., 2001b; Xue and Del Bigio, 2000).

It is known that the β2 integrins (CD11/CD18) are expressed in leukocytes. A recent study that used $CD18^{-/-}$ mice revealed a significant decrease in brain edema and mortality after collagenase-induced ICH; the attenuation of brain injury in $CD18^{-/-}$ mice was associated with a concomitant decrease in myeloperoxidase and nitrotyrosine immunoreactivity in the hemorrhagic brain (Titova et al., 2008). Although neuroprotection can be obtained with anti-neutrophil strategies in animal models of cerebral ischemia (Zheng and Yenari, 2004), the beneficial effects have not been observed in an acute human ischemic stroke trial (Enlimomab Acute Stroke Trial Investigators, 2001). The data for such strategies in experimental ICH are lacking.

3.1.2. Clinical studies—Clinical studies further confirm the toxic role of leukocytes in ICH. In patients with ICH, the number of leukocytes present in the cerebrospinal fluid is frequently elevated, and elevated peripheral leukocyte counts have been reported to be positively correlated with hematoma size (Bestue-Cardiel et al., 1999). In fact, a high peripheral leukocyte count is considered to be an independent predictor of early clinical worsening in primary ICH (Leira et al., 2004; Silva et al., 2005). To date, three histopathologic studies have examined leukocyte infiltration in the human ICH brain. The first study showed infiltration of neutrophils into the hematoma 2–4 days after ICH (Wisniewski, 1961). Prior to this infiltration, neutrophil accumulation was observed in blood vessels that bordered the hematoma as early as 6–12 h after ICH. In the second study, investigators examined the early cellular response in postmortem brain tissue and observed that leukocyte infiltration in the peri-ICH region appeared as early as 5–8 h and disappeared by 72 h (MacKenzie and Clayton, 1999). The third study examined inflammatory responses and neuronal death in the perihematomal tissue obtained during craniotomy. In that study, infiltration of neutrophils, macrophages, and lymphocytes appeared at 6–12 h and increased further at 12–24 h; the infiltration of these inflammatory cells was correlated with TUNEL-positive cells and expression of Bax protein (Guo et al., 2006). Thus, available clinical evidence supports a role for leukocytes in ICH pathogenesis (Giaume et al., 2010), although the underlying mechanisms involved remain to be determined.

3.2. Microglia/macrophages

Microglia are the primary immune effector cells in the brain and are often referred to as the brain's macrophage. In normal brains, resting microglia exhibit ramified morphology and constitute approximately 5–20% of the total glial population. The processes and protrusions of microglia have been observed to interact dynamically with neighboring neurons, astrocytes, and blood vessels (Nimmerjahn et al., 2005). In response to various types of brain damage, microglia become activated (also referred to as reactive) and undergo morphologic and functional transformations. Specifically, the cell body becomes enlarged with thick processes, pro-inflammatory proteins are upregulated, and the cells become migratory, proliferative, and phagocytotic (reviewed in Wang and Tsirka, 2005a). Activated microglia are believed to have both neurotoxic and neuroprotective properties. The overall effect depends on pathologic conditions and brain injury severity (van Rossum and Hanisch, 2004).

3.2.1. Preclinical studies—Increasing evidence has been obtained from preclinical studies regarding the role of microglia/macrophages in ICH. Activated microglia/macrophages can be identified by microscopy. Both resting and activated microglia/macrophages can be visualized by immunohistochemistry, and activated cells can be easily identified by their hypertrophic morphology. One should keep in mind that although ED1 antibody is claimed to be specific for activated microglia/macrophages in rats, ED1 is not expressed in all reactive cells. We and others have used antibody to 5D4 (an epitope of keratan sulfate proteoglycan) for the detection of activated microglia in mice (Fan et al., 2007; Miao et al., 2005; Wang and Tsirka, 2005c).

Accumulating evidence indicates that microglia/macrophages are activated early after ICH and that this activation contributes to ICH-induced secondary brain injury (Gao et al., 2008; Wang and Doré, 2007b; Wang and Tsirka, 2005a). After ICH, reactive microglia appear rod-like, spherical, or amoeboid, with short, strong processes, intense immunoreactivity, and a cell body usually more than 7.5 μm in diameter. In contrast, resting microglia are characterized by small cell bodies with a diameter less than 7.5 μm, ramified processes, and mild immunoreactivity (Wang and Doré, 2007a; Wang et al., 2008). Although the major role for microglia/macrophages after ICH is to clear the hematoma and the tissue debris, reactive

microglia/macrophages also express and release a variety of potentially toxic factors, such as cytokines, chemokines, ROS, proteases, cyclooxygenase-2, prostaglandins, and HO-1 and its metabolites (Wang and Doré, 2007a,b; Wang and Tsirka, 2005a). In addition, the degree of microglia/macrophage activation has been shown to be greater in aged rats after ICH than in young rats (Gong et al., 2004; Lee et al., 2009). It remains unclear how to differentiate activated microglia from macrophages recruited from the circulation.

Many studies have suggested that activated microglia/macrophages contribute to ICH-induced early brain injury (Aronowski and Hall, 2005; Keep et al., 2005; Wang and Doré, 2007b). We have recently demonstrated that after collagenase-induced ICH, activation of microglia occurs much earlier than infiltration of neutrophils in and around the hematoma; the former occurs within 1 h, whereas the latter occurs after 4–5 h (Fig. 1A and Fig. 2F) (Wang and Doré, 2007a). The early response of microglia after ICH is consistent with the observation that focal microglia are activated immediately after BBB disruption by laser lesions (Nimmerjahn et al., 2005). We have reported that reactive microglia are prominent in the perihematomal region on day 1 after ICH, reach a maximum on day 7, and return to normal by 21 days (Wang et al., 2003; Wang and Tsirka, 2005c). Similarly, reactive microglia were observed in the perihematomal regionasearlyas1–4 h after whole blood infusion into the rat striatum (Zhao et al., 2007b), peaked at 3–7 days, and persisted for 4 weeks (Gong et al., 2000; Xue and Del Bigio, 2000). We have additional data to demonstrate the presence of reactive microglia/macrophages in the perihematomal region 1–3 days after intrastrial infusion of autologous whole blood (Wang et al., 2008). A recent ICH study in rats demonstrated an increase in immunoreactivity of chemokine macrophage inflammatory protein (MIP)-2 that appeared as early as 2 h and peaked at 2 days; the changes in MIP-2 level correlated with nuclear factor-κB activation and brain water content (Wu et al., 2009a).

Therapeutic strategies based on the inhibition of microglial activation have been tested in preclinical trials. In two of our early studies, we pretreated (2 days before) or post-treated (2 h after) mice with MIF (microglia/macrophage inhibitory factor, tuftsin fragment 1–3, Thr-Lys-Pro) to inhibit microglial activation (Wang et al., 2003; Wang and Tsirka, 2005c). MIF-treated mice showed reduced brain injury and improved neurologic function. Similarly, inhibition of microglial activation with minocycline, which also inhibits MMPs, was shown to protect the BBB, reduce brain edema (Wasserman and Schlichter, 2007a), and thereby improve functional recovery in collagenase-induced ICH in rats (Power et al., 2003), although neuronal loss was not decreased (Wasserman and Schlichter, 2007b). Neuroprotection conferred by minocycline also was reported in rodents subjected to the whole-blood model (Wu et al., 2009b; Xue et al., 2010) and will be discussed in depth below. Interestingly, negative results have also been reported (Szymanska et al., 2006). Although cumulatively the data suggest that strategies to target early inhibition of microglia/macrophage activation after ICH could be therapeutic, long-term inhibition may not be fully beneficial because inhibition could potentially abolish neuroprotective benefits of microglia/macrophages as phagocytes and suppliers of neuroreactive molecules (Nakajima et al., 2001; Wang and Tsirka, 2005c).

3.3. Astrocytes

In the human brain, astrocytes are star-shaped glial cells that generally outnumber neurons by tenfold. Astrocytes are vital for normal brain functions and are considered to be active elements of the brain circuitry. Calcium signaling in activated astrocytes has been proposed to trigger the release of gliotransmitters, such as glutamate, ATP, TNF-α, and D-serine, which can modulate neuronal excitability, synaptic activity, and plasticity (Giaume et al., 2010). Astrocytes react to many central nervous system injuries and undergo profound morphologic and functional remodeling that is dependent on the type and timing of injury

and the distance from the injury site. Disturbance of astrocytic function by brain injury or disease can compromise neuronal functionality and viability. The presence of reactive astrocytes is a hallmark of various neuropathologic conditions (Anderson et al., 2003; Miller, 2005). Although astrocytes are recognized to be important in both the initiation and propagation of secondary ischemic brain injury, their contribution to the process of hemorrhagic brain injury has not been clearly defined, and very limited data are available from preclinical or clinical studies.

3.3.1. Preclinical studies—Astrocytes, like microglia, are capable of secreting inflammatory mediators. After ICH, brain astrocytes become activated in concert with microglial activation and increase production of glial fibrillary acidic protein (GFAP), causing a so-called “reactive gliosis.” Reactive astrogliosis after brain injury could inhibit axonal regeneration. In our two recent studies, we observed a robust activation of astrocytes in the perihematomal region early after ICH that was greatest in regions closest to the injury site and gradually decreased with distance (Fig. 3C) (Wang and Dore, 2008;Wang et al., 2008); this pattern was similar to that of microglial activation. Astrocytes were shown to participate in the brain inflammatory process by expressing MMPs after ICH in both the collagenase model (Wang and Tsirka, 2005b) and whole-blood model (Tejima et al., 2007). Interestingly, the presence of blood plasma proteins seems to be an important mediator for activation of astrocytes given that the injection of whole blood induces greater activation of astrocytes than does injection of purified red blood cells (Koeppen et al., 1995). Consistent with the fact that astrocytes have stronger antioxidative potential than do neurons, cultured astrocytes are highly resistant to ROS, and we found that astrocytes are more resistant than neurons to ICH-induced brain injury (Wang and Doré, 2007a). The functional significance of the astrocytic response surrounding the hematoma is not totally clear at present. Reactive astrocytes may protect neurons by promoting the secretion of neurotrophic factors (Brahmachari et al., 2006) or by modulating the expression of microglial inflammatory mediators (Pyo et al., 2003) and microglial ROS production (Min et al., 2006). However, in cases of rapid and severe activation, for example early after ICH, astrocytes are more likely to mediate an inflammatory response to become neurotoxic. Interestingly, reactive astrocytes and microglia can interact to cause local MMP activation (Rosenberg et al., 2001). Therefore, controlling microglia–astrocyte interactions could be considered as a potential means to minimize ICH-induced brain injury (Wang and Doré, 2007b).

3.3.2. Clinical studies—Very few clinical studies have focused on astrocytes after ICH in the human brain. In one early study, researchers investigated brain biopsy specimens from six patients with cerebral amyloid anigiopathy-associated ICH. They found that reactive astrocytes in some patients were labeled with Alzheimer A4 and gamma-trace peptides, suggesting that astrocytes themselves may have the ability to generate the amyloid peptides (Yong et al., 1992). In patients with subarachnoid hemorrhage, GFAP concentrations in the cerebrospinal fluid were found to correlate with secondary brain damage caused by delayed cerebral ischemia (Petzold et al., 2006). Tejima et al. (2007) demonstrated the astrocytic induction of MMP-9 in the perihematomal areas in a postmortem human ICH brain, further supporting the detrimental role of astrocytic overactivation early after ICH. Thrombin, its endogenous inhibitor protease nexin-1, and water channel protein aquaporin-4 have also been strongly detected by immunohistochemistry in astrocytes, neurons, and cerebral vasculature of the ipsilateral hippocampus at 5–96 h post-ICH in postmortem human brain (Wu et al., 2008).

