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Intercellular Cross-talk in Intracerebral Hemorrhage

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Abstract

Intracerebral hemorrhage (ICH) is a devastating cerebrovascular disorder with high mortality and morbidity. Currently, there are few treatment strategies for ICH-induced brain injury. A recent increase in interest in the pathophysiology of ICH, has led to elucidation of the pathways underlying ICH-induced brain injury, pathways where intercellular and hematoma to cell signaling play important roles. In this review, we summarize recent advances in ICH research focusing on intercellular and hematoma:cell cross-talk related to brain injury and recovery after ICH.

Keywords

brain injury; experimental models; intracerebral hemorrhage; injury mechanisms; intercellular signaling

1. Introduction

Intracerebral hemorrhage (ICH) is a devastating form of cerebrovascular disorder with a particularly high mortality (about 40% at one month) (Feigin et al., 2009), and typically, it causes severe neurological deficits in those who survive. Thus, only 20% of ICH survivors are able to live their life independently at six months after onset (Adeoye and Broderick, 2010). The annual incidence of ICH is 10 to 30 per 100,000 population, with about 2 million cases occurring in the world annually (Qureshi et al., 2009; Xi and Keep, 2012). Furthermore, because ICH occurrence is strongly age-related, the rate of ICH is expected to increase as a result of the aging population (Qureshi et al., 2001; Qureshi et al., 2009). Few effective medical and surgical treatment strategies are currently available, and ICH-induced mortality at one month has not decreased in the past three decades (van Asch et al., 2010). With increasing interest in the mechanisms of ICH-induced brain injury, many potential injury pathways have been elucidated in animal and clinical studies (Keep et al., 2012; Xi et al., 2014). In this review, we aim to describe recent advances in ICH research, mainly

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focusing intercellular communication and hematoma:cell signaling related to brain injury, consequent pathogenesis, and brain recovery.

2. Primary and Secondary Brain Injury after ICH

2.1. Primary brain injury

An ICH causes immediate physical disruption to the brain cellular architecture adjacent to the hemorrhage, a mass effect. Little can be done to ameliorate this initial damage (Keep et al., 2012; Xi et al., 2006). After the first ictus of bleeding, about 20–40% of patients experience hematoma enlargement during the first day (Balami and Buchan, 2012; Delgado Almandoz et al., 2010; Dowlatshahi et al., 2011). The precise mechanisms of hematoma growth are still not known. Hypertension may influence hematoma growth (Anderson et al., 2010; Qureshi et al., 2010). Only a few experimental studies have investigated of the effects of blood pressure on hematoma growth. Wu et al. (Wu et al., 2011) found that the volume of ICH induced by collagenase injection was the same in spontaneous hypertensive and normotensive rats. However, interestingly, more severe neuronal death, microglial activation, ferritin upregulation, and neurological deficits were observed in spontaneous hypertensive than in normotensive rats, suggesting that hypertension may contribute to brain injury other than by expansion of hematoma (Keep et al., 2012).

Whether perihematomal ischemia occurs and contributes to ICH-induced neuronal injury is controversial both in humans and animals. Interpretation of blood flow reductions in perihematomal tissue is complicated by changes in metabolism and edema formation (Keep et al., 2012; Xi et al., 2006). Zazulia and colleagues reported reduced perihematomal blood flow after ICH and that this area corresponded with reduced, rather than increased, oxygen extraction fraction (Zazulia et al., 2001). This finding suggests that perihematomal blood flow reductions are matched with reduced metabolic demand in tissue damaged by the ICH. Other clinical and experimental studies have reported reduced perihematomal blood flows. However, the reductions seem insufficient to cause ischemic injury and they were combined with perifocal edema (Herweh et al., 2007; Mayer et al., 1998; Qureshi et al., 1999).

2.2 Secondary brain injury

Secondary brain injury after ICH is caused by a cascade of events. Immediately after ICH, activation of hemostatic cascade occurs as an initial reaction to limit bleeding. Thrombin, an essential component of the hemostatic cascade, is produced in the brain immediately after hemorrhage (Hua et al., 2007). Thrombin can affect many type of cells including endothelial cells, astrocytes, neurons, and microglia (Keep et al., 2012). Much evidence suggests that thrombin contributes to early blood-brain barrier (BBB) disruption and edema formation after ICH (Keep et al., 2014; Lee et al., 1996; Lee et al., 1997; Xi et al., 1998).

Release of hemoglobin and iron from the hematoma is considered another major contributor to ICH-induced brain injury (Keep et al., 2012; Wagner et al., 2003; Xi et al., 2006). Erythrocyte lysis occurs within 24 hours after ICH, peaking at day 1 to 7 (Nakamura et al., 2004; Wu et al., 2003; Xi et al., 2006), and hemoglobin- and iron-mediated brain injuries occur along with lysis of extravasated erythrocytes. Injection of hemoglobin and its

degradation products into striatum also cause brain damage (Hua et al., 2003; Huang et al., 2002).

