*Critical Review***Exploring Neuroprotective Drug Therapies for Intracerebral Hemorrhage**Hiroshi Katsuki^{1,*}¹*Department of Chemico-Pharmacological Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan**Received August 20, 2010; Accepted October 4, 2010*

Abstract. Intracerebral hemorrhage (ICH) is a devastating neurological disorder with high mortality and poor prognosis, for which virtually no effective drug therapies are available at present. Experimental animal models, based on intrastriatal injection of collagenase or autologous blood, have enabled great advances in elucidation of cellular/molecular events contributing to brain pathogenesis associated with ICH. Many lines of evidence indicate that blood constituents, including hemoglobin-derived products as well as proteases such as thrombin, play important roles in the pathogenic events. Inflammatory reactions involving neutrophils, activated microglia, and production of proinflammatory cytokines also constitute a critical aspect of pathology leading to neurodegeneration and tissue damage. Efforts are continuing to find drugs that potentially alleviate pathological and neurological outcomes of ICH. Various drugs that possess antioxidative, anti-inflammatory or neurotrophic/neuroprotective properties have been demonstrated to produce therapeutic effects on ICH animal models. Drugs already in clinical use such as minocycline, statins, and several nuclear receptor ligands are among the list of effective drugs, but whether they also show therapeutic efficacy in human ICH patients remains unproven. Here, current knowledge of ICH pathogenesis and problems arising with respect to exploration of new drug candidates are discussed.

Keywords: hemorrhagic stroke, matrix metalloproteinase, heme, peroxisome proliferator-activated receptor, retinoid

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1. Introduction

Intracerebral hemorrhage (ICH) is a type of acute stroke characterized by extravasation of blood into brain

parenchyma and formation of hematoma, leading to edema and tissue damage in the brain. ICH represents approximately 15% of all strokes: hemorrhage resulting from denaturalization of vascular walls such as microaneurysm rupture occurs preferentially in several brain regions including the striatum (caudate-putamen) and the thalamus (1). No satisfactory treatments have been developed against this disorder, and patients suffer from high mortality and poor prognosis accompanied by en-

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during brain dysfunction. In addition, ischemic stroke is frequently associated with secondary hemorrhagic transformation that aggravates brain injury and neurological symptoms (2). Overall, hemorrhagic events within the brain tissue are serious medical problems. Current strategies for clinical management of ICH are recently reviewed elsewhere (3).

Experimental animal models of ICH, based on injection of either collagenase (that disrupts basal lamina of blood vessels) or autologous blood into brain parenchyma (usually into the striatum), have been developed (4), which greatly contributed to advances in the understanding of complicated pathogenic mechanisms of ICH. These experimental models have also been used to explore candidates for drug therapies for ICH. Here, an overview about the cellular and molecular mechanisms involved in pathogenic events after ICH is given in this review. Potential therapeutic strategies proven to be effective in animal models are also outlined, with special emphasis on strategies achieved by drug treatments.

2. Molecules involved in ICH pathogenesis

Formation of hematoma within the brain parenchyma after ICH inevitably brings physical damage to brain tissue. The 'mass effect' is an important factor in the pathogenic events in ICH (1), although it may be difficult to manage this effect directly by drug therapies. Perhaps equally important factors that contribute to ICH pathogenesis are several blood constituents such as thrombin and hemoglobin as well as its degradation products, which exert biological actions or toxic influences on brain cells. These events, primarily resulting from blood extravasation, also trigger secondary reactions in the brain parenchyma including recruitment of additional proteases, inflammatory responses, and intrinsic cytoprotective systems.

2.1. Thrombin

Thrombin is a serine protease that plays a pivotal role in the blood coagulation cascade. Besides its physiological role, substantial lines of evidence indicate that thrombin participates in various pathological conditions in the brain (5). In the case of ICH, a large amount of blood-derived thrombin invades the brain tissue and exerts biological actions through its proteolytic activity (Fig. 1A). The substrates for thrombin include proteinase-activated receptors that transduce intracellular signals via trimeric G proteins (6).

The role of thrombin in ICH pathogenesis was first suggested by its possible involvement in edema formation. That is, injection of whole blood into the striatum of rats induced edema, which was prevented by addition of

hirudin, a thrombin inhibitor (7). Edema induced by autologous blood injection into the striatum was attenuated also by argatroban, another inhibitor of thrombin, even when the drug was systemically administered from 6 h after blood injection (8). ICH-associated edema results from disruption of the blood-brain barrier and death of brain parenchymal cells, both of which may be induced by thrombin (9).