4. Inflammatory mediators

4.1. Matrix metalloproteinases

MMPs are a large family of zinc-dependent endopeptidases involved in extracellular remodeling as well as the neuroinflammatory response. To date, 23 MMPs have been identified in humans (Gueders et al., 2006). MMPs are normally located in the cytosol in a pro- or inactivated state; however, under pathologic conditions, they are cleaved by proteases, such as plasmin, tissue plasminogen activator (tPA), or other MMPs, to their active state (Wang and Doré,2007b; Xue et al., 2009a). Accordingly, expression of MMPs in the normal brain is very low, but many MMPs are upregulated and activated in response to various brain injuries.

4.1.1. Preclinical studies—Preclinical studies of MMPs in ICH have focused on MMP-2, -3, -9, and -12 (Wang and Doré, 2007b; Xue and Yong, 2008). The first such study, by Rosenberg and Navratil (1997), demonstrated that gelatinases MMP-2 and MMP-9 were activated within 24 h of collagenase-induced ICH in rats. Later, using the same model in mice, we observed a similar increase in gelatinase activity at 24 h post-ICH (Fig. 4A) (Wang et al., 2003). Extending this work, we demonstrated a dramatic increase in MMP-9 activity by gel and in situ zymography at 24–72 h post-ICH (Wang and Tsirka, 2005b). Other investigators observed increases in mRNA levels of MMP-3 and/or -12 (Power et al., 2003; Wells et al., 2005). Early increases in MMP-9 mRNA, protein, and/or activity have been confirmed in other ICH studies that have used different animal models (Lee et al., 2003; Lu et al., 2006; Mun-Bryce et al., 2004; Wu et al., 2010b; Xue et al., 2006). Recently, Xue and colleagues (Xue et al., 2009a, 2006) observed that human recombinant MMP-3 and -9 can directly kill human fetal neurons. In support of this finding, mice with genetic deletions for MMP-3, -9, and -12 were reported to have less hemorrhagic brain injury than wild-type (WT) mice after ICH (Wells et al., 2005; Xue et al., 2009a, 2006), although contrary results for MMP-9 were reported in one study (Tang et al., 2004). Furthermore, MMP-3 and -9 were reported to act synergistically with thrombin (Xue et al., 2009a).

Given the proposed detrimental effect of MMP activity early after ICH, several investigators have used inhibitors to block MMP activity in preclinical studies. Rosenberg and Navratil (1997) reported that rats treated with BB-1101 (a broad-spectrum hydroxamic acid-based MMP inhibitor) reduced brain edema and mortality when administrated 6 h after the onset of ICH. In our own studies, treatment of mice with the broad-spectrum MMP inhibitor GM6001 attenuated gelatinase activity, neutrophil infiltration, and ROS production, and thereby decreased early brain injury and improved neurologic function (Wang and Tsirka, 2005b). Neuroprotection conferred by GM6001 was further confirmed in a recent mouse study that used the whole-blood model of ICH (Xue et al., 2009b). Two early studies reported that MMP inhibition with BB-94 reduced recombinant tPA-induced hemorrhage after thromboembolic stroke (Lapchak et al., 2000) and recombinant tPA-related mortality after middle cerebral artery occlusion (Pfefferkorn and Rosenberg, 2003). In contrast, Grossetete and Rosenberg (2008) reported that MMP inhibition with BB-94 increased cell death and hemorrhage volume in a collagenase-induced ICH mouse model.

Minocycline, a semi-synthetic tetracycline, has shown promise as a MMP inhibitor and a neuroprotectant (Yong et al., 2004). Power et al. (2003) reported that inhibition of MMP-12 with minocycline protected the morphology of neurons and improved functional recovery after ICH. Although delayed administration of minocycline (6 h post-ICH) failed to reduce neuronal death or striatal tissue loss at day 7 (Wasserman and Schlichter, 2007b), it did decrease early upregulation of TNF-α and MMP-12 (Wasserman and Schlichter, 2007a; Wasserman et al., 2007), thereby protecting the BBB and reducing brain edema (Wasserman and Schlichter, 2007a). Contrary to these positive results, one study reported that

minocycline provided neither histologic nor functional protection when administrated 3 h after ICH, even though the number of microglia/macrophages was reduced in the perihematomal areas (Szymanska et al., 2006). Together, most of the preclinical findings support the view that MMP-3, -9, and -12 might play a detrimental role in the pathophysiology of ICH and that intervention with MMP inhibitors might decrease ICH-induced early brain injury. However, the timing of MMP inhibition is critical, and long-term inhibition can be deleterious because MMPs do exert beneficial effects during neurovascular remodeling (Cunningham et al., 2005; Yong, 2005; Zhao et al., 2006a) and recovery in the later phases of ICH.

4.1.2. Clinical studies—Several clinical studies have reported that blood MMP-9 levels are increased in patients with acute spontaneous ICH (Abilleira et al., 2003; Alvarez-Sabin et al., 2004; Castellazzi et al., 2010; Silva et al., 2005). This increase in blood MMP-9 levels within 12 or 24 h after stroke onset is associated with subsequent enlargement of the hematoma (Silva et al., 2005), perihematomal edema, and worsening of neurologic function (Abilleira et al., 2003). Alvarez-Sabin et al. (2004) systematically investigated the temporal profile of MMPs and their natural inhibitors in patients with acute ICH and found that an increase in blood MMP-9 levels correlated with perihematomal edema and neurologic deterioration during the acute stage, whereas elevated blood MMP-3 levels were associated with mortality. Moreover, increasing evidence identifies an association between elevated MMP-9 concentration and subsequent hemorrhagic transformation or thrombolytic-induced hemorrhage in ischemic stroke patients (Castellanos et al., 2003, 2007; Montaner et al., 2001a,b, 2003; Ning et al., 2006). In one recent study, investigators observed an association between plasma levels of MMP-9 and BBB disruption after stroke in humans (Barr et al., 2010). To date, only two histopathologic studies have been identified in which MMPs were examined in human ICH brain within the first 6 h after death (Rosell et al., 2006; Tejima et al., 2007). The results showed that MMP-9 was upregulated in neurons and reactive astrocytes surrounding the hematoma; brain tissue around the hemotoma had higher MMP-9 levels than did the contralateral hemisphere, supporting a contribution of MMP-9 to perihematomal edema. We have recently reported in a histopathologic case-control study (Wu et al., 2010a) that expression levels of MMP-9, nuclear factor-kappa B/p65 subunit, and macrophage inflammatory protein-2 were each upregulated on the injured side of the hippocampus at times ranging from 2 h to 5 days post-ICH. Interestingly, the expression of all three markers was also upregulated on the uninjured side of the hippocampus and in the cerebellum, although to a lesser extent. Taken together, available clinical data support detrimental roles for MMP-9 in ICH and hemorrhagic transformation after ischemic stroke; however, the roles of MMP-3 and -12 still need to be studied in clinical settings.

4.2. Nrf2/heme oxygenase/iron

Nrf2 is a key transcriptional factor that regulates antioxidant genes that act together to remove ROS (Chen and Kunsch, 2004; Nguyen et al., 2009). These Nrf2-regulated genes constitute the phase II antioxidant and detoxification response and code for antioxidant enzymes such as NAD(P)H:quinine oxidoreductase 1, glutathione S transferases, glutamate–cysteine ligase, glutathione peroxidase, and HO-1. Nrf2 is now regarded as a protector for brain and many other organs (reviewed in Lee et al., 2005). During ICH, substantial amounts of heme are released from extravasated blood and dying cells. The HO enzymes (HO-1 and HO-2) degrade this heme into biliverdin, carbon monoxide, and iron (Ryter and Tyrrell, 2000). Increasing evidence suggests that iron-induced oxidative stress can cause neurodegeneration (Zecca et al., 2004).

4.2.1. Preclinical studies—In the brain, Nrf2 is present in astrocytes, neurons, and microglia, where it participates in redox homeostasis by regulating the expression of

antioxidant genes. Studies have shown that activation of Nrf2 is neuroprotective in a variety of in vitro models (Johnson et al., 2008). Specifically, astrocytic Nrf2 activation is sufficient to protect neurons from cytotoxicity both in vitro (Shih et al., 2005) and in vivo (Jakel et al., 2007). In terms of stroke, administration of an Nrf2 inducer, either tert-butylhydroquinone or sulforaphane, significantly improved stroke outcomes in rodents (Shih et al., 2005; Zhao et al., 2006b). Conversely, Nrf2$^{-/-}$ mice suffered more stroke damage than did WT controls in permanent and transient stroke models (Shah et al., 2007; Shih et al., 2005). The cellular localization of Nrf2 in the hemorrhagic brain is not clear. We first demonstrated that mice lacking Nrf2 are more susceptible than WT control mice to collagenase-induced hemorrhagic brain injury (Fig. 5A–C). Additionally, we showed that the exacerbation of brain injury in Nrf2$^{-/-}$ mice was associated with increases in leukocyte infiltration, ROS production, and cytochrome c release (Wang et al., 2007). Consistent with our results, Zhao et al. (2007a) reported that Nrf2$^{-/-}$ mice subjected to the whole-blood model of ICH exhibited more severe neurologic deficits than did WT control mice and that the Nrf2 inducer sulforaphane administered 30 min after ICH was able to reduce neutrophil count, oxidative damage, and behavioral deficits in WT but not in Nrf2$^{-/-}$ mice.

Nrf2 is known to regulate the transcription of HO-1; however, the brain contains two HO isoforms: inducible HO-1 and constitutive HO-2. Both isoforms catalyze the rate-limiting reaction by which heme is degraded into biliverdin, carbon monoxide, and iron (Ryter and Tyrrell, 2000). In rodent brain after ICH, HO-1 is mainly induced in glial cells, whereas HO-2 is expressed in neurons throughout the brain (Koeppen et al., 2004; Matz et al., 1997; Nakaso et al.,2000; Wang and Doré, 2007a). Such different cellular expression of HO-1 and HO-2 suggests that they might have distinct roles in ICH. Although some evidence indicates that HO-1 and HO2 have cytoprotective functions (Parfenova and Leffler, 2008; Takahashi et al., 2004), several preclinical studies have demonstrated that non-selective HO inhibitors are neuroprotective in blood ICH models (Huang et al., 2002; Koeppen et al., 2004; Wagner et al., 2000). Presumably, HO-1 does not exert a direct neuroprotective effect early after ICH because it selectively localizes to microglia/macrophages. Although activation of microglia/macrophages contributes to hematoma resolution (Wang et al., 2003), it is involved in early brain injury after ICH (Wang et al., 2003; Wang and Tsirka, 2005c). Furthermore, HO-1 activation is not neuroprotective in ischemia, as stroke damage was not exacerbated in HO-1$^{-/-}$ mice (Doré et al., 1999; Shah et al., 2006). These findings challenge the belief that HO-1 confers neuroprotection early after ICH. Actually, evidence suggests that the effects of HO-1 depend on the relative activity of the enzyme, and excessively high levels of HO-1 could be cytotoxic (Suttner and Dennery, 1999). In ICH, the heme-induced upregulation of HO-1 might exceed the protection threshold and result in brain injury. To elucidate the role of HO-1 after ICH, we compared the outcomes in WT and HO-1$^{-/-}$ mice subjected to the collagenase-induced ICH model and found that HO-1 protein was highly expressed in the perihematomal region, mostly in microglia/macrophages and endothelial cells. The injury volume was smaller in HO-1$^{-/-}$ mice than in WT mice early after ICH, and the protection in HO-1$^{-/-}$ mice was associated with decreased inflammation and free radical levels (Fig. 1 and Fig. 2) (Wang and Doré, 2007a). A correlation between HO-1 induction and oxidative brain injury also has been reported in whole-blood ICH models by other investigators (Chen and Regan, 2007; Wagner et al., 2002; Wu et al., 2003). In contrast, early studies suggested that HO-2 expression is cytoprotective (Doré et al., 2000; Doré and Snyder, 1999; Parfenova and Leffler, 2008) and that constitutive HO-2 protects the brain against ischemia and traumatic brain injury (Chang et al., 2003; Doré et al., 2000,1999; Goto et al., 2003). We have shown that HO-2 deletion renders primary cultured neurons more vulnerable to hemin (oxidized heme)-induced toxicity (Wang et al., 2006) and that HO-2$^{-/-}$mice are more vulnerable than WT mice to collagenase-induced ICH (Wang and Dore, 2008; Wang et al., 2006). Furthermore, the exacerbation of brain injury in HO-2$^{-/-}$ mice is associated with increases in neuroinflammation and ROS production (Fig. 3) (Wang

and Dore, 2008). Conflicting data were reported by Qu et al. (2007). The differences may reside in the ICH model used (collagenase vs. single-blood infusion) and the use of anticoagulant heparin in the blood injected.