It was reported that neurotoxic effects of hemoglobin are mainly due to heme (Lara et al., 2009). Degradation of heme by heme oxygenases (HO) after erythrocyte lysis results in the release of iron, carbon monoxide, and biliverdin, which is then converted to bilirubin (Maines, 1988). Iron overload in the brain starts within 24 hours to peak at 7 days after hemorrhage, and remains at least a month (Chaudhary et al., 2013; Wu et al., 2010; Wu et al., 2003). Iron toxicity may be based on the Fenton chemistry reaction that can produce highly reactive free radicals, and it can cause neurodegeneration via oxidative injury (Zecca et al., 2004). In neurons, iron overload results in increased intracellular free iron by saturation of iron binding, transport and storage proteins (Gaasch et al., 2007). In experimental ICH models, it was confirmed that oxidative DNA damage caused by free radicals occurs after ICH, and it leads to neuronal death (Nakamura et al., 2005; Nakamura et al., 2006; Xi et al., 2006). The cellular response in the brain to iron toxicity varies by cell type. Astrocytes are more resistant to iron-induced toxicity than neurons, which are highly vulnerable to oxidative stress (Kress et al., 2002). *In vitro* experiments suggest that astrocyte activation may be related to iron-induced oxidative stress (Macco et al., 2013).

2.3. Endogenous defense mechanisms

It should be noted that as well as inducing primary and secondary brain injury, an ICH upregulates a variety of potential endogenous defense mechanisms, including variety of iron-handling proteins, that may limit ICH-induced injury (Keep et al., 2012). For example, blood- and brain-derived haptoglobin plays an important role in neutralizing toxicity of extracellular hemoglobin (Yang et al., 2003).

It has been proposed that some endogenous responses are deleterious after a large ICH, however, the same response is protective in the settings of small hematomas (Keep et al., 2005). For example, the effects of HO-1 depend on the levels of activity (Suttner and Dennery, 1999). It is possible that the iron generated by HO-1 could be handled by iron-handling proteins in the setting of a small hematoma, however, in a large ICH, generated iron may exceed the handling capacity in the parenchymal cells and result in brain injury (Keep et al., 2005).

3. Effects of ICH on neural cell types

3.1. Neuronal death and brain atrophy

ICH causes significant death of brain cells (Hua et al., 2006; Xi et al., 2014). In experimental ICH models, necrotic, apoptotic and autophagic cell death occurs in the perihematoma region (He et al., 2008; Jin et al., 2013; Matsushita et al., 2000; Xi et al., 2014). Necrosis around the hematoma has been observed in both human and experimental ICH (Jin et al., 2013; Suzuki and Ebina, 1980), and it may be caused by mechanical disruption during hematoma formation or blood clot-derived neurotoxic factors. Perihematoma apoptotic cell death has also been observed (Matsushita et al., 2000; Qureshi et al., 2003). It remains unclear which cell-death pathway is predominant process of ICH-mediated neuronal injury (Keep et al., 2012). Another experimental study showed that

programmed necrosis (necroptosis) occurs after ICH, and this may be a novel therapeutic target for ICH (Zhu et al., 2012). It was also reported that autophagy partly plays a role in ICH-induced cell death (He et al., 2008). Autophagy is considered as an important process to maintain cellular homeostasis and it occurs in other neurological disorders (Adhami et al., 2006; Komatsu et al., 2006; Rubinshtein et al., 2005). The precise roles of autophagy in ICH-mediated injury are not yet elucidated. Further investigations are needed to determine whether autophagy is protective or harmful in ICH (Xi et al., 2014).

Long-term, such cell death in animal models results in brain atrophy and ventricular dilation as well as prolonged neurological deficits (Hua et al., 2002; Hua et al., 2006). Iron overload after hemorrhage is a potential cause of such brain atrophy (Hua et al., 2006; Okauchi et al., 2009).