To elucidate the mechanisms of thrombin-induced cell death and tissue injury in the brain parenchyma, we set out for the experiments using an organotypic slice culture preparation (10). Application of thrombin to rat corticostriatal cultures induced delayed neuron death in the cortical region and shrinkage of the striatal region. Various pharmacological examinations revealed distinct properties of the mechanisms of injury between the cerebral cortex and the striatum. For example, extracellular signal-regulated kinase (ERK), p38, and c-Jun *N*-terminal kinase (JNK), three major members of mitogen-activated protein kinase (MAPK) family, all contributed to thrombin-induced injury of the striatum, whereas ERK, but not p38 or JNK, was involved in cortical injury. In addition, depletion of microglia from slice cultures rescued striatal tissue, but not cortical cells, from thrombin-induced injury, suggesting that microglia participate only in striatal tissue injury (10). Involvement of MAPKs and activated microglia in striatal tissue injury was confirmed by an *in vivo* study where thrombin was directly injected into the striatum of adult rats (11). On the other hand, plasminogen was found to cooperate with thrombin in inducing cortical injury but not striatal injury (12).

Thrombin-mediated cellular injury may also be mediated by activation of matrix metalloproteinase (MMP)-9 (see below). That is, concurrent application of MMP-9 exacerbates cytotoxicity of thrombin in neurons in primary culture, and thrombin cytotoxicity is partially attenuated by MMP inhibitors. Moreover, brain damage induced by autologous blood injection is synergistically attenuated by deletion of the gene encoding MMP-9 and administration of the thrombin inhibitor hirudin (13).

2.2. Matrix metalloproteinases

MMPs are a large group of proteases that act as cell surface sheddases, thereby regulating intercellular signaling (14). After hemorrhagic events in the brain, several members of the MMP family are recruited and involved in pathogenic processes. An early study on collagenase injection model (15) reported that the MMP inhibitor BB-1101 could reduce brain edema when administered 6 h after induction of ICH. In human ICH patients, expression of MMP-9 and MMP-3 increases after the incident (16). MMP-9 expression is induced in astrocytes and neurons in the perihematomal area, possibly by an action

Fig. 1. Putative mechanisms of ICH pathogenesis. A) Mechanisms with emphasis on the roles of proteases. Hemorrhage results in invasion of blood constituents such as prothrombin into the brain. Prothrombin is converted to its active form thrombin, and the protease disrupts the blood–brain barrier (BBB), thereby promoting formation of edema and infiltration of neutrophils. In addition, thrombin stimulates proteinase-activated receptors (PARs) on microglia/macrophages, activates these cells via recruitment of mitogen-activated protein kinases (MAPKs), and enhances production of several inflammatory mediators including TNF- α , IL-1 β , and nitric oxide (NO). These mediators contribute to neuron damage and edema formation. On the other hand, MMP-9 derived from both blood and brain parenchymal cells (neurons, astrocytes, and vascular endothelial cells) also contribute to edema formation, presumably via disruption of BBB and via actions (either directly or indirectly) onto microglia/macrophages. Expression of MMP-9 within the brain can be upregulated by hemoglobin. Aside from MMP-9, involvement of other members of MMPs such as MMP-3 (not shown here) and MMP-12 has been suggested in several studies. Expression of MMP-12 in microglia/macrophages is stimulated by thrombin. Argatroban inhibits thrombin, whereas BB-1101 and GM6001 inhibit MMPs, thereby lessening pathological events associated with ICH such as edema formation. B) Mechanisms with relation to hemolysis. Recruitment of complement cascade results in lysis of red blood cells. Hemolysis causes release of hemoglobin and heme, both of which are taken up by brain cells such as microglia and neurons. ICH is also associated with robust induction of HO-1 in microglia/macrophages, and the enzyme catalyzes degradation of heme into biliverdin (which is converted to bilirubin), carbon monoxide (CO), and iron. Iron (and presumably other products also) contributes to pathological changes such as increase in oxidative stress, formation of edema, infiltration of neutrophils, and induction of neuron death. Moreover, HO-1 may support survival of microglia/macrophages and consequently, may sustain deleterious inflammatory reactions brought about by these cells. Neurons express HO-2, the constitutive HO isoform, which might be able to participate in heme catabolism. C3a- and C5a-receptor antagonists (C3aRA and C5aRA) interrupt the complement cascade, whereas deferoxamine and clioquinol chelate free iron, all of which have been reported to alleviate pathological events in ICH animal models.