Hemolysis of red blood cells leads to hemoglobin degradation and the formation of products such as non-heme iron. In a rat model of ICH, brain hemorrhage led to iron deposition and a threefold increase in non-heme iron (Wu et al., 2003). Excess iron in the brain can result in lipid peroxidation and the formation of free radicals (Gutteridge, 1994), which damage neurons in many disease states (Thompson et al., 2001; Zecca et al., 2004). Hemolysis and heme/iron-mediated toxicity occur 2–3 days after ICH (Wagner et al., 2003). Considerable evidence suggests that hemoglobin breakdown and subsequent iron accumulation within the brain mediates secondary brain injury after ICH (Wagner et al., 2003; Xi et al., 2006). Brain atrophy and neurologic deficits observed up to 2 months after ICH have been attributed to iron deposition within the striatum (Hua et al., 2006). Interestingly, and in support of iron-mediated toxicity after ICH, treatment with an iron chelator such as deferoxamine was shown to provide neuroprotection in a whole-blood ICH model in rats (Hua et al., 2006; Nakamura et al., 2004a; Okauchi et al., 2009; Song et al., 2007) and in piglets (Fig. 6) (Gu et al., 2009), but not in a collagenase-induced ICH model in rats (Warkentin et al., 2010).

4.2.2. Clinical studies—Currently, no histopathologic data are available on Nrf2 in human ICH brain. Two studies have shown induction of HO-1 in microglia/macrophages and increased iron content associated with the hemorrhagic lesion (Beschorner et al., 2000; Lou et al., 2009). Interestingly, increased HO-1 concentration was reported to be associated with worse neurologic outcome after traumatic brain injury in infants and children (Cousar et al., 2006). In a small clinical study, it was reported that the levels of nonprotein-bound iron were elevated in the cerebrospinal fluid from preterm infants with intraventricular hemorrhage compared with control infants (Savman et al., 2001). In patients with spontaneous ICH, an association between serum ferritin level and perihematomal edema volume on days 3–4 has been reported (Mehdiratta et al., 2008). High serum ferritin levels did not correlate with acute phase reactions and were found to be associated with poor outcomes (de la Ossa et al., 2010). Recently, magnetic resonance imaging revealed a relationship between iron content within the hematoma and perihematomal edema in the human brain after ICH (Lou et al., 2009), further linking iron-mediated toxicity to brain edema formation and delayed neuronal death after ICH.

5. Stem cell therapy

5.1. Preclinical studies

Neural stem cell (NSC) transplantation has been proposed as a means to repair brain damage, and related brain repair has been shown in several preclinical models of neurologic disorders (Miller, 2006). Using rats with collagenase-induced ICH, Jeong et al. (2003) found that the intravenous transplantation of human NSCs, which differentiated into neurons and astrocytes, improved neurologic function. The same group examined the effects of systemic NSC transplantation on brain and spleen inflammatory reactions during the acute phase of ICH. They observed that early intravenous NSC injection had an important “bystander” anti-inflammatory effect on the spleen-macrophage system that promoted brain repair (Lee et al., 2008). Although stem cell therapy seems to be very promising, questions regarding NSC differentiation, migration, and integration as well as the optimal injection routes, cell doses, and timing of transplantation should be answered before any translation to the clinic can be considered.

6. Summary

Preclinical and clinical studies have provided evidence to indicate that various cellular and molecular components of inflammation are involved in hemorrhagic brain injury. As discussed, microglial activation after ICH occurs much earlier than neutrophil infiltration. Inflammation is now recognized as a key player in the pathologic progression of ICH and could affect ICH outcome. Considering the limitations of preclinical ICH models and numerous difficulties in translating experimental data to clinical use, human histopathologic studies are critically needed to confirm the findings from preclinical studies. Although it remains uncertain whether and when anti-inflammatory strategies might be successfully translated into clinical practice, additional strategies that target newly identified signaling pathways or molecules could offer a promising therapeutic approach to ICH.

Abbreviations

BBB	blood-brain barrier
GFAP	glial fibrillary acidic protein
HO	heme oxygenase
ICH	intracerebral hemorrhage
MIF	microglia/macrophage inhibitory factor
MIP	macrophage inflammatory protein
MMP	matrix metalloproteinase
Nrf2	nuclear factor erythroid 2-related factor 2
NSC	neural stem cell
ROS	reactive oxygen species
TNF	tumor necrosis factor
tPA	tissue plasminogen activator
WT	wild-type

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References

Abilleira S, Montaner J, Molina CA, Monasterio J, Castillo J, Alvarez-Sabin J. Matrix metalloproteinase-9 concentration after spontaneous intracerebral hemorrhage. J Neurosurg 2003;99:65–70. [PubMed: 12854746]

Alvarez-Sabin J, Delgado P, Abilleira S, Molina CA, Arenillas J, Ribo M, Santamarina E, Quintana M, Monasterio J, Montaner J. Temporal profile of matrix metalloproteinases and their inhibitors after spontaneous intracerebral hemorrhage: relationship to clinical and radiological outcome. Stroke 2004;35:1316–1322. [PubMed: 15087562]

Andaluz N, Zuccarello M, Wagner KR. Experimental animal models of intracerebral hemorrhage. Neurosurg Clin N Am 2002;13:385–393. [PubMed: 12486927]

Anderson MF, Blomstrand F, Blomstrand C, Eriksson PS, Nilsson M. Astrocytes and stroke: networking for survival? Neurochem Res 2003;28:293–305. [PubMed: 12608702]

Arima H, Wang JG, Huang Y, Heeley E, Skulina C, Parsons MW, Peng B, Li Q, Su S, Tao QL, Li YC, Jiang JD, Tai LW, Zhang JL, Xu E, Cheng Y, Morgenstern LB, Chalmers J, Anderson CS. Significance of perihematomal edemain acute intracerebral hemorrhage: the INTERACT trial. Neurology 2009;73:1963–1968. [PubMed: 19996072]

Aronowski J, Hall CE. New horizons for primary intracerebral hemorrhage treatment: experience from preclinical studies. Neurol Res 2005;27:268–279. [PubMed: 15845210]

Barr TL, Latour LL, Lee KY, Schaewe TJ, Luby M, Chang GS, El-Zammar Z, Alam S, Hallenbeck JM, Kidwell CS, Warach S. Blood-brain barrier disruption in humans is independently associated with increased matrix metalloproteinase-9. Stroke 2010;41:e123–e128. [PubMed: 20035078]

Beschorner R, Adjodah D, Schwab JM, Mittelbronn M, Pedal I, Mattern R, Schluesener HJ, Meyermann R. Long-term expression of heme oxygenase-1 (HO-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. Acta Neuropathol (Berl) 2000;100:377–384. [PubMed: 10985695]

Bestue-Cardiel M, Martin-Martinez J, Iturriaga-Heras C, Ara-Callizo JR, Oliveros-Juste A. Leukocytes and primary intracerebral hemorrhage. Rev Neurol 1999;29:968–971. [PubMed: 10637848]

Brahmachari S, Fung YK, Pahan K. Induction of glial fibrillary acidic protein expression in astrocytes by nitric oxide. J Neurosci 2006;26:4930–4939. [PubMed: 16672668]

Brott T, Broderick J, Kothari R, Barsan W, Tomsick T, Sauerbeck L, Spilker J, Duldner J, Khoury J. Early hemorrhage growth in patients with intracerebral hemorrhage. Stroke 1997;28:1–5. [PubMed: 8996478]

Carmichael ST, Vespa PM, Saver JL, Coppola G, Geschwind DH, Starkman S, Miller CM, Kidwell CS, Liebeskind DS, Martin NA. Genomic profiles of damage and protection in human intracerebral hemorrhage. J Cereb Blood Flow Metab 2008;28:1860–1875. [PubMed: 18628781]

Castellanos M, Leira R, Serena J, Pumar JM, Lizasoain I, Castillo J, Davalos A. Plasma metalloproteinase-9 concentration predicts hemorrhagic transformation in acute ischemic stroke. Stroke 2003;34:40–46. [PubMed: 12511748]

Castellanos M, Sobrino T, Millan M, Garcia M, Arenillas J, Nombela F, Brea D, Perez de la Ossa N, Serena J, Vivancos J, Castillo J, Davalos A. Serum cellular fibronectin and matrix metalloproteinase-9asscreening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: a multicenter confirmatory study. Stroke 2007;38:1855–1859. [PubMed: 17478737]

Castellazzi M, Tamborino C, De Santis G, Garofano F, Lupato A, Ramponi V, Trentini A, Casetta I, Bellini T, Fainardi E. Timing of serum active MMP-9 and MMP-2 levels in acute and subacute phases after spontaneous intracerebral hemorrhage. Acta Neurochir Suppl 2010;106:137–140. [PubMed: 19812936]

Chang EF, Wong RJ, Vreman HJ, Igarashi T, Galo E, Sharp FR, Stevenson DK, Noble-Haeusslein LJ. Heme oxygenase-2 protects against lipid peroxidation-mediated cell loss and impaired motor recovery after traumatic brain injury. J Neurosci 2003;23:3689–3696. [PubMed: 12736340]

Chen M, Regan RF. Time course of increased heme oxygenase activity and expression after experimental intracerebral hemorrhage: correlation with oxidative injury. J Neurochem 2007;103:2015–2021. [PubMed: 17760860]

Chen XL, Kunsch C. Induction of cytoprotective genes through Nrf2/ antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. Curr Pharm Des 2004;10:879–891. [PubMed: 15032691]

Chu K, Jeong SW, Jung KH, Han SY, Lee ST, Kim M, Roh JK. Celecoxib induces functional recovery after intracerebral hemorrhage with reduction of brain edema and perihematomal cell death. J Cereb Blood Flow Metab 2004;24:926–933. [PubMed: 15362723]

Cousar JL, Lai Y, Marco CD, Bayir H, Adelson PD, Janesko-Feldman KL, Kochanek PM, Clark RS. Heme oxygenase 1 in cerebrospinal fluid from infants and children after severe traumatic brain injury. Dev Neurosci 2006;28:342–347. [PubMed: 16943657]

Cunningham LA, Wetzel M, Rosenberg GA. Multiple roles for MMPs and TIMPs in cerebral ischemia. Glia 2005;50:329–339. [PubMed: 15846802]

de la Ossa NP, Sobrino T, Silva Y, Blanco M, Millan M, Gomis M, Agulla J, Araya P, Reverte S, Serena J, Davalos A. Iron-related brain damage in patients with intracerebral hemorrhage. Stroke 2010;41:810–813. [PubMed: 20185788]