3.2. Activation of microglia/macrophages

Microglia are among the first cells to respond to various brain injuries. Activation of resident microglia occurs very early after ICH (~1 hour) and persists for several weeks, with a peaking at 3 to 7 days (Figure 1) (Keep et al., 2012). There is also an infiltration of blood-derived macrophages into the perihematomal brain tissue (Hammond et al., 2014) and activated microglia/macrophages are thought to play a major role in hematoma clearance after ICH (Zhao et al., 2007). However, they may also contribute to ICH-induced brain injury through the secretion of various pro-inflammatory factors and production of reactive oxygen species (ROS) (Wang and Dore, 2007b; Xi et al., 2006) and microglia/macrophage activation is more prominent in aged than in young rats after ICH (Gong et al., 2004). It has been reported that classically activated (M1 polarized) microglia/macrophages release various pro-inflammatory factors and ROS, whereas alternatively activated (M2 polarized) microglia/macrophages have phagocytotic activity and release various protective and trophic factors (David and Kroner, 2011; Gordon and Taylor, 2005; Hu et al., 2014; Martinez et al., 2008). Phenotypic and functional shifts can be triggered and occur even in fully polarized M1 or M2 subpopulation by extra- or intra-cellular signaling (Chhor et al., 2013; Hu et al., 2012).

3.3. Activation of astrocytes

Many types of brain injury, including ICH, cause a prominent astrocyte reaction (Figure 2) (Xi and Keep, 2012). Extravasated blood-derived factors, especially thrombin, are crucial for astrocyte activation. Thrombin drastically upregulates transient receptor potential canonical 3, and it contributes to the excessive astrocyte proliferation and leads to astrogliosis (Shirakawa et al., 2010). Activated astrocytes may be both harmful and protective in ICH. They participate in inflammatory processes by secreting matrix metalloproteinases (MMPs) after experimental ICH and may aggravate neuronal injury (Tejima et al., 2007; Wang and Tsirka, 2005), but they may also protect neurons by promoting the secretion of angiogenic and neurotrophic factors (Brahmachari et al., 2006). In contrast to microglia/macrophages activation, astrocytic reaction is stronger in young than in aged rats with ICH (Gong et al., 2004).

3.4. Oligodendrocytes and white matter injury

White matter components, axons and myelin-producing oligodendrocytes, are highly vulnerable to damage in a various neurological diseases (Dewar et al., 2003). Thus, in a rat collagenase model, ICH caused demyelination and axonal damage around the hematoma within a day, and this damage spreads to surrounding brain parenchyma (Wasserman and Schlichter, 2008). Several potential mechanisms for ICH-induced white matter injury were elucidated in recent studies. Upregulation of receptor-interacting protein kinase 1 (RIPK1), as well as increased tumor necrosis factor- α (TNF- α) levels, were observed in white matter in a piglet ICH model (Xie et al., 2014). TNF- α can induce necroptotic cell death through RIPK1 upregulation (Degterev et al., 2005). Deferoxamine treatment suppressed TNF- α and RIPK1 upregulation and attenuated white matter edema after ICH. These results suggested that necroptosis is one potential mechanism of ICH-induced white matter injury (Xie et al., 2014). The effects of blood breakdown products on ICH-induced white matter injury have recently been investigated (Lakovic et al., 2014). According to that study, bilirubin affects white matter structural integrity and function, and bilirubin oxidation products are responsible for the bilirubin toxicity. Marked microglia activation has been observed in damaged white matter at post-ICH day 3 to 7 (Lakovic et al., 2014; Wasserman and Schlichter, 2008).

Haptoglobin, an acute-phase response protein, plays an important role in protecting neuron from hemoglobin-induced damage after ICH (Zhao et al., 2009b). Haptoglobin tightly binds to hemoglobin and stabilizes and shields heme iron within the hydrophobic pocket of hemoglobin, and preventing oxidative damage induced by hemolysis (Yang et al., 2003). It was reported that the brain-derived haptoglobin is synthesized and released into extracellular space by oligodendrocytes. Haptoglobin expression in oligodendrocytes is upregulated by nuclear factor-erythroid 2-related factor (Nrf2) activator, sulforaphane (Zhao et al., 2009b). These processes may be involved in endogenous defense mechanism against ICH (Aronowski and Zhao, 2011).

4. Role of intercellular and hematoma:cell signaling in ICH-induced injury

Figure 3 shows the schema of cellular interactions and hematoma cell interaction after ICH. Various types of cell play a role in ICH-induced brain injury, as well as neuroprotection and brain repair. Important examples of such interactions are described in the text below.

4.1. Early hematoma to brain signaling

It has been suggested that thrombin plays a coordinating role in the endogenous response of the brain to ICH (Keep et al., 2011). Following ICH, a sequential endogenous responses including clot formation, vascular repair, and clot resolution may occur. After vessel rupture in the brain, thrombin is produced almost instantaneously from prothrombin as a result of activation of the coagulation cascade, and clot formation is initiated (Hua et al., 2007). This early production of thrombin also seems important for the subsequent initiation of vascular repair, hematoma resolution, and upregulating endogenous defense mechanisms such as iron-handling proteins (Keep et al., 2011).