of hemoglobin (17). Increased MMP-9 is associated with the extent of perihematomal edema, whereas increased MMP-3 is associated with high mortality (16). Experiments using MMP-9-deficient mice (17) demonstrated that MMP-9 derived from both blood and brain parenchyma contributes to edema formation after autologous blood injection (Fig. 1A). In addition, MMP-9-deficient mice displayed lower levels of neurodegeneration, neutrophil infiltration, and microglia/macrophage reactions than wild-type mice (13). In the collagenase injection model, MMP-9 expression was found mainly in neurons and vascular endothelial cells, and administration of the MMP inhibitor GM6001, beginning 2 h after induction of ICH, attenuated neutrophil infiltration, oxidative stress, brain edema, neurodegeneration, and neurological impairment (18). With regard to MMP-3, early induction of this enzyme may contribute to brain damage in combination with other proteases such as MMP-9 and thrombin (19). Other lines of evidence indicate that MMP-12 may play a key role in the pathogenesis of ICH. That is, MMP-12 was induced most prominently among MMP isozymes in the collagenase-injection model in rats and mice (20, 21), and MMP-12-deficient mice showed better functional recovery after ICH as well as reduced levels of recruitment of microglia/macrophages in the perihematomal region (21).

It should be noted that several studies reported conflicting results. For example, a study on MMP-9-deficient mice showed that collagenase injection into the striatum of the mutant mice resulted in enhanced bleeding, increased mortality, and exacerbated neurological deficits. These changes may be attributable to heightened expression of MMP-2 and MMP-3 in response to ICH, together with lowered levels of collagen in the brain of MMP-9-deficient mice (22). In addition, systemic administration of BB-94, a broad spectrum MMP inhibitor, from 30 min before collagenase injection increased hemorrhagic volume and the number of cells exhibiting DNA fragmentation (23).

2.3. Heme and iron

Aside from proteases, important blood constituents contributing to ICH pathogenesis are hemoglobin and its degradation products. Following ICH, a large number of red blood cells penetrate into brain parenchyma. Hemolysis does not occur promptly after ICH, but rather, proceeds slowly, taking several days to weeks (24). Hemoglobin is released from lysed red blood cells, heme is liberated from hemoglobin, and hemoglobin as well as heme is taken up by brain parenchymal cells such as microglia and neurons (24, 25).

Heme is degraded by heme oxygenases (HOs) into biliverdin, iron, and carbon monoxide, and heme itself or

its degradation products may contribute to formation of brain edema (26). The major isoforms of HOs are HO-1 and HO-2, the former of which is an inducible isoform. After induction of ICH by collagenase or injection of autologous blood, robust expression of HO-1 is induced predominantly in non-neural cells such as microglia/macrophages and endothelial cells (27, 28). Earlier studies on ICH models in pigs and rabbits demonstrated that tin-mesoporphyrin, an isoform-nonselective HO inhibitor, attenuated edema formation and neuron loss (27, 29). A later study on HO-1-deficient mice also suggested a deleterious role of HO-1 activity in ICH pathogenesis. That is, ablation of the HO-1 gene reduced injury volume, neutrophil infiltration, microglia/macrophage activation, and oxidative damage associated with ICH (28).

We recently proposed a novel role of HO-1 in ICH pathogenesis. As was the case with thrombin cytotoxicity *in vivo* (11), neuroprotective effects of MAPK inhibitors suggested involvement of MAPKs in the pathogenesis of rat ICH model based on collagenase injection (30). Interestingly, we also found that administration of MAPK inhibitors triggered apoptotic cell death of microglia in the perihematomal region. Consistent with the occurrence of microglial cell death, the number of activated microglia was decreased by MAPK inhibition. Similar results were obtained in organotypic slice cultures treated with thrombin, where MAPK inhibition resulted in induction of apoptotic cell death in striatal microglia (31). Further examinations revealed that HO-1 expression was induced in activated microglia in a p38-dependent manner and was involved in maintenance of survival of these cells during thrombin insults (32). Therefore, one of the roles of HO-1 in ICH pathogenesis may be that the enzyme supports the survival of activated microglia, thereby sustaining and expanding deleterious influences of these cells onto other brain cells such as neurons and oligodendrocytes.