Dinkel K, Dhabhar FS, Sapolsky RM. Neurotoxic effects of polymorphonuclear granulocytes on hippocampal primary cultures. Proc Natl Acad Sci U S A 2004;101:331–336. [PubMed: 14684829]

Donnan GA, Hankey GJ, Davis SM. Intracerebral haemorrhage: a need for more data and new research directions. Lancet Neurol 2010;9:133–134. [PubMed: 20056490]

Doré S, Goto S, Sampei K, Blackshaw S, Hester LD, Ingi T, Sawa A, Traystman RJ, Koehler RC, Snyder SH. Heme oxygenase-2 acts to prevent neuronal death in brain cultures and following transient cerebral ischemia. Neuroscience 2000;99:587–592. [PubMed: 10974422]

Doré S, Sampei K, Goto S, Alkayed NJ, Guastella D, Blackshaw S, Gallagher M, Traystman RJ, Hurn PD, Koehler RC, Snyder SH. Heme oxygenase-2 is neuroprotective in cerebral ischemia. Mol Med 1999;5:656–663. [PubMed: 10602774]

Doré S, Snyder SH. Neuroprotective action of bilirubin against oxidative stress in primary hippocampal cultures. Ann N Y Acad Sci 1999;890:167–172. [PubMed: 10668423]

Ducruet AF, Zacharia BE, Hickman ZL, Grobelny BT, Yeh ML, Sosunov SA, Connolly ES. The complement cascade as a therapeutic target in intracerebral hemorrhage. Exp Neurol 2009;219:398–403. [PubMed: 19632224]

Enlimomab Acute Stroke Trial Investigators. Use of anti-ICAM-1 therapy in ischemic stroke: results of the enlimomab acute stroke trial. Neurology 2001;57:1428–1434. [PubMed: 11673584]

Fan R, Xu F, Previti ML, Davis J, Grande AM, Robinson JK, Van Nostrand WE. Minocycline reduces microglial activation and improves behavioral deficits in a transgenic model of cerebral microvascular amyloid. J Neurosci 2007;27:3057–3063. [PubMed: 17376966]

Gao Z, Wang J, Thiex R, Rogove AD, Heppner FL, Tsirka SE. Microglial activation and intracerebral hemorrhage. Acta Neurochir Suppl 2008;105:51–53. [PubMed: 19066082]

Garrett MC, Otten ML, Starke RM, Komotar RJ, Magotti P, Lambris JD, Rynkowski MA, Connolly ES. Synergistic neuroprotective effects of C3a and C5a receptor blockade following intracerebral hemorrhage. Brain Res 2009;1298:171–177. [PubMed: 19410563]

Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N. Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 2010;11:87–99. [PubMed: 20087359]

Gong C, Hoff JT, Keep RF. Acute inflammatory reaction following experimental intracerebral hemorrhage in rat. Brain Res 2000;871:57–65. [PubMed: 10882783]

Gong Y, Hua Y, Keep RF, Hoff JT, Xi G. Intracerebral hemorrhage: effects of aging on brain edema and neurological deficits. Stroke 2004;35:2571–2575. [PubMed: 15472083]

Gong Y, Tian H, Xi G, Keep RF, Hoff JT, Hua Y. Systemic zinc protoporphyrin administration reduces intracerebral hemorrhage-induced brain injury. Acta Neurochir 2006;96 Suppl:232–236.

Goto S, Sampei K, Alkayed NJ, Doré S, Koehler RC. Characterization of a new double-filament model of focal cerebral ischemia in heme oxygenase-2-deficient mice. Am J Physiol Regul Integr Comp Physiol 2003;285:R222–R230. [PubMed: 12663258]

Grossetete M, Rosenberg GA. Matrix metalloproteinase inhibition facilitates cell death in intracerebral hemorrhage in mouse. J Cereb Blood Flow Metab 2008;28:752–763. [PubMed: 17971790]

Gu Y, Hua Y, Keep RF, Morgenstern LB, Xi G. Deferoxamine reduces intracerebral hematoma-induced iron accumulation and neuronal death in piglets. Stroke 2009;40:2241–2243. [PubMed: 19372448]

Gueders MM, Foidart JM, Noel A, Cataldo DD. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: potential implications in asthma and other lung diseases. Eur J Pharmacol 2006;533:133–144. [PubMed: 16487964]

Guo FQ, Li XJ, Chen LY, Yang H, Dai HY, Wei YS, Huang YL, Yang YS, Sun HB, Xu YC, Yang ZL. Study of relationship between inflammatory response and apoptosis in perihematoma region in patients with intracerebral hemorrhage. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2006;18:290–293. [PubMed: 16700995]

Gutteridge JM. Hydroxyl radicals, iron, oxidative stress, and neurodegeneration. Ann N Y Acad Sci 1994;738:201–213. [PubMed: 7832429]

Hua Y, Nakamura T, Keep RF, Wu J, Schallert T, Hoff JT, Xi G. Long-term effects of experimental intracerebral hemorrhage: the role of iron. J Neurosurg 2006;104:305–312. [PubMed: 16509506]

Huang FP, Xi G, Keep RF, Hua Y, Nemoianu A, Hoff JT. Brain edema after experimental intracerebral hemorrhage: role of hemoglobin degradation products. J Neurosurg 2002;96:287–293. [PubMed: 11838803]

Iida S, Baumbach GL, Lavoie JL, Faraci FM, Sigmund CD, Heistad DD. Spontaneous stroke in a genetic model of hypertension in mice. Stroke 2005;36:1253–1258. [PubMed: 15914769]

Jakel RJ, Townsend JA, Kraft AD, Johnson JA. Nrf2-mediated protection against 6-hydroxydopamine. Brain Res 2007;1144:192–201. [PubMed: 17336276]

Jeong SW, Chu K, Jung KH, Kim SU, Kim M, Roh JK. Human neural stem cell transplantation promotes functional recovery in rats with experimental intracerebral hemorrhage. Stroke 2003;34:2258–2263. [PubMed: 12881607]

Johnson JA, Johnson DA, Kraft AD, Calkins MJ, Jakel RJ, Vargas MR, Chen PC. The Nrf2-ARE pathway: an indicator and modulator of oxidative stress in neurodegeneration. Ann N Y Acad Sci 2008;1147:61–69. [PubMed: 19076431]

Joice SL, Mydeen F, Couraud PO, Weksler BB, Romero IA, Fraser PA, Easton AS. Modulation of blood-brain barrier permeability by neutrophils: in vitro and in vivo studies. Brain Res 2009;1298:13–23. [PubMed: 19728990]

Kane PJ, Modha P, Strachan RD, Cook S, Chambers IR, Clayton CB, Mendelow AD. The effect of immunosuppression on the development of cerebral oedema in an experimental model of intracerebral haemorrhage: whole body and regional irradiation. J Neurol Neurosurg Psychiatr 1992;55:781–786. [PubMed: 1402968]

Kazui S, Naritomi H, Yamamoto H, Sawada T, Yamaguchi T. Enlargement of spontaneous intracerebral hemorrhage: incidence and time course. Stroke 1996;27:1783–1787. [PubMed: 8841330]

Keep RF, Xi G, Hua Y, Hoff JT. The deleterious or beneficial effects of different agents in intracerebral hemorrhage: think big, think small, or is hematoma size important? Stroke 2005;36:1594–1596. [PubMed: 15933250]

Koeppen AH, Dickson AC, McEvoy JA. The cellular reactions to experimental intracerebral hemorrhage. J Neurol Sci 1995;134 Suppl:102–112. [PubMed: 8847540]

Koeppen AH, Dickson AC, Smith J. Heme oxygenase in experimental intracerebral hemorrhage: the benefit of tin-mesoporphyrin. J Neuropathol Exp Neurol 2004;63:587–597. [PubMed: 15217087]

Lapchak PA, Chapman DF, Zivin JA. Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced hemorrhage after thromboembolic stroke. Stroke 2000;31:3034–3040. [PubMed: 11108768]

Lee JC, Cho GS, Choi BO, Kim HC, Kim WK. Aging exacerbates intracerebral hemorrhage-induced brain injury. J Neurotrauma 2009;26:1567–1576. [PubMed: 19473060]

Lee JM, Li J, Johnson DA, Stein TD, Kraft AD, Calkins MJ, Jakel RJ, Johnson JA. Nrf2, a multi-organ protector? Faseb J 2005;19:1061–1066. [PubMed: 15985529]

Lee JM, Yin KJ, Hsin I, Chen S, Fryer JD, Holtzman DM, Hsu CY, Xu J. Matrix metalloproteinase-9 and spontaneous hemorrhage in an animal model of cerebral amyloid angiopathy. Ann Neurol 2003;54:379–382. [PubMed: 12953271]

Lee ST, Chu K, Jung KH, Kim SJ, Kim DH, Kang KM, Hong NH, Kim JH, Ban JJ, Park HK, Kim SU, Park CG, Lee SK, Kim M, Roh JK. Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. Brain 2008;131:616–629. [PubMed: 18156155]

Leira R, Davalos A, Silva Y, Gil-Peralta A, Tejada J, Garcia M, Castillo J. Early neurologic deterioration in intracerebral hemorrhage: predictors and associated factors. Neurology 2004;63:461–467. [PubMed: 15304576]

Lindsberg PJ, Strbian D, Karjalainen-Lindsberg ML. Mast cells as early responders in the regulation of acute blood-brain barrier changes after cerebral ischemia and hemorrhage. J Cereb Blood Flow Metab 2010;30:689–702. [PubMed: 20087366]

Lou M, Lieb K, Selim M. The relationship between hematoma iron content and perihematoma edema: an MRI study. Cerebrovasc Dis 2009;27:266–271. [PubMed: 19202331]

Lu A, Tang Y, Ran R, Ardizzone TL, Wagner KR, Sharp FR. Brain genomics of intracerebral hemorrhage. J Cereb Blood Flow Metab 2006;26:230–252. [PubMed: 16034371]

Lyden PD, Shuaib A, Lees KR, Davalos A, Davis SM, Diener HC, Grotta JC, Ashwood TJ, Hardemark HG, Svensson HH, Rodichok L, Wasiewski WW, Ahlberg G. Safety and tolerability of NXY-059 for acute intracerebral hemorrhage: the CHANT trial. Stroke 2007;38:2262–2269. [PubMed: 17569876]

MacKenzie JM, Clayton JA. Early cellular events in the penumbra of human spontaneous intracerebral hemorrhage. J Stroke Cerebrovasc Dis 1999;8:1–8. [PubMed: 17895130]

MacLellan CL, Clark DL, Silasi G, Colbourne F. Use of prolonged hypothermia to treat ischemic and hemorrhagic stroke. J Neurotrauma 2009;26:313–323. [PubMed: 19216634]

Masada T, Hua Y, Xi G, Yang GY, Hoff JT, Keep RF. Attenuation of intracerebral hemorrhage and thrombin-induced brain edema by overexpression of interleukin-1 receptor antagonist. J Neurosurg 2001;95:680–686. [PubMed: 11596963]

Masada T, Hua Y, Xi G, Yang GY, Hoff JT, Keep RF, Nagao S. Over-expression of interleukin-1 receptor antagonist reduces brain edema induced by intracerebral hemorrhage and thrombin. Acta Neurochir 2003;86 Suppl:463–467.