Although very low concentrations of thrombin can be neuroprotective (Masada et al., 2000; Striggow et al., 2000; Xi et al., 1999), it is well known that high concentrations of thrombin in the brain are harmful (Jiang et al., 2002; Xi et al., 2003). Both *in vivo* and *in vitro* experiments showed that thrombin causes cell death or injury in endothelial cells and astrocytes, and leads to increase BBB permeability (Liu et al., 2010). Thrombin mainly acts on protease-activated receptors (PARs) that mediate thrombin signaling (Wang and Reiser, 2003), and PARs modulate the activation of intracellular Src family kinases (SFKs). Thus, thrombin causes SFKs activation via PARs signaling, and it was reported that SFK inhibitors reduced thrombin-induced BBB breakdown (Ardizzone et al., 2007; Liu et al., 2010). Taken together, it is thought that SFKs mediate thrombin-induced BBB injury (Liu et al., 2010). Thrombin receptors are expressed and function in neurons (Niclou et al., 1994; Weinstein et al., 1995), and above-mentioned PARs-mediated SFKs activation results in neuronal death via multiple downstream cascades (Coughlin, 2000; Fujimoto et al., 2007; Liu et al., 2008). It has been reported that inhibition of PARs, especially PAR-1, or deletion of PAR-1 reduces ICH-induced neuronal death (Cheng et al., 2014; Xue et al., 2006). A recent study also showed that inhibiting SFKs ameliorates hippocampal neuronal death without increasing hemorrhage in experimental traumatic brain injury (Liu et al., 2014a). Thrombin is reported as a crucial factor for activating astrocytes (Nishino et al., 1993). Thrombin markedly upregulates transient receptor potential canonical 3, and it contributes to excessive astrocyte proliferation, and leads to astrogliosis (Shirakawa et al., 2010). Thrombin also can activate and stimulate microglia to secrete inflammatory cytokines, such as interleukin (IL)-1 β , or tumor necrosis factor- α (TNF- α) (Moller et al., 2000; Wu et al., 2008). Activation of the Rho-associated protein kinase pathway, downstream of SFK signaling, was suggested as a potential mechanism of microglial inflammatory activation caused by thrombin (Cui et al., 2013). A recent study showed that thrombin induces autophagy in both brain after ICH and cultured astrocytes (Hu et al., 2011). Thrombin-induced astrocyte death was aggravated by an autophagy inhibitor, 3-methyladenine. The role of thrombin in autophagy and its contribution to ICH-induced cell death needs further investigation.

4.2. Signaling in inflammation after ICH

Intracerebral hemorrhage induces brain inflammation with microglia activation, cytokine and chemokine production, BBB disruption and leukocyte infiltration (Keep et al., 2012; Keep et al., 2014; Wang and Dore, 2007b). The latter involves extravasation of neutrophils, macrophages and lymphocytes into brain. Brain inflammation is a highly regulated process involving multiple signaling pathways and mediators (see Figure 4 for schema for ICH). Many preclinical studies have shown that targeting such signaling can affect ICH-induced brain injury, with inflammation being involved in early ICH-induced brain injury but also in tissue repair (Keep et al., 2012; Wang and Dore, 2007b; Zhou et al., 2014b).

In ICH, an initial inflammatory response is induced by the presence of blood within the brain (hematoma to cell signaling), with the production of thrombin playing a prominent role. Thus, thrombin can activate microglia and astrocytes, induce BBB hyperpermeability and cause leukocyte infiltration via mechanisms that are PAR dependent or independent (Liu et al., 2010; Xi et al., 2003). The latter may involve the cleavage of fibrinogen to fibrin

as there is evidence from multiple sclerosis that extravascular fibrin is important in neuroinflammation and particularly microglia activation (Adams et al., 2004; Davalos et al., 2012). Hemoglobin, which is released when erythrocytes lyse in the hematoma, is also a potent activator of microglia via toll-like receptors (TLRs) (Wang et al., 2014; Zhou et al., 2014b). Bilirubin, a hemoglobin breakdown product, can also induce neutrophil extravasation into brain but does not activate microglia (Loftspring et al., 2011).

Other potent initiators of inflammation are the so-called danger associated molecular pattern molecules (DAMPs), such as high mobility group box 1 (HMGB1), which are released from dying cells (Lei et al., 2013; Zhou et al., 2014b). These act through receptors, such as the TLRs in effector cells, to induce inflammation. Thus, cell injury caused by ICH-induced physical disruption or secondary mechanisms (such as inflammation (Keep et al., 2012; Xi et al., 2006)) may enhance the inflammatory response after ICH.