Iron derived from heme degradation may also play a key role in ICH pathogenesis, presumably via acceleration of oxidative stress. After autologous blood injection in rats, induction of HO-1 is followed by a gradual increase in tissue levels of non-heme iron (33). A recent clinical study showed that high serum levels of ferritin, a soluble protein for iron storage, are associated with poor outcome in ICH patients, suggesting that iron is involved in brain damage by ICH (34). Consistent with this idea, several studies on animal models of ICH showed that the iron chelator deferoxamine attenuated tissue damage and neurological deficits. For instance, intraperitoneal administration of deferoxamine starting from 2 or 6 h after autologous blood injection lessened brain edema, oxidative stress, and motor function deficits in rats (35). Another study on aged rats showed that deferoxamine was

effective in reducing brain edema and motor dysfunction even when administration of the drug was delayed 12 and 48 h, respectively, after autologous blood injection (36). However, the therapeutic efficacy of deferoxamine might depend on the type of ICH model. Reports mentioned above demonstrating beneficial effects of deferoxamine are based on the model made by autologous blood injection, whereas deferoxamine did not improve the outcome of the collagenase-induced ICH model in rats (37). On the other hand, a study on clioquinol, another kind of metal chelator, has demonstrated that oral administration of the drug, starting 6 h after induction of hemorrhage near the internal capsule by collagenase, alleviated motor dysfunction of rats (38).

2.4. Glutamate

Glutamate has long been recognized as the major excitatory neurotransmitter in the central nervous system (CNS). This amino acid is also well known as an important player in various CNS disorders, since over-activation of ionotropic glutamate receptors causes neuronal damage via processes called excitotoxicity. Several lines of evidence suggest that glutamate is involved in the pathogenesis of ICH. Transient elevation of the extracellular concentration of glutamate in the perihematomal region was demonstrated in rabbits following injection of autologous blood into the gray matter of the cerebrum (39). Subsequently, the effect of memantine, a low-affinity blocker of the *N*-methyl-D-aspartate subtype of glutamate receptor-associated channels, was investigated in the collagenase-injection model in rats. Daily intraperitoneal administration of memantine, starting from 30 min after induction of ICH, reduced hemorrhage volume, apoptotic cell death, neutrophil infiltration, and the number of microglia/macrophages in the periphery of hematoma. Neurological deficits associated with ICH were also ameliorated by this drug (40).

2.5. Complement

The complement cascade, consisting of interactions and associations of various serum and membrane-bound proteins, regulates inflammation, opsonization, and cytotoxicity and plays a key role in various disorders. This cascade may be involved in the pathogenesis of ICH (41), in part via participation in hemolysis (Fig. 1B). In the autologous blood-injection model in rats, perihematomal immunoreactivity against C9 was markedly elevated (42). Application of *N*-acetylhepstatin, an inhibitor of complement activation, suppressed edema formation (42). Moreover, C3-deficient mice that underwent autologous blood injection exhibited less brain edema, lower HO-1 levels, less microglia activation, and neutrophil infiltration, concomitantly with alleviated deficits in

motor functions, compared to wild-type mice (43). The deleterious role of C3 is also supported by a study using a C3a-receptor antagonist. Administration of a C3a-receptor antagonist beginning 6 h after autologous blood injection in mice significantly reduced neurological dysfunction and tended to decrease brain edema (44). In contrast, the role of C5 in the pathogenesis of ICH is controversial. A study on genetically modified mice suggests that C5 deficiency exacerbates brain edema triggered by autologous blood injection (45), whereas a C5a-receptor antagonist, either alone or in combination with a C3a-receptor antagonist, has been shown to reduce neutrophil infiltration and to improve neurological and spatial memory functions after autologous blood injection in mice (46).