Matsushita K, Meng W, Wang X, Asahi M, Asahi K, Moskowitz MA, Lo EH. Evidence for apoptosis after intercerebral hemorrhage in rat striatum. J Cereb Blood Flow Metab 2000;20:396–404. [PubMed: 10698078]

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 1997;40:152–160. discussion 160–2. [PubMed: 8971837]

Mayer SA, Rincon F. Treatment of intracerebral haemorrhage. Lancet Neurol 2005;4:662–672. [PubMed: 16168935]

Mayne M, Fotheringham J, Yan HJ, Power C, Del Bigio MR, Peeling J, Geiger JD. Adenosine A2A receptor activation reduces proinflammatory events and decreases cell death following intracerebral hemorrhage. Ann Neurol 2001a;49:727–735. [PubMed: 11409424]

Mayne M, Ni W, Yan HJ, Xue M, Johnston JB, Del Bigio MR, Peeling J, Power C. Antisense oligodeoxynucleotide inhibition of tumor necrosis factor-alpha expression is neuroprotective after intracerebral hemorrhage. Stroke 2001b;32:240–248. [PubMed: 11136943]

Mehdiratta M, Kumar S, Hackney D, Schlaug G, Selim M. Association between serum ferritin level and perihematoma edema volume in patients with spontaneous intracerebral hemorrhage. Stroke 2008;39:1165–1170. [PubMed: 18292378]

Miao J, Xu F, Davis J, Otte-Holler I, Verbeek MM, Van Nostrand WE. Cerebral microvascular amyloid beta protein deposition induces vascular degeneration and neuroinflammation in transgenic mice expressing human vasculotropic mutant amyloid beta precursor protein. Am J Pathol 2005;167:505–515. [PubMed: 16049335]

Miller G. Neuroscience. The dark side of glia. Science 2005;308:778–781. [PubMed: 15879185]

Miller RH. The promise of stem cells for neural repair. Brain Res 2006;1091:258–264. [PubMed: 16563359]

Min KJ, Yang MS, Kim SU, Jou I, Joe EH. Astrocytes induce hemeoxygenase-1 expression in microglia: a feasible mechanism for preventing excessive brain inflammation. J Neurosci 2006;26:1880–1887. [PubMed: 16467537]

Montaner J, Alvarez-Sabin J, Molina C, Angles A, Abilleira S, Arenillas J, Gonzalez MA, Monasterio J. Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 2001a;32:1759–1766. [PubMed: 11486102]

Montaner J, Alvarez-Sabin J, Molina CA, Angles A, Abilleira S, Arenillas J, Monasterio J. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. Stroke 2001b;32:2762–2767. [PubMed: 11739970]

Montaner J, Molina CA, Monasterio J, Abilleira S, Arenillas JF, Ribo M, Quintana M, Alvarez-Sabin J. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. Circulation 2003;107:598–603. [PubMed: 12566373]

Mun-Bryce S, Wilkerson A, Pacheco B, Zhang T, Rai S, Wang Y, Okada Y. Depressed cortical excitability and elevated matrix metalloproteinases in remote brain regions following intracerebral hemorrhage. Brain Res 2004;1026:227–234. [PubMed: 15488484]

Nakajima K, Honda S, Tohyama Y, Imai Y, Kohsaka S, Kurihara T. Neurotrophin secretion from cultured microglia. J Neurosci Res 2001;65:322–331. [PubMed: 11494368]

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 2004a; 100:672–678. [PubMed: 15070122]

Nakamura T, Xi G, Hua Y, Schallert T, Hoff JT, Keep RF. Intracerebral hemorrhage in mice: model characterization and application for genetically modified mice. J Cereb Blood Flow Metab 2004b; 24:487–494. [PubMed: 15129180]

Nakaso K, Kitayama M, Mizuta E, Fukuda H, Ishii T, Nakashima K, Yamada K. Co-induction of heme oxygenase-1 and peroxiredoxin I in astrocytes and microglia around hemorrhagic region in the rat brain. Neurosci Lett 2000;293:49–52. [PubMed: 11065135]

Nguyen HX, O'Barr TJ, Anderson AJ. Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. J Neurochem 2007;102:900–912. [PubMed: 17561941]

Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem 2009;284:13291–13295. [PubMed: 19182219]

Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005;308:1314–1318. [PubMed: 15831717]

NINDS ICH Workshop Participants. Priorities for clinical research in intracerebral hemorrhage: report from a National Institute of Neurological Disorders and Stroke workshop. Stroke 2005;36:e23–e41. [PubMed: 15692109]

Ning M, Furie KL, Koroshetz WJ, Lee H, Barron M, Lederer M, Wang X, Zhu M, Sorensen AG, Lo EH, Kelly PJ. Association between tPA therapy and raised early matrix metalloproteinase-9 in acute stroke. Neurology 2006;66:1550–1555. [PubMed: 16717217]

Okauchi M, Hua Y, Keep RF, Morgenstern LB, Xi G. Effects of deferoxamine on intracerebral hemorrhage-induced brain injury in aged rats. Stroke 2009;40:1858–1863. [PubMed: 19286595]

Parfenova H, Leffler CW. Cerebroprotective functions of HO-2. Curr Pharm Des 2008;14:443–453. [PubMed: 18289071]

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 2001a;40:433–439. [PubMed: 11166336]

Peeling J, Yan HJ, Chen SG, Campbell M, Del Bigio MR. Protective effects of free radical inhibitors in intracerebral hemorrhage in rat. Brain Res 1998;795:63–70. [PubMed: 9622595]

Peeling J, Yan HJ, Corbett D, Xue M, Del Bigio MR. Effect of FK-506 on inflammation and behavioral outcome following intracerebral hemorrhage in rat. Exp Neurol 2001b;167:341–347. [PubMed: 11161622]

Petzold A, Keir G, Kerr M, Kay A, Kitchen N, Smith M, Thompson EJ. Early identification of secondary brain damage in subarachnoid hemorrhage: a role for glial fibrillary acidic protein. J Neurotrauma 2006;23:1179–1184. [PubMed: 16866629]

Pfefferkorn T, Rosenberg GA. Closure of the blood-brain barrier by matrix metalloproteinase inhibition reduces rtPA-mediated mortality in cerebral ischemia with delayed reperfusion. Stroke 2003;34:2025–2030. [PubMed: 12855824]

Power C, Henry S, Del Bigio MR, Larsen PH, Corbett D, Imai Y, Yong VW, Peeling J. Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. Ann Neurol 2003;53:731–742. [PubMed: 12783419]

Pyo H, Yang MS, Jou I, Joe EH. Wortmannin enhances lipopolysaccharide-induced inducible nitric oxide synthase expression in microglia in the presence of astrocytes in rats. Neurosci Lett 2003;346:141–144. [PubMed: 12853104]

Qu Y, Chen-Roetling J, Benvenisti-Zarom L, Regan RF. Attenuation of oxidative injury after induction of experimental intracerebral hemorrhage in heme oxygenase-2 knockout mice. J Neurosurg 2007;106:428–435. [PubMed: 17367065]

Qureshi AI, Mendelow AD, Hanley DF. Intracerebral haemorrhage. Lancet 2009;373:1632–1644. [PubMed: 19427958]

Qureshi AI, Suri MF, Ling GS, Khan J, Guterman LR, Hopkins LN. Absence of early proinflammatory cytokine expression in experimental intracerebral hemorrhage. Neurosurgery 2001;49:416–420. discussion 421. [PubMed: 11504118]

Ribo M, Grotta JC. Latest advances in intracerebral hemorrhage. Curr Neurol Neurosci Rep 2006;6:17–22. [PubMed: 16469266]

Rosell A, Ortega-Aznar A, Alvarez-Sabin J, Fernandez-Cadenas I, Ribo M, Molina CA, Lo EH, Montaner J. Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. Stroke 2006;37:1399–1406. [PubMed: 16690896]

Rosenberg GA, Cunningham LA, Wallace J, Alexander S, Estrada EY, Grossetete M, Razhagi A, Miller K, Gearing A. Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures. Brain Res 2001;893:104–112. [PubMed: 11222998]

Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. Stroke 1990;21:801–807. [PubMed: 2160142]

Rosenberg GA, Navratil M. Metalloproteinase inhibition blocks edema in intracerebral hemorrhage in the rat. Neurology 1997;48:921–926. [PubMed: 9109878]

Rynkowski MA, Kim GH, Garrett MC, Zacharia BE, Otten ML, Sosunov SA, Komotar RJ, Hassid BG, Ducruet AF, Lambris JD, Connolly ES. C3a receptor antagonist attenuates brain injury after intracerebral hemorrhage. J Cereb Blood Flow Metab 2009;29:98–107. [PubMed: 18728680]

Rynkowski MA, Kim GH, Komotar RJ, Otten ML, Ducruet AF, Zacharia BE, Kellner CP, Hahn DK, Merkow MB, Garrett MC, Starke RM, Cho BM, Sosunov SA, Connolly ES. A mouse model of intracerebral hemorrhage using autologous blood infusion. Nat Protoc 2008;3:122–128. [PubMed: 18193028]

Ryter SW, Tyrrell RM. The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. Free Radic Biol Med 2000;28:289–309. [PubMed: 11281297]

Savill, J.; Haslett, C. Resolution of inflammation. In: Ley, K., editor. Physiology of Inflammation. New York: Oxford University Press; 2001. p. 496-525.

Savman K, Nilsson UA, Blennow M, Kjellmer I, Whitelaw A. Non-protein-bound iron is elevated in cerebrospinal fluid from preterm infants with post-hemorrhagic ventricular dilatation. Pediatr Res 2001;49:208–212. [PubMed: 11158515]

Shah ZA, Li RC, Thimmulappa RK, Kensler TW, Yamamoto M, Biswal S, Dore S. Role of reactive oxygen species in modulation of Nrf2 following ischemic reperfusion injury. Neuroscience 2007;147:53–59. [PubMed: 17507167]

Shah ZA, Namiranian K, Klaus J, Kibler K, Dore S. Use of an optimized transient occlusion of the middle cerebral artery protocol for the mouse stroke model. J Stroke Cerebrovasc Dis 2006;15:133–138. [PubMed: 17904065]

Shih AY, Li P, Murphy TH. A small-molecule-inducible Nrf2-mediated antioxidant response provides effective prophylaxis against cerebral ischemia in vivo. J Neurosci 2005;25:10321–10335. [PubMed: 16267240]

Silva Y, Leira R, Tejada J, Lainez JM, Castillo J, Davalos A. Molecular signatures of vascular injury are associated with early growth of intracerebral hemorrhage. Stroke 2005;36:86–91. [PubMed: 15550687]

Song S, Hua Y, Keep RF, Hoff JT, Xi G. A new hippocampal model for examining intracerebral hemorrhage-related neuronal death: effects of deferoxamine on hemoglobin-induced neuronal death. Stroke 2007;38:2861–2863. [PubMed: 17761912]

Strbian D, Durukan A, Tatlisumak T. Rodent models of hemorrhagic stroke. Curr Pharm Des 2008;14:352–358. [PubMed: 18289061]

Strbian D, Tatlisumak T, Ramadan UA, Lindsberg PJ. Mast cell blocking reduces brain edema and hematoma volume and improves outcome after experimental intracerebral hemorrhage. J Cereb Blood Flow Metab 2007;27:795–802. [PubMed: 16926842]

Sutherland GR, Auer RN. Primary intracerebral hemorrhage. J Clin Neurosci 2006;13:511–517. [PubMed: 16769513]

Suttner DM, Dennery PA. Reversal of HO-1 related cytoprotection with increased expression is due to reactive iron. Faseb J 1999;13:1800–1809. [PubMed: 10506583]

Szymanska A, Biernaskie J, Laidley D, Granter-Button S, Corbett D. Minocycline and intracerebral hemorrhage: influence of injury severity and delay to treatment. Exp Neurol 2006;197:189–196. [PubMed: 16259983]

Takahashi T, Morita K, Akagi R, Sassa S. Heme oxygenase-1: a novel therapeutic target in oxidative tissue injuries. Curr Med Chem 2004;11:1545–1561. [PubMed: 15180563]