As well as signaling induced by the hematoma and dying cells, cell to cell signaling has a prominent role in ICH-induced inflammation and such signaling may be an important therapeutic target. Microglial activation is a very early event after ICH (Taylor and Sansing, 2013; Zhao et al., 2009a) and activated microglia can produce many pro- and anti-inflammatory mediators. Thus, for example, pro-inflammatory M1 microglial cells produce IL-1 β , TNF- α , and ROS (Cherry et al., 2014). Such factors are known to activate other microglia and astrocytes (Cherry et al., 2014; Dunn et al., 2002; Morgan et al., 1997). They also can induce BBB disruption and cause the expression of adhesion molecules on the luminal surface of brain endothelial cells (Obermeier et al., 2013), important steps for leukocytes to migrate into brain. In contrast, microglial cells with a M2 phenotype are primarily anti-inflammatory (e.g. secreting IL-10 and transforming growth factor- β (TGF- β), and expressing arginase-1 which reduces nitric oxide production), phagocytic and involved in tissue repair (Cherry et al., 2014; Taylor and Sansing, 2013; Zhao et al., 2009a).

There has been considerable interest in targeting microglia activation as a very early event in the inflammatory cascade or altering microglia phenotypes to reduce ICH-injury. Several studies have shown that microglia inhibition reduces ICH-induced brain injury (Taylor and Sansing, 2013; Wang and Dore, 2007a). However, in ICH and other neurological conditions, microglia can have beneficial as well as detrimental effects (Taylor and Sansing, 2013; Wang and Dore, 2007b) and the effects of microglia inhibition are time-dependent with benefit early but not late after ICH (Taylor and Sansing, 2013). As noted above, an alternate therapeutic approach may be to modify microglial activation to promote potential beneficial effects (phagocytosis, tissue repair). Peroxisome proliferator activated receptor- γ (PPAR γ) agonists promote the M2 phagocytic phenotype and PPAR γ agonists have shown benefit in animal ICH models (Zhao et al., 2009a) and a PPAR γ agonist, is now in clinical trial for ICH (Gonzales et al., 2013).

Astrocytes are activated after ICH as the result of clot-derived factors and microglia activation. However, the precise role of that activation in ICH-induced brain injury is unclear. Astrocytes are important regulators of BBB function, helping induce the BBB phenotype by secreting factors such as TGF- β , glial-derived neurotrophic factor, basic fibroblast growth factor, angiopoietin (Ang)-1 (Abbott et al., 2006). Astrocytes also can

reduce the production of proinflammatory cytokines by microglia (Aloisi et al., 1997; Wang and Dore, 2007b). However, in ischemic stroke and other neurological conditions, reactive astrocytes can also have detrimental effects (Gleichman and Carmichael, 2014; Pekny and Nilsson, 2005) and the overall effect of astrocyte activation in ICH needs to be clarified.

It should be noted that another cell of the neurovascular unit, the pericyte, is also involved in BBB induction and maintenance (Armulik et al., 2010; Daneman et al., 2010; Engelhardt et al., 2014). The role of pericytes in ICH-induced BBB dysfunction has not been directly examined. There is, though, evidence the cerebral ischemia and reperfusion causes migration of pericytes away from the endothelium and the loss of pericyte:endothelium signaling under those conditions might contribute to BBB disruption (Liu et al., 2012). The role of the pericyte in ICH merits investigation.

Activation of the cerebral endothelium in response to inflammatory mediators results in remodeling of the endothelium, the expression of adhesion molecules on the endothelial luminal surface, and disruption of endothelial tight junctions (Carman, 2009; Man et al., 2007). The expression of adhesion molecules is necessary for adhesion and transmigration of leukocytes across the endothelium. The remodeling of the tight junctions may also participate in this process, although there is a long-standing argument over whether leukocyte migration is para- or transcellular (or both) (Carman, 2009). The mechanism of leukocyte extravasation after ICH has received much less attention than in diseases such as ischemic stroke or multiple sclerosis. However, there is evidence of increased expression of involves endothelial activation with the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular active peptide-1 (VAP-1) after ICH (Gong et al., 2000; Ma et al., 2011; Rolland et al., 2013).

It should be noted that infiltrating leukocytes also secrete inflammatory mediators that will further amplify/modify the response of other inflammatory cells, including further leukocyte infiltration. Thus, for example, inhibiting monocyte entry into brain after ICH by genetic deletion of the chemokine CCL2 (monocyte-chemoattractant protein 1; MCP-1) or its receptor CCR2, also affected neutrophil infiltration and microglia activation (Yao and Tsirka, 2012). Similarly, depleting mice of neutrophils also reduces monocyte entry after ICH (Sansing et al., 2011).