2.6. Proinflammatory cytokines and other inflammatory mediators

Pathogenic events in ICH are characterized by occurrence of various inflammatory events including microglial activation, neutrophil infiltration, increase in oxidative stress, and production of proinflammatory cytokines (47). Expression levels of tumor necrosis factor (TNF)- α are increased in response to injection of autologous blood or thrombin into the striatum (48) and in response to induction of ICH by collagenase (49). Suppression of TNF- α expression by administration of antisense oligonucleotide into the striatum reduced cell death within the hematoma and improved neurological deficits after collagenase-induced ICH in rats (49). In this context, we have recently proposed that TNF- α produced from activated microglia contributes to striatal tissue injury by thrombin in organotypic slice culture (31). Interleukin (IL)-1 β may also be involved in the pathogenesis of ICH, since over-expression of IL-1-receptor antagonist significantly reduced brain edema induced by injection of autologous blood or thrombin into rat striatum (50).

Nuclear factor (NF)- κ B is a transcription factor that plays a key role in inflammatory reactions by regulating expression of various proinflammatory factors. Activation of NF- κ B is triggered promptly after experimental ICH by autologous blood injection and increased expression levels of NF- κ B subunits are sustained for 7 days (51). Expression of inducible nitric oxide synthase (iNOS), a product of NF- κ B-regulated genes, is also upregulated following ICH (51). Contribution of iNOS to ICH pathogenesis is suggested by a finding that iNOS-knockout mice exhibited reduced edema formation following collagenase-induced ICH, compared to wild-type mice (52).

2.7. Protective factors

In addition to the various harmful factors mentioned

above, several intrinsic cytoprotective systems are also mobilized in response to ICH, which may act to limit hemorrhage-associated tissue damage. For example, expression of heat shock protein 70 (HSP70), a molecular chaperone, is induced after ICH in neurons and glial cells (53). Prior induction of HSP70 by administration of geranylgeranylacetone in rats has been reported to afford neuroprotection and lessen neurological deficits associated with ICH induced by collagenase injection (54).

NF-E2-related factor 2 (Nrf2) may also act as an important component of the cytoprotective systems. Nrf2-deficient mice exhibit worsened outcome after collagenase injection as compared to wild-type mice, characterized by increases in injury volume, neuron death, neutrophil infiltration, oxidative stress, and neurological deficit score (55). Because expression of Nrf2 can be upregulated by various small compounds with electrophilic properties, this protein may serve as a potential molecular target for drug therapy. Indeed, intraperitoneal injection of the Nrf2 activator sulforaphane at 30 min after autologous blood injection into the striatum upregulated expression of several Nrf2-regulated antioxidant and detoxifying enzymes such as glutathione *S*-transferase and NAD(P)H:quinone oxidoreductase 1 and lessened neurological deficit score, neutrophil infiltration, as well as oxidative stress (56). A follow-up study has demonstrated that Nrf2 activation leads to upregulation of haptoglobin expression in oligodendrocytes. Haptoglobin can bind and neutralize hemoglobin, thereby protecting neurons and oligodendrocytes from hemoglobin-related damage (57).

3. Potential pharmacological interventions

Several research groups have been engaged in exploration of future candidates for therapeutic drugs for ICH; and indeed, various promising results have been reported, at least in animal models. Most of the drugs showing efficacy in ICH models have already been shown to exert beneficial effects on other CNS disorders and to have antioxidative, anti-inflammatory, or neurotrophic/neuroprotective actions.

3.1. Free radical scavengers

An early study on a rat ICH model demonstrated that neurological deficits were significantly attenuated by intraperitoneal administration of dimethylthiourea (a scavenger of hydroxyl radical) or α -phenyl-*N*-tert-butyl nitron (a spin trapping agent) beginning 2 h after collagenase injection. However, these agents did not prevent ICH-associated neuron loss (58).

Edaravone is a radical scavenger used clinically for treatment of ischemic stroke in Japan. A study assessing

the effect of edaravone showed that subcutaneous administration of this drug immediately after autologous blood injection in rats reduced edema and neurological deficits, together with reduction in oxidative DNA damage. Concerning reduction of edema, edaravone was effective even when administration was started 2 h after autologous blood injection (59).

Another free radical trapping agent, NXY-059, has been shown to exert beneficial effects in the collagenase-induced ICH model in rats. Subcutaneous injection of NXY-059 starting from 30 min after collagenase injection diminished neurological impairment, neutrophil infiltration in the vicinity of hematoma, and occurrence of apoptotic cell death in the perihematomal region (60). Unfortunately, a clinical trial employing intravenous injection of NXY-059 starting within 6 h after ICH revealed no significant effect of the drug on clinical outcome (61).