Tang J, Liu J, Zhou C, Alexander JS, Nanda A, Granger DN, Zhang JH. Mmp-9 deficiency enhances collagenase-induced intracerebral hemorrhage and brain injury in mutant mice. J Cereb Blood Flow Metab 2004;24:1133–1145. [PubMed: 15529013]

Tejima E, Zhao BQ, Tsuji K, Rosell A, van Leyen K, Gonzalez RG, Montaner J, Wang X, Lo EH. Astrocytic induction of matrix metalloproteinase-9 and edema in brain hemorrhage. J Cereb Blood Flow Metab 2007;27:460–468. [PubMed: 16788715]

Teng W, Wang L, Xue W, Guan C. Activation of TLR4-mediated NFkappaB signaling in hemorrhagic brain in rats. Mediators Inflamm 2009:473276. [PubMed: 20150961]

Thompson KJ, Shoham S, Connor JR. Iron and neurodegenerative disorders. Brain Res Bull 2001;55:155–164. [PubMed: 11470311]

Titova E, Ostrowski RP, Kevil CG, Tong W, Rojas H, Sowers LC, Zhang JH, Tang J. Reduced brain injury in CD18-deficient mice after experimental intracerebral hemorrhage. J Neurosci Res 2008;86:3240–3245. [PubMed: 18615643]

van Rossum D, Hanisch UK. Microglia. Metab Brain Dis 2004;19:393–411. [PubMed: 15554430]

Wagner KR. Modeling intracerebral hemorrhage: glutamate, nuclear factor-kappa B signaling and cytokines. Stroke 2007;38:753–758. [PubMed: 17261732]

Wagner KR, Hua Y, de Courten-Myers GM, Broderick JP, Nishimura RN, Lu SY, Dwyer BE. Tin-mesoporphyrin, a potent heme oxygenase inhibitor, for treatment of intracerebral hemorrhage: in vivo and in vitro studies. Cell Mol Biol (Noisy-le-grand) 2000;46:597–608. [PubMed: 10872746]

Wagner KR, Packard BA, Hall CL, Smulian AG, Linke MJ, De Courten-Myers GM, Packard LM, Hall NC. Protein oxidation and heme oxygenase-1 induction in porcine white matter following intracerebral infusions of whole blood or plasma. Dev Neurosci 2002;24:154–160. [PubMed: 12401953]

Wagner KR, Sharp FR, Ardizzone TD, Lu A, Clark JF. Heme and iron metabolism: role in cerebral hemorrhage. J Cereb Blood Flow Metab 2003;23:629–652. [PubMed: 12796711]

Wakisaka Y, Chu Y, Miller JD, Rosenberg GA, Heistad DD. Spontaneous intracerebral hemorrhage during acute and chronic hypertension in mice. J Cereb Blood Flow Metab 2010;30:56–69. [PubMed: 19724290]

Wang J, Dore S. Heme oxygenase 2 deficiency increases brain swelling and inflammation after intracerebral hemorrhage. Neuroscience 2008;155:1133–1141. [PubMed: 18674596]

Wang J, Dore S. Heme oxygenase-1 exacerbates early brain injury after intracerebral haemorrhage. Brain 2007a;130:1643–1652. [PubMed: 17525142]

Wang J, Doré S. Inflammation after intracerebral hemorrhage. J Cereb Blood Flow Metab 2007b; 27:894–908. [PubMed: 17033693]

Wang J, Fields J, Dore S. The development of an improved preclinical mouse model of intracerebral hemorrhage using double infusion of autologous whole blood. Brain Res 2008;1222:214–221. [PubMed: 18586227]

Wang J, Fields J, Zhao C, Langer J, Thimmulappa RK, Kensler TW, Yamamoto M, Biswal S, Dore S. Role of Nrf2 in protection against intracerebral hemorrhage injury in mice. Free Radic Biol Med 2007;43:408–414. [PubMed: 17602956]

Wang J, Rogove AD, Tsirka AE, Tsirka SE. Protective role of tuftsin fragment 1–3 in an animal model of intracerebral hemorrhage. Ann Neurol 2003;54:655–664. [PubMed: 14595655]

Wang J, Tsirka SE. Contribution of extracellular proteolysis and microglia to intracerebral hemorrhage. Neurocrit Care 2005a;3:77–85. [PubMed: 16159103]

Wang J, Tsirka SE. Neuroprotection by inhibition of matrix metalloproteinases in a mouse model of intracerebral haemorrhage. Brain 2005b;128:1622–1633. [PubMed: 15800021]

Wang J, Tsirka SE. Tuftsin fragment 1–3 is beneficial when delivered after the induction of intracerebral hemorrhage. Stroke 2005c;36:613–618. [PubMed: 15692122]

Wang J, Zhuang H, Doré S. Heme oxygenase 2 is neuroprotective against intracerebral hemorrhage. Neurobiol Dis 2006;22:473–476. [PubMed: 16459095]

Warkentin LM, Auriat AM, Wowk S, Colbourne F. Failure of deferoxamine, an iron chelator, to improve outcome after collagenase-induced intracerebral hemorrhage in rats. Brain Res 2010;1309:95–103. [PubMed: 19879860]

Wasserman JK, Schlichter LC. Minocycline protects the blood-brain barrier and reduces edema following intracerebral hemorrhage in the rat. Exp Neurol 2007a;207:227–237. [PubMed: 17698063]

Wasserman JK, Schlichter LC. Neuron death and inflammation in a rat model of intracerebral hemorrhage: effects of delayed minocycline treatment. Brain Res 2007b;1136:208–218. [PubMed: 17223087]

Wasserman JK, Zhu X, Schlichter LC. Evolution of the inflammatory response in the brain following intracerebral hemorrhage and effects of delayed minocycline treatment. Brain Res 2007;1180:140–154. [PubMed: 17919462]

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 2005;21:187–196. [PubMed: 15654856]

Wisniewski H. The pathogenesis of some cases of cerebral hemorrhage (a morphologic study of the margins of hemorrhagic foci and areas of the brain distant form the hemorrhage). Acta Med Pol 1961;2:379–390. [PubMed: 14007694]

Wu H, Cong Y, Wang D, Zhao R, Qi J. Correlation of macrophage inflammatory protein-2 expression and brain edema in rats after intracerebral hemorrhage. Int J Clin Exp Pathol 2009a;2:83–90. [PubMed: 18830379]

Wu H, Zhang Z, Hu X, Zhao R, Song Y, Ban X, Qi J, Wang J. Dynamic changes of inflammatory markers in brain after hemorrhagic stroke in humans: a postmortem study. Brain Res 2010a; 1342:111–117. [PubMed: 20420814]

Wu H, Zhang Z, Li Y, Zhao R, Li H, Song Y, Qi J, Wang J. Time course of upregulation of inflammatory mediators in the hemorrhagic brain in rats: correlation with brain edema. Neurochem Int 2010b;57:248–253. [PubMed: 20541575]

Wu H, Zhao R, Qi J, Cong Y, Wang D, Liu T, Gu Y, Ban X, Huang Q. The expression and the role of protease nexin-1 on brain edema after intracerebral hemorrhage. J Neurol Sci 2008;270:172–183. [PubMed: 18442833]

Wu J, Hua Y, Keep RF, Nakamura T, Hoff JT, Xi G. Iron and iron-handling proteins in the brain after intracerebral hemorrhage. Stroke 2003;34:2964–2969. [PubMed: 14615611]

Wu J, Yang S, Xi G, Fu G, Keep RF, Hua Y. Minocycline reduces intracerebral hemorrhage-induced brain injury. Neurol Res 2009b;31:183–188. [PubMed: 19061541]

Xi G, Keep RF, Hoff JT. Mechanisms of brain injury after intracerebral haemorrhage. Lancet Neurol 2006;5:53–63. [PubMed: 16361023]

Xue M, Del Bigio MR. Intracerebral injection of autologous whole blood in rats: time course of inflammation and cell death. Neurosci Lett 2000;283:230–232. [PubMed: 10754230]

Xue M, Fan Y, Liu S, Zygun DA, Demchuk A, Yong VW. Contributions of multiple proteases to neurotoxicity in a mouse model of intracerebral haemorrhage. Brain 2009a;132:26–36. [PubMed: 18772219]

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 2009b;40:2199–2204. [PubMed: 19359644]

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 2006;26:10281–10291. [PubMed: 17021183]

Xue M, Mikliaeva EI, Casha S, Zygun D, Demchuk A, Yong VW. Improving outcomes of neuroprotection by minocycline. guides from cell culture and intracerebral hemorrhage in mice. Am J Pathol 2010;176:1193–1202. [PubMed: 20110416]

Xue M, Yong VW. Matrix metalloproteinases in intracerebral hemorrhage. Neurol Res 2008;30:775–782. [PubMed: 18826803]

Yong VW. Metalloproteinases: mediators of pathology and regeneration in the CNS. Nat Rev Neurosci 2005;6:931–944. [PubMed: 16288297]

Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM. The promise of minocycline in neurology. Lancet Neurol 2004;3:744–751. [PubMed: 15556807]

Yong WH, Robert ME, Secor DL, Kleikamp TJ, Vinters HV. Cerebral hemorrhage with biopsy-proved amyloid angiopathy. Arch Neurol 1992;49:51–58. [PubMed: 1728264]

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR. Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 2004;5:863–873. [PubMed: 15496864]

Zhang D, Hu X, Qian L, Wilson B, Lee C, Flood P, Langenbach R, Hong JS. Prostaglandin E2 released from activated microglia enhances astrocyte proliferation in vitro. Toxicol Appl Pharmacol 2009;238:64–70. [PubMed: 19397918]

Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, Wang X, Lo EH. Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 2006a;12:441–445. [PubMed: 16565723]

Zhao J, Kobori N, Aronowski J, Dash PK. Sulforaphane reduces infarct volume following focal cerebral ischemia in rodents. Neurosci Lett 2006b;393:108–112. [PubMed: 16233958]

Zhao X, Sun G, Zhang J, Strong R, Dash PK, Kan YW, Grotta JC, Aronowski J. Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. Stroke 2007a; 38:3280–3286. [PubMed: 17962605]

Zhao X, Sun G, Zhang J, Strong R, Song W, Gonzales N, Grotta JC, Aronowski J. Hematoma resolution as a target for intracerebral hemorrhage treatment: Role for peroxisome proliferator-activated receptor gamma in microglia/macrophages. Ann Neurol 2007b;61:352–362. [PubMed: 17457822]

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 2006c;26:811–820. [PubMed: 16208315]

Zheng Z, Yenari MA. Post-ischemic inflammation: molecular mechanisms and therapeutic implications. Neurol Res 2004;26:884–892. [PubMed: 15727272]

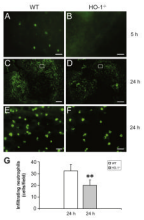

Fig. 1.

Effect of HO-1 on leukocyte infiltration after ICH. Infiltrating neutrophils (myeloperoxidase-positive cells) were apparent in the injury site 5 h post-ICH in WT mice (A), but not in HO-1$^{-/-}$ mice (B). At 24 h post-ICH, many more infiltrating neutrophils were present in and around the injury site in WT mice (C, E) than in HO-1$^{-/-}$ mice (D, F). The images in E and F represent higher magnification of the boxed area in C and D. (G) Quantification analysis indicated that HO-1$^{-/-}$ mice had significantly fewer infiltrating neutrophils than did WT mice at 24 h post-ICH (n = 5/group, $^{**}p < 0.01$). Scale bar, A, B, E, F, 20 μm; (C, D) 300 μm. From Wang and Doré (2007a).