4.3. Interactions between microglia/astrocytes and neurons

The direct interactions between activated astrocytes, or microglia/macrophages and neurons have been little studied in ICH, while it is well known that these activated cell types contribute to both ICH-induced neuronal death and neuroprotection via indirect pathways by the secretion of various inflammatory or trophic factors. A recent *in vitro* study showed that reactive astrocytes secrete lipocalin 2 (LCN2), and LCN2 is selectively toxic to neurons (Bi et al., 2013). Accumulating evidence suggests that LCN2 is mainly secreted astrocytes, and it has been implicated as having a role in brain injury after ICH and subarachnoid hemorrhage (Figure 5) (Dong et al., 2013; Egashira et al., 2014). Interestingly, after ischemic stroke, neurons in the penumbra express LCN2, and LCN2 produced by neurons act as a “help me” signal for astrocytes and microglia (Jin et al., 2014; Xing et al., 2014).

The role of LCN2 mediated cellular interaction in ICH-induced brain injury merits investigation.

Reactive microglia/macrophages, particularly of the M1 phenotype, may damage neurons by releasing oxidative metabolites and proteases (Kigerl et al., 2009). A recent study showed that TNF- α and increased intracellular iron in macrophages induce a rapid switch towards M1 polarization after spinal cord injury (Kroner et al., 2014). These phenomena are thought to occur after ICH and this is, therefore, a potential mechanism of microglia/ macrophage mediated neuronal death after ICH. Minocycline, a selective inhibitor of M1 microglia/ macrophages, attenuates iron accumulation and early neuronal death after ICH in rats (Zhao et al., 2011).

Activated astrocytes can modulate neuronal survival and neurogenesis in various brain injuries by producing angiogenic and neurotrophic factors (e.g. erythropoietin) (Swanson et al., 2004). M2 polarized microglia/macrophages are also considered to generally act as an anti-inflammatory phenotype and they also have potentials of secretion of protective or trophic factors such as insulin-like growth factor-1 (IGF-1) (Choi et al., 2008). Especially in the later phase of stroke, they seem to play an important role in brain recovery, including neural repair, neurogenesis, angiogenesis, and oligodendrogenesis (Hu et al., 2014; Swanson et al., 2004).

Interactions between astrocytes and microglia have been proposed as a potential regulator of ICH-induced brain injury (Wang and Dore, 2007b; Wang, 2010). Reactive astrocytes may modulate microglial ROS production and prevent excessive inflammation (Min et al., 2006). However, after ICH, a rapid and severe astrogliosis may occur. In such cases, astrocytes can accelerate the inflammatory response in coordination with activated microglia/macrophages (Wang, 2010). Indeed, it was reported that reactive astrocytes and microglia could cooperate to induce MMP activation (Rosenberg et al., 2001). To establish potential strategies to modulate the activity of astrocytes and microglia/macrophages in order to limit ICH-induced injury, further preclinical studies are needed.

4.4. Erythrocyte-microglia/macrophage signaling and hematoma clearance

After ICH, extravasated erythrocytes are cleared from brain parenchyma by microglia/ macrophages. Figure 6 demonstrates hematoma resolution process by phagocytosis of erythrocytes by microglia/macrophages after experimental ICH. Faster and more effective clearance may lead to less ICH-induced secondary brain injury, although activated microglia/macrophages may also contribute to ICH-induced brain injury (Wang and Dore, 2007b; Xi et al., 2006).

Several cell-surface scavenger receptors on the microglia/macrophages that relate to phagocytosis of erythrocytes or the products of erythrocyte have been identified. Cluster of differentiation (CD) 36 is known as a receptor for erythrocytes. Clearance of erythrocytes via CD36 prevents their lysis and subsequent release of toxic contents into brain tissue. If hemolysis occurs, blood- and brain-derived haptoglobin strongly binds to hemoglobin, and the complex is endocytosed via the scavenger receptor, CD163. Free heme is also detoxified

by binding hemopexin, and then endocytosed via the CD91 receptor (Aronowski and Zhao, 2011).

After ICH, microglia/macrophages strongly express HO-1, the inducible isozyme of HO (Wang and Dore, 2007a), that converts heme to iron, carbon monoxide, and biliverdin (Maines, 1988). To prevent excessive ROS production, iron is temporarily stored within the phagocytes by iron-binding proteins, such as ferritin (Aronowski and Zhao, 2011). This clean-up process persists 3 to 4 weeks after ICH onset (Zhao et al., 2007). Limiting extracellular hemoglobin degradation by early inhibition of HO-1 effectively ameliorated hemoglobin-induced brain edema in rats (Huang et al., 2002). However, particularly in the later phase, HO-1 inhibition may interfere with phagocyte activity and the clean-up process after ICH (Aronowski and Zhao, 2011).