3.2. Growth factors and cytokines

A prospective study on primary ICH patients has demonstrated that high serum levels of growth factors such as vascular endothelial growth factor, granulocyte-colony stimulating factor (G-CSF), and angiopoietin 1 are associated with good functional outcome and reduced lesion volume (62). With regard to G-CSF, daily intraperitoneal injection of this cytokine from 2 h after induction of ICH by collagenase in rats resulted in improved recovery from neurological deficits. G-CSF administration also produced beneficial effects on various pathological parameters, including reduction in edema and blood-brain barrier permeability and decreases in apoptosis, neutrophil infiltration, and the number of activated microglia/macrophages (63). A recent study suggested possible contribution of neurogenesis to the beneficial effect of G-CSF against ICH-induced neurological deficits. Indeed, G-CSF treatment increased the number of stem cells in the hemorrhagic region and promoted mobilization of circulating hematopoietic stem cells to the damaged brain areas (64).

Erythropoietin is also noteworthy as this pleiotropic cytokine exhibits neuroprotective activities in several CNS disease models. Concerning ICH, daily intraperitoneal administration of erythropoietin starting from 2 h after ICH induction by collagenase resulted in improved functional recovery and reduction in edema, hemorrhage volume, apoptotic cell death, and the appearance of neutrophils and activated microglia/macrophages. Erythropoietin also attenuated expression of TNF- α as well as a death receptor and its ligand Fas and Fas-L and prevented activation of caspases (65). Protective effects of erythropoietin and its long-acting analog darbepoietin have also been demonstrated in the autologous blood-

injection model in rats (66).

3.3. Minocycline

Minocycline is originally known as a tetracycline type of antibiotic. However, many lines of evidence show that, independently of its antibiotic activities, this compound possesses neuroprotective properties. Neuroprotective activities of minocycline may be attributable to its anti-apoptotic, antioxidative, and anti-inflammatory actions.

The beneficial effect of minocycline on brains after ICH was first demonstrated by Power et al. (20). *In vitro*, minocycline attenuated expression of IL-1 β and MMP-12 in monocytoid cells; and *in vivo*, systemic treatment of rats with minocycline at 1 and 12 h after induction of ICH by collagenase injection reduced macrophage infiltration, microglia hypertrophy, and appearance of apoptotic cells, concomitantly with inhibition of MMP-12 expression. Moreover, minocycline treatment improved behavioral performance (20). Another study demonstrated that minocycline treatment starting immediately after autologous blood injection attenuated brain edema, brain tissue atrophy, and behavioral deficits in rats (67). Minocycline was ineffective in reducing brain infarct and behavioral deficits, despite suppressing activation of microglia/macrophages, when administration was started 3 h after ICH induction by collagenase (68). However, other investigators reported that minocycline administration beginning 6 h after collagenase-induced ICH in rats ameliorated brain tissue damage via reduction in edema, microvessel loss, neutrophil infiltration, and expression of TNF- α and MMP-12 (69), but had no beneficial effect on striatal tissue loss and neuron loss outside the hematoma (70). A recent publication proposed that delivery of a high concentration of minocycline locally into the brain, with supplementation via systemic administration, achieved better neuroprotection in the autologous blood-injection model in mice (71).

3.4. Statins

Statins refer to a category of drugs used for treatment of hypercholesterolemia. Based on their inhibitory actions on hydroxymethylglutaryl-CoA reductase, these drugs interfere with cholesterol biosynthesis in the liver and lower serum cholesterol levels. Similar to the case with minocycline, potential applications of statins for the treatment of neurological disorders have been a topic of recent investigations.

In a rat autologous blood injection model, oral administration of atorvastatin starting from 24 h after ICH significantly reduced the severity of neurological deficits and the area of cell loss in the striatum (72). The authors attributed these effects of atorvastatin to the ability of

this drug to increase synaptic plasticity, since expression of markers of synaptogenesis, immature neurons and neuronal migration was enhanced by the treatment (72). A later study by the same group (73) has demonstrated that simvastatin, in addition to atorvastatin, produced beneficial effects against ICH pathology. Simvastatin tended to produce greater effects in reducing hematoma volume and tissue loss than atorvastatin. Another group using a collagenase injection model also demonstrated that two-week administration of atorvastatin starting 6 h after induction of ICH reduced edema, brain atrophy, and occurrence of apoptosis. Expression of iNOS and the number of neutrophils and microglia/macrophages were also reduced by atorvastatin, and reduction in these pathological changes was accompanied by improved performance in sensorimotor activities in treated animals (74).