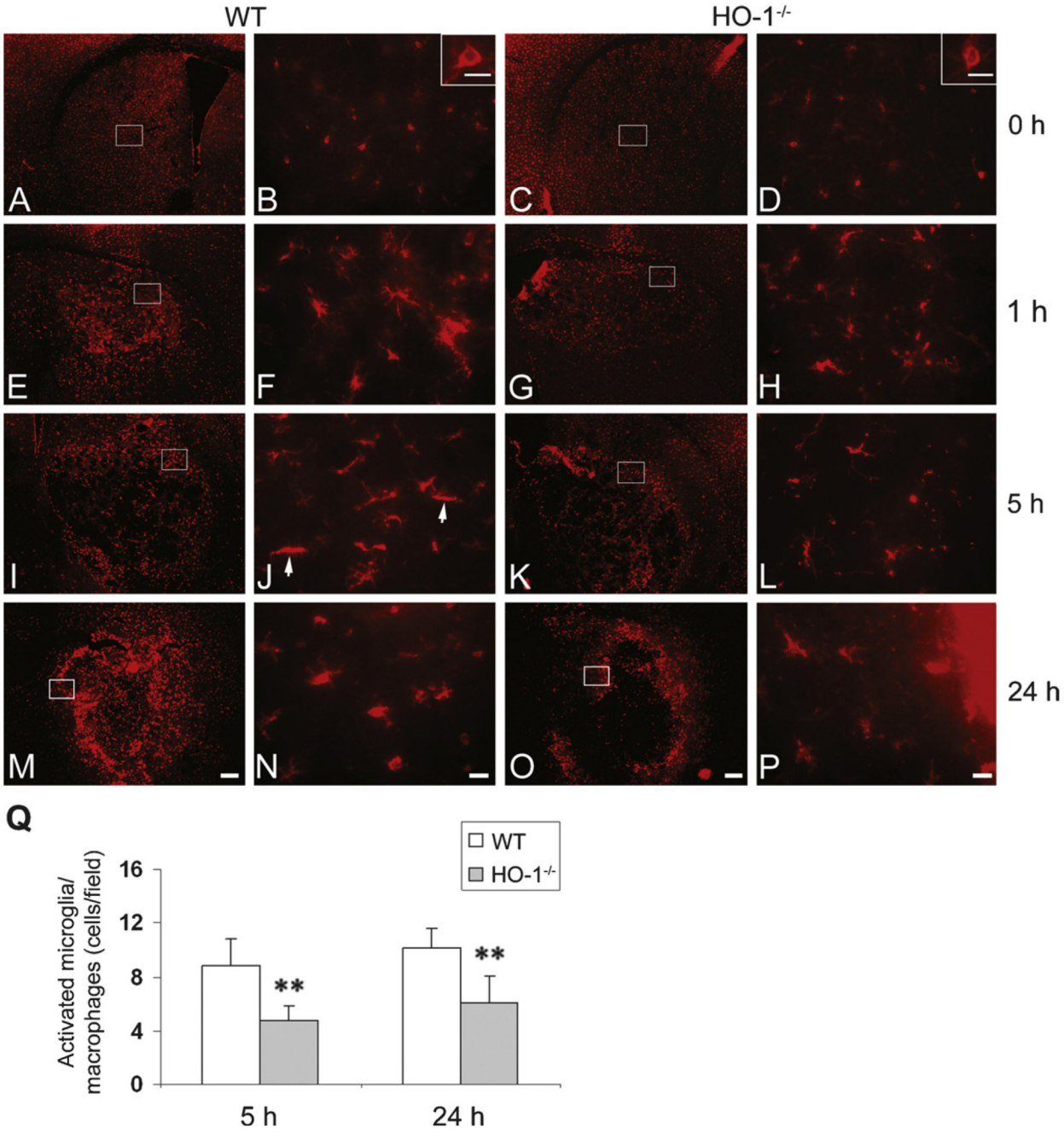

Fig. 2.

Effect of HO-1 on microglial/macrophage activation after ICH. The distribution and morphology of microglia/macrophages (Iba1-positive) are shown in coronal sections collected at different time-points in WT (A, B, E, F, I,J, M, N) and HO-$1^{-/-}$ (C, D, G, H,K, L, O, P) mice. (A–D) Images shown at 0 h are from sham-operated mice. The images in B, F, J, N, D, H, L, P (scale bar: 20 μm) represent higher magnification of the boxed areas in A, E, I, M, C, G, K, and O (scale bar: 200 μm), respectively. In sham-operated WT (A, B) and HO-$1^{-/-}$ (C, D) mice, resting microglial cells were sparsely distributed. Insets in B and D (scale bar: 5 μm) illustrate Iba1-positive resting microglial cells at higher magnification. Microglial activation appeared as early as 1 h after ICH in WT (E, F) and HO-$1^{-/-}$ (G, H)

mice, but more intensely stained, activated cells (with large cell bodies and short processes) were observed in and around the ICH region in WT mice. This tendency persisted at 5 h (I–L) and up to 24 h (M–P) after ICH. (J) In a WT section 5 h post-ICH, two typical activated microglia/macrophages (elongated, rod cells) are indicated by arrows. (Q) Quantification of activated microglia/macrophages around the border region of injury. HO-$1^{-/-}$ mice had significantly fewer activated microglia/macrophages than did WT mice at 5 and 24 h post-ICH (n = 5/group, $^{**}p < 0.01$). Values represent means ± SD. From Wang and Doré (2007a).

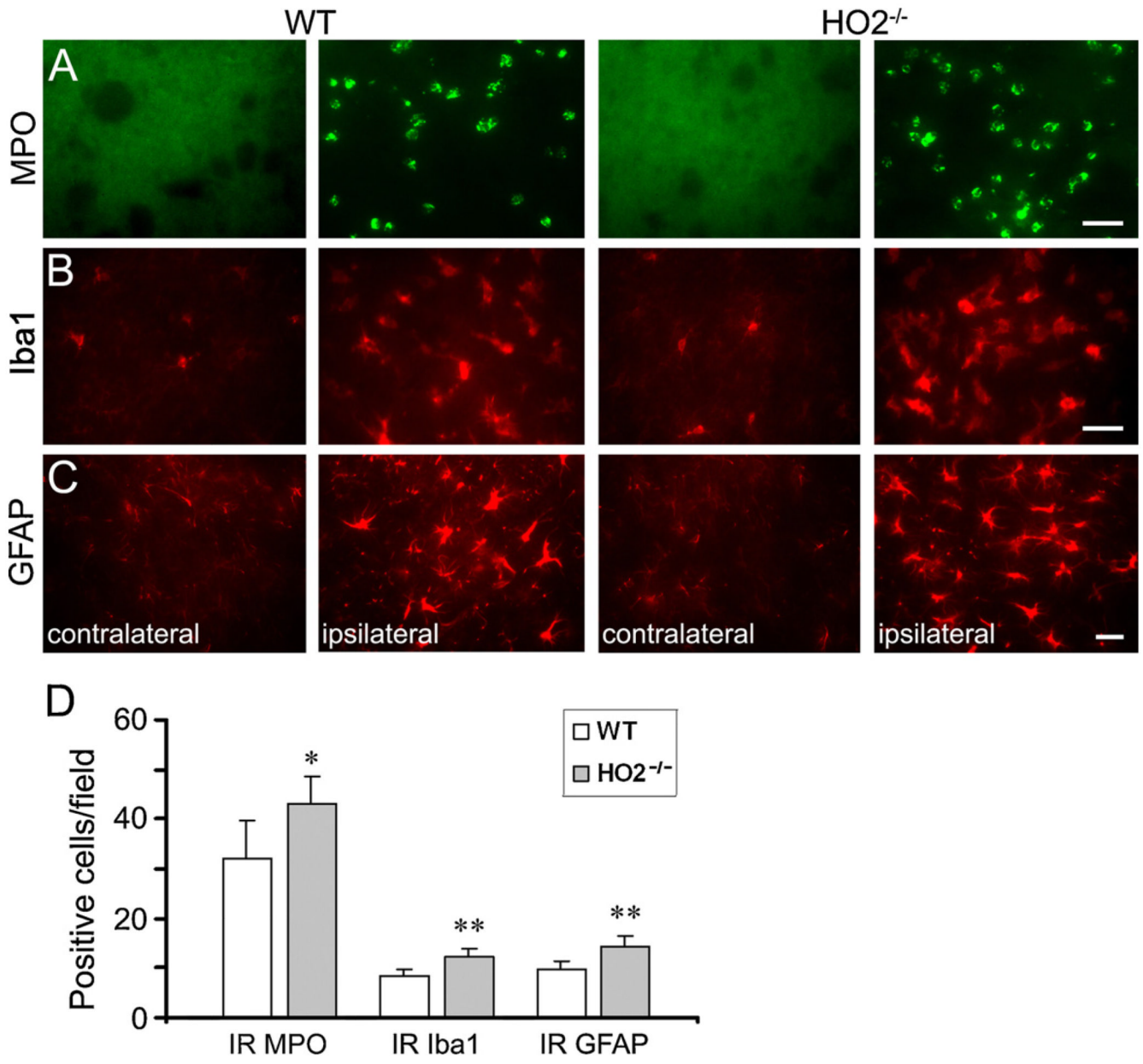

Fig. 3.

Deletion of HO2 increases neuroinflammation in mice subjected to intracerebral hemorrhage (ICH). (A) Infiltrating neutrophils (myeloperoxidase-positive cells), (B) activated microglia/macrophages (Iba1-immunoreactive cells), and (C) reactive astrocytes (GFAP-positive cells) were apparent in or around the injury site in WT and $HO2^{-/-}$ mice 72 h post-ICH. (**D**) Three sections per mouse with similar brain injury size were chosen from six WT and six $HO2^{-/-}$ mice. Positive cells were counted randomly from 12 locations per animal (4 fields per section × 3 sections per animal) and the numbers were averaged and expressed as positive cells/field. Cell count analysis indicated that $HO2^{-/-}$ mice had significantly more infiltrating neutrophils, activated microglia/macrophages and astrocytes than did WT mice at 72 h post-ICH (all $n = 6$/group, $*p < 0.05$, $**p < 0.01$). Scale bar = 30 μm for A, B, C; IR, immunoreactive. Values are means ± SD. From Wang and Dore (2008).