As mentioned above, it is possible that microglia/macrophage polarization is key to determining the effect of these cells in ICH, and modulation of phenotypic shifts could be an important therapeutic strategy for various brain injuries including ICH (Hu et al., 2014). For example, PPAR γ is one transcription factor that modulates microglia/macrophage phenotypes, and mediates monocyte differentiation toward a M2 phenotype (Bouhlef et al., 2007). In the experimental ICH, administration of a PPAR γ agonist promotes hematoma resolution through microglia/macrophage activation, and knockdown or inhibition of PPAR γ significantly inhibits this phagocytotic process (Zhao et al., 2007; Zhao et al., 2009a). Many extracellular signaling pathways that lead to M1 or M2 polarization have been identified. Inhibition or promotion of these signaling could be another strategy for modulating microglia/macrophages phenotypes (Hu et al., 2014).

Very recently, it was reported that brain CD47 levels in perihematoma region are increased after ICH in pig, and deferoxamine treatment reduces this CD47 upregulation (Zhou et al., 2014a). CD47 is known as a regulator of phagocytosis of erythrocytes (Matozaki et al., 2009), as well as an inflammatory mediator. Under normal conditions, CD47 on the erythrocyte inhibits phagocytosis via providing macrophages a “don’t eat me” signal by interaction with its receptor SIRP α on the macrophage. In contrast, the conformation change of CD47, by binding its ligand thrombospondin-1, promotes phagocytosis via providing macrophages an “eat me” signal (Burger et al., 2012). These findings suggest that the modulation of phagocytic signals on erythrocytes could be another potential therapeutic target for promoting hematoma clearance after ICH.

4.5. Neurogenesis and angiogenesis after ICH

Neural progenitor cells are found in the subventricular zone and the hippocampal subgranular zone in adult mammalian brain (Gage, 2000). In response to various neuronal injuries including ICH, these endogenous progenitor cells migrate toward injured lesions and start to differentiate into mature neurons and form synapses (Yamashita et al., 2006). It was reported that neurogenesis occurs after ICH, and may lead to better functional outcome (Masuda et al., 2007; Shen et al., 2008). M2 polarized microglia/macrophages have a potential to promote the proliferation and migration of neural progenitor cells through the secretion of specific trophic factors, such as galectin-3 or IGF-1 (Choi et al., 2008; Yan et al., 2009). Erythropoietin, albumin, and statins are reported as potential agents that enhance

endogenous neurogenesis and lead to better functional after experimental ICH (Belayev et al., 2007; Indraswari et al., 2012; Karki et al., 2009; Seyfried et al., 2009).

Transplantation of various exogenous stem cells are also reported as a potential therapeutic strategy for ICH, as for ischemic stroke (Frantzias et al., 2011). However, how these cells participate in neuronal repair and functional recovery is still unclear. Other than neuronal replacement, these cells may provide cytoprotective or trophic factors to cells surrounding the injured tissue (Shen et al., 2008) or modulate the effects of inflammatory cells.

Brain angiogenesis is considered to one recovery process after ICH (Tang et al., 2007). Many trophic factors, such as hypoxia-inducible factor-1 α , vascular endothelial growth factor (VEGF), Ang-1, and Ang-2 are upregulated after ICH. Thrombin can initiate the endothelial production of these trophic factors, and it promotes angiogenesis after ICH (Zhou et al., 2012). M2 microglia/macrophages also can produce pro-angiogenic factors, such as VEGF and IL-8, and are also thought as a potential contributor for angiogenesis after ICH. However, it has also been reported that the M2 type cytokine TGF- β inhibits endothelial proliferation while the M1 type cytokine TNF- α promotes this process (Welser et al., 2010). The precise role microglia/macrophage polarization in angiogenesis after ICH needs further investigation.

A recent randomized controlled trial showed that rehabilitation improves survival and functional outcomes in patients with ICH. Rehabilitation can also enhance functional recovery after experimental ICH (Liu et al., 2014b). However, the underlying mechanisms by which rehabilitation affects delayed neuronal death or neurogenesis have not been fully elucidated (Caliaperumal and Colbourne, 2014; MacLellan et al., 2011).

5. Conclusions

Recent advances from basic and clinical research have improved our understanding of ICH-related brain injury. In this review, we have focused on some of the intercellular and hematoma to brain signaling that occurs after ICH. There is still much to learn about the precise effects of such signaling, the principal mediators involved, and how such signaling changes temporally during hemorrhage and hematoma resolution. Hopefully, such information will provide novel therapeutic strategies to reduce ICH-induced brain injury. As with ischemic stroke, it has proven very difficult to translate advances in our understanding the mechanisms underlying ICH-induced brain injury into the clinic. One criticism that has been made of past preclinical stroke studies is that they have been too 'neuron-centric' and that multiple cell types are impacted by the injury. Growing evidence in ICH and cerebral ischemia also now indicate that studies and therapies should also be focused not only on individual cell types but also how they interact after stroke.