In contrast to these promising results in animal experiments, epidemiological studies draw controversial conclusions as to whether or not statin use brings beneficial influences on the clinical outcome of ICH. Retrospective studies on ICH patients in Johns Hopkins Hospital from 1999 to 2006 suggested association of prior statin use with decreased mortality (75) and decreased perihematomal edema (76). On the other hand, a prospective cohort study did not find association between prior use of statins and improvement in functional outcome or mortality after ICH (77).

3.5. Nuclear receptor ligands

Peroxisome proliferator-activated receptor- γ (PPAR γ) is a member of the nuclear receptor superfamily, regulating transcription of various genes including those related to lipid and glucose metabolism. With relation to CNS pathology, stimulation of PPAR γ by ligands such as 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) has been reported to prevent microglial activation (78). Consistent with this fact, administration of 15d-PGJ₂ directly into the hematoma reduced inflammatory responses associated with autologous blood injection, as shown by decreased NF- κ B activation and neutrophil infiltration (79). These effects were accompanied by reduced apoptosis and improved behavioral functions in rats. In addition, stimulation of PPAR γ by rosiglitazone, another agonist, enhanced the phagocytic activity of microglia in primary culture (80). Enhanced phagocytic activity of microglia toward red blood cells may promote hematoma resolution *in vivo*, thereby attenuating brain cell damage associated with ICH (Fig. 2A). Indeed, intraperitoneal administration of rosiglitazone starting from 24 h after autologous blood injection reduced hematoma volume and improved behavioral performance in mice (80).

There are several reports suggesting beneficial effects

Fig. 2. Proposed mechanisms of action of nuclear receptor ligands in the brain after ICH. A) Peroxisome proliferator-activated receptor- γ (PPAR γ) acts as a transcriptional regulator in combination with retinoid X receptor (RXR). Stimulation of PPAR γ in microglia/macrophages by ligands such as 15d-PGJ₂ and rosiglitazone (a) results in reduced production of inflammatory mediators (b) and enhanced phagocytic activity toward red blood cells in the hematoma (c). B) Similarly to PPAR γ , retinoic acid receptor (RAR) forms a heterodimer with RXR to regulate gene transcription, although RXR-independent mechanisms of RAR signaling may also exist. Stimulation of RAR in microglia/macrophages by the specific agonist Am80 (a) prevents activation of these cell populations and reduces inflammatory reactions (b). RAR agonist might also act on neurons (c) and enhance production of neurotrophic factors such as brain-derived neurotrophic factor that protects neurons in an autocrine/paracrine manner (d).

of the glucocorticoid dexamethasone in ICH animal models (81, 82), although an earlier clinical study casted caution on the usage of this drug for the treatment of primary supratentorial ICH (83). Intraperitoneal injection of dexamethasone in rats at 2 and 24 h after collagenase injection significantly reduced hematoma volume, neutrophil infiltration, and neurological deficits (82). Modulation of aquaporin-4 expression by dexamethasone might result in reduced edema formation after ICH (84).

We explored the possibility that retinoic acid receptors (RARs) may become a novel target of neuroprotective therapy for ICH. This assumption was based on the fact that RAR stimulation suppressed inflammatory responses of microglia (85). In addition, we recently found that RAR stimulation by the specific agonist Am80 (tamibarotene) increased expression of brain-derived neu-

rotrophic factor in brain tissues and protected midbrain dopaminergic neurons from inflammatory degeneration (86, 87). Therefore, both the anti-inflammatory action and neurotrophic action of the RAR agonist might exert beneficial influences on the integrity of brain tissue after ICH (Fig. 2B). We examined the effect of Am80 on the collagenase-based ICH model in mice. Daily oral administration of Am80, starting from up to 6 h after induction of ICH, effectively prevented neuron loss within the hematoma. Am80 treatment had no effect on injury volume or the extent of brain edema, but significantly reduced the number of activated microglia/macrophages and expansion of oxidative stress in the perihematomal region. Moreover, Am80 treatment improved recovery of mice from neurological deficits after ICH (88).