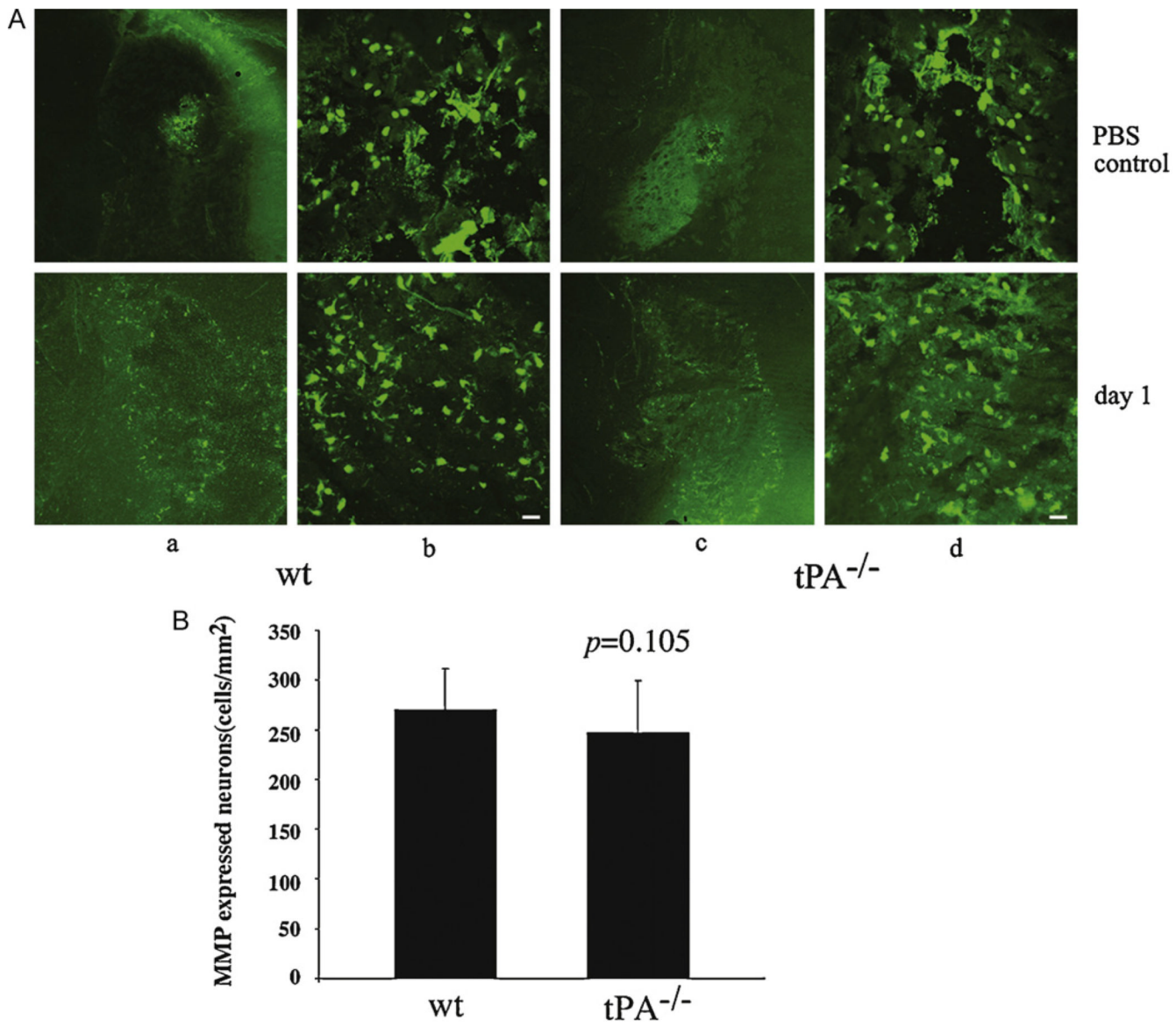

Fig. 4.

Increased in situ gelatinolytic activity after ICH on day 1 in control, wild-type (wt) and tPA knockout ($^{-/-}$) mice. (A) Gelatinolytic activity-positive cells are present in the injury area in all animals. Column a, c: 100 × magnification; Column b, d: 400 × magnification. Scale bar, 20 μm. (B) Quantification of gelatinolytic activity-positive cells on day 1 in wt and tPA$^{-/-}$ mice. No significant difference was observed between the two genotypes of mice (n = 5). Values shown are means ± SD. Modified from Wang et al. (2003).

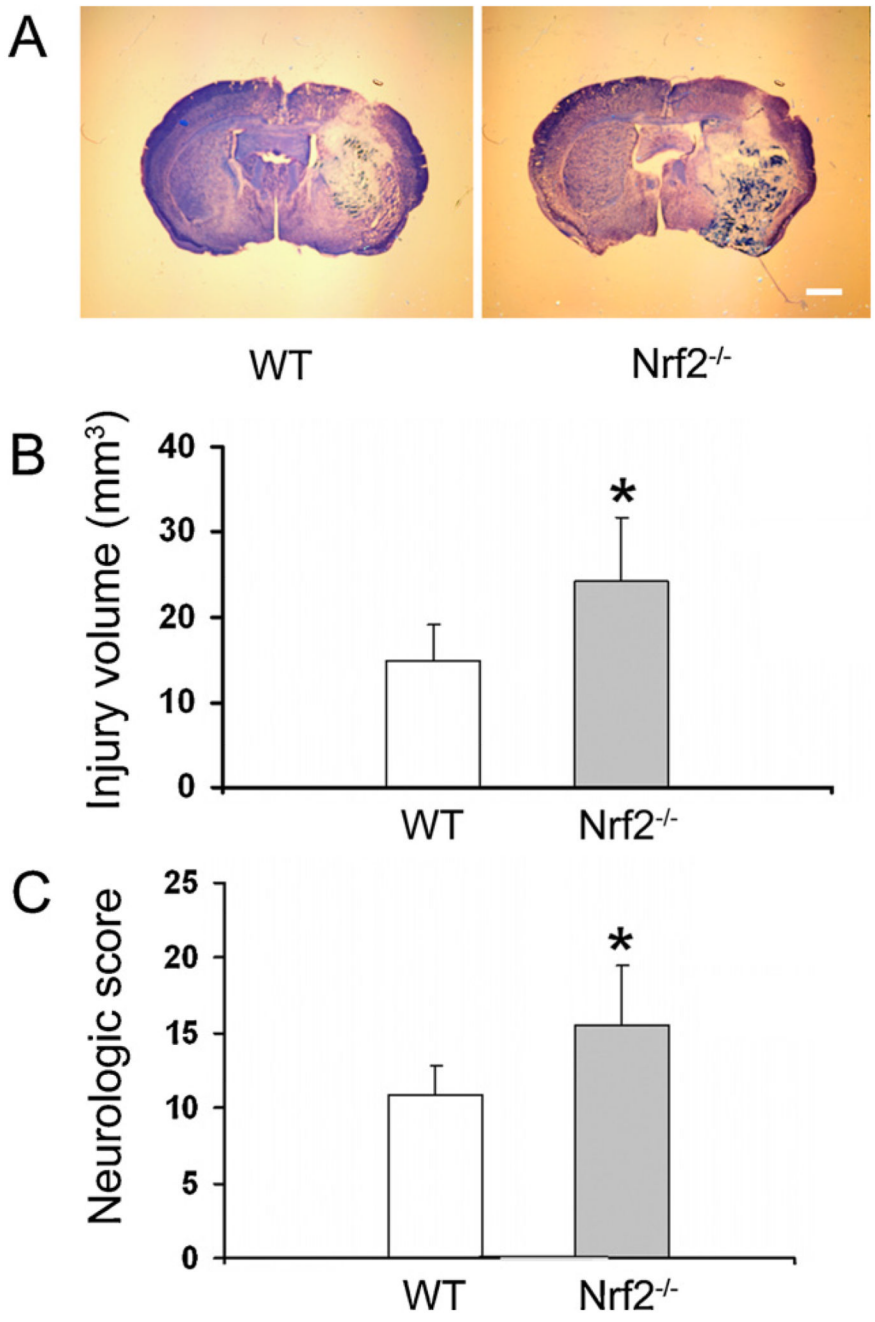

Fig. 5.

Deletion of Nrf2 increases brain injury volume and neurologic deficits in mice subjected to intracerebral hemorrhage (ICH). Age- and weight-matched Nrf2 knockout ($Nrf2^{-/-}$) and wild-type (WT) mice were subjected to ICH, and brains were sectioned and stained with Luxol fast blue/Cresyl Violet. (**A**) Representative sections from $Nrf2^{-/-}$ and WT mice 24 h after collagenase injection showing different areas of injury as represented by lack of staining. Scale bar =100 μm. (**B**) Quantification shows significantly larger brain injury volume in $Nrf2^{-/-}$ mice (n = 7) compared with WT mice (n = 10) 24 h after collagenase injection. (**C**) An investigator blinded to genotype assessed the neurologic deficits of $Nrf2^{-/-}$ and WT mice with a 24-point neurologic scoring system 24 h after collagenase

injection. Neurologic deficits were significantly more severe in $Nrf2^{-/-}$ mice ($n = 7$) than in WT mice ($n = 10$). Values are means ± SD. *$p < 0.05$. From Wang et al. (2007).

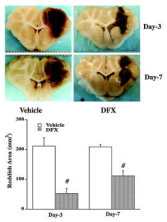

Fig. 6.
Deferoxamine reduces reddish zone around hematoma at day 3 and day 7 in a pig ICH model. Values are means ± SD, $n = 4$, $\#p < 0.01$ vs vehicle. From Gu et al. (2009).

Table 1

Preclinical and clinical trials of various anti-inflammatory strategies in ICH.

Target	Model/strain	Result[a]	Reference
Leukocyte infiltration			
Global depletion of circulating leukocytes and platelets	Microballoon/rat	+	(Kane et al., 1992)
Microglial activation			
Microglia/macrophage inhibitory factor (MIF)	Collagenase/mouse	+	(Wang et al., 2003; Wang and Tsirka, 2005c)
Minocycline[b]	Collagenase/rat	+	(Power et al., 2003)
Minocycline	Collagenase/rat	+	(Wasserman and Schlichter, 2007a)
Minocycline	Blood/rat	+	(Wu et al., 2009b)
Minocycline	Blood/mouse	+	(Xue et al., 2010)
Minocycline	Collagenase/rat	−	(Szymanska et al., 2006)
Mast cell stabilization			
Sodium cromoglycate	Blood/rat	+	(Strbian et al., 2007)
Cytokines			
TNFα-specific antisense oligodeoxynucleotide	Collagenase/rat	+	(Mayne et al., 2001b)
Adenosine A_{2A} receptor agonist	Collagenase/rat	+	(Mayne et al., 2001a)
Overexpression of IL-1ra	Blood/rat	+	(Masada et al., 2001, 2003)
Matrix metalloproteinases			
BB-1101	Collagenase/rat	+	(Rosenberg and Navratil, 1997)
GM6001	Collagenase/mouse	+	(Wang and Tsirka, 2005b)
GM6001	Blood/mouse	+	(Xue et al., 2009b)
BB-94	Collagenase/mouse	−	(Grossetete and Rosenberg, 2008)
Reactive oxygen species			
Alpha-phenyl-N-tert-butyl nitrone	Collagenase/rat	+	(Peeling et al., 1998)
Alpha-phenyl-N-tert-butyl nitrone	Blood/rat	+	(Aronowski and Hall, 2005)
NXY-059	Collagenase/rat	+	(Peeling et al., 2001a)
NXY-059	Phase II clinical trial	−	(Lyden et al., 2007)
Nrf2			
Nrf2 inducer sulforaphane	Blood/rat	+	(Zhao et al., 2007a)
Heme oxygenases			
Tin-mesoporphyrin IX	Blood/rabbit	+	(Koeppen et al., 2004)
Tin-mesoporphyrin IX	Blood/pig	+	(Wagner et al., 2000)
Tin-protoporphyrin	Blood/rat	+	(Huang et al., 2002)
Zinc protoporphyrin	Blood/rat	+	(Gong et al., 2006)
Ferric iron			
Deferoxamine	Blood/rat	+	(Hua et al., 2006; Nakamura et al., 2004a; Okauchi et al., 2009; Song et al., 2007)
Deferoxamine	Blood/pig	+	(Gu et al., 2009)
Deferoxamine	Collagenase/rat	−	(Warkentin et al., 2010)
Complement			
C3a receptor antagonist	Blood/mouse	+	(Rynkowski et al., 2009)

Target	Model/strain	Result[a]	Reference
C5a receptor antagonist	Blood/mouse	+	(Garrett et al., 2009)
Others			
PPARγ agonist rosiglitazone	Blood/mouse	+	(Zhao et al., 2007b)
15d–PGJ2	Blood/rat	+	(Zhao et al., 2006c)
FK-506	Collagenase/rat	+	(Peeling et al., 2001b)
Neural stem cell transplantation	Collagenase/rat	+	(Jeong et al., 2003; Lee et al., 2008)

[a] + Indicates positive result; − indicates negative result.

[b] Minocycline also targets MMPs.