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Abbreviations

Ang	angiopoietin
BBB	blood-brain barrier
CD	cluster of differentiation
DAMP	danger associated molecular pattern molecule
HMGB1	high morbidity group box 1
HO	heme oxygenase
ICAM-1	intercellular adhesion molecule-1
ICH	intracerebral hemorrhage
IL	interleukin
LCN2	lipocalin 2
MCP-1	monocyte-chemoattachment protein-1
MMP	matrix metalloproteinase
Nrf2	nuclear factor-erythroid 2-related factor
PAR	protease-activated receptor
PPARγ	peroxisome proliferator-activated receptor- γ
RIPK1	receptor-interacting protein kinase 1
ROS	reactive oxygen species
SFK	Src family kinase
TGF-β	transforming growth factor- β
TLR	toll-like receptor
TNF-α	tumor necrosis factor- α
VAP-1	vascular active peptide-1
VEGF	vascular endothelial growth factor

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Highlights

- Recent studies have elucidated many potential injury pathways after ICH.
- Various hematoma- and cell-cell interactions play a role in ICH-related injury.
- Such interactions are also important in regulating hematoma resolution.
- We summarize recent advances in ICH research focusing on intercellular cross-talk.

Figure 1.

Example of activation of microglia/macrophages in an aged (18 months old) rat 3 days after ICH (100 μ L injection of autologous whole blood). Immunoreactivity for OX-42, a microglia/macrophages marker, prominently increased around the hematoma. Dashed line indicates the hematoma edge. Scale bar = 100 μ m. Insets are higher magnification views. Scale bar = 20 μ m.

Figure 2. Example of activation of astrocytes in a young (3 months old) rat 3 days after ICH (100 μL injection of autologous whole blood). Immunoreactivity for glial fibrillary acidic protein (GFAP) was prominently observed around the hematoma. Dashed line indicates the hematoma edge. Scale bar = 100 μm. Insets are higher magnification views. Scale bar = 20 μm.

Figure 3.

Schematic drawing of hematoma:cell and intercellular signaling after intracerebral hemorrhage (ICH). ICH-derived factors (e.g. thrombin and iron) affect many types of brain cell (black solid arrow), causing activation or death in those cells. Brain cells interact and affect each other through various intercellular signaling pathways (black dashed arrow), and contribute to neuroprotection (blue arrow) or neuronal injury (red arrow) via many direct and indirect pathways. It is suggested that injured neurons have the potential to activate other types of brain cells as a help-me signal (yellow solid arrow).

Figure 4.

Schematic of the pro-inflammatory process after ICH. While clot derived factors are involved in initiating inflammation after ICH, factors derived from cells involved in the inflammatory cascade are involved in regulating (and amplifying or dampening) inflammation by impacting other cell types (intercellular communication). Some of those factors are outlined in red. Note that microglia can produce anti- as well as pro-inflammatory mediators (see text). DAMPs, danger associated molecular pattern molecules; Hb, hemoglobin; HMGB1, high mobility group box 1; ICAM-1, intercellular adhesion molecule-1; IL-1 β , interleukin 1 β ; MMPs, matrix metalloproteinases; ROS, reactive oxygen species; TNF α , tumor necrosis factor α ; VAP-1, vascular adhesion protein-1. A '?' is inserted for factors derived from reactive astrocytes. Although astrocyte derived factors have been identified that modulate endothelial and microglia function, the overall effect of such factors in ICH is unclear.

Figure 5.

Time course showing LCN2 immunoreactivity in the ipsi- and contralateral basal ganglia (A), and the protein levels of LCN2 and β -actin in the ipsilateral basal ganglia (B) and cortex (C) after an injection of 100 μ l autologous blood into the right caudate in the rat. While there was very little LCN2 immunoreactivity in the contralateral hemisphere, there was marked LCN2 expression in the ipsilateral hemisphere after ICH. That upregulation gradually waned over two weeks. Scale bar=100 μ m.

Figure 6. During hematoma resolution after ICH, there is phagocytosis of erythrocytes by invading macrophages/microglia. Hematoxylin & eosin stained section from a pig ICH model at day 7 after blood injection. Note the phagocytes engulfing erythrocytes and the production of hemosiderin, a hemoglobin breakdown product (yellow staining).

Figure 7.

Normal cells, including erythrocytes, express the cell surface protein CD47. The interaction of that protein with SIRP α on myeloid cells (microglia and macrophages) results in inhibiting phagocytosis. The CD47 acts as a 'don't eat me' signal. However, if CD47 is absent (X) from the erythrocyte or is blocked (square cap), phagocytosis may proceed. Thrombospondin-1 (TSP-1) also affects CD47 signaling by altering protein conformation (Burger et al., 2012).