3.6. Others

Other miscellaneous targets and pharmacological interventions have been reported to produce therapeutic effects against ICH-associated pathology and neurological dysfunction. Tauroursodeoxycholic acid (TUDCA) is a derivative of the endogenous bile acid ursodeoxycholic acid, which has anti-apoptotic properties and alleviates endoplasmic reticulum stress. Administration of TUDCA through the internal carotid artery from up to 3 h after collagenase injection into the striatum reduced ICH-induced apoptotic events and improved motor performance in rats (89). Inhibition of apoptotic events and upregulation of neuroprotective gene products are also achieved by intraperitoneal injection of valproic acid, an anti-epileptic drug and histone deacetylase inhibitor. Indeed, valproic acid treatment reduced hematoma volume, inflammatory events, and neurological deficits, concomitantly with upregulation of HSP70 and antiapoptotic proteins Bcl-2 and Bcl-x1 (90). Inhibitors of tissue-type transglutaminase (cystamine) and the ubiquitin-proteasome system (bortezomib) as well as an antagonist at AT1-angiotensin receptors (telmisartan) have also been reported to improve various pathological parameters associated with ICH (91–93), although exactly how these molecular systems contribute to ICH pathogenesis remains to be determined.

Table 1 presents a list of drugs shown to be effective in ICH animal models. The list includes only drugs that showed significant beneficial effects, when systemic (not intracerebral) administration of the drug was performed in a post-treatment regimen, where the first dose was given at or more than 1 h after induction of ICH.

4. Perspectives

Recent advances in basic research on ICH animal models have been elucidating key aspects of the patho-

Table 1. List of drugs reported to show beneficial effects on ICH animal models in post-treatment regimen *¹

Drug name	Model * ²	Route	Timing of administration	Parameters assessed * ³									Ref.
				Behavior	Edema	Volume of injury or hematoma	Brain atrophy	Apoptosis /Neurode-generation	Microglia /Macro-phages	Neutro-phil	Cyto-kines	Oxidative stress	
Argatroban	rat/B	i.p.	6 h post, continuous infusion		Yes	No							8
BB-1101	rat/C	i.p.	6 h post		Yes								15
GM6001	mouse/C	i.p.	2 h post, then every 24 h	Yes	Yes	Yes		Yes	No	Yes		Yes	18
Deferoxamine	rat/B	i.p.	2 or 6 h post, then every 12 h	Yes * ⁴	Yes							Yes * ⁴	35
Deferoxamine	rat/B	i.p.	2 h post, then every 12 h	Yes			Yes						100
Deferoxamine	rat/B	i.m.	up to 48 h post, second injection 4 h later, then every 12 h	Yes	Yes * ⁵		Yes * ⁶						36
Clioquinol	rat/C	p.o.	6 h post, then every 24 h	Yes				Yes					38
C3aRA	mouse/B	i.p.	6 and 12 h post, then twice daily	Yes		No			No	Yes			44
C5aRA	mouse/B	i.p.	6 and 12 h post, then twice daily	Yes	No				No	Yes			46
C3aRA/C5aRA	mouse/B	i.p.	6 and 12 h post, then twice daily	Yes	Yes				No	Yes			46
Dimethylthiourea	rat/C	i.p.	2 h post, then twice daily	Yes		No	No	No					58
PBN	rat/C	i.p.	2 h post, then twice daily	Yes		No	No	No					58
G-CSF	rat/C	i.p.	2 h post, then daily	Yes	Yes	No	Yes	Yes	Yes	Yes			63
G-CSF	rat/C	i.p.	24 h post, then daily	Yes									64
Erythropoietin	rat/C	i.p.	2 h post, then daily	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		65
Minocycline	rat/C	i.p.	1 and 12 h post, then twice daily for 1 week, and once daily for another week	Yes				Yes	Yes	No	Yes		20
Minocycline	rat/C	i.p.	3 h post, then twice daily	No		No			Yes				68
Minocycline	rat/C	i.p.	6 h, 1 d and 2 d post		Yes					Yes	Yes		69
Minocycline	rat/C	i.v. /i.p.	6, 24, 48, and 72 h post			No		No	Yes	Yes			70
Atorvastatin	rat/C	p.o.	6 h post, then daily	Yes	Yes	No	Yes	Yes	Yes	Yes			74
Atorvastatin	rat/B	p.o.	24 h post, then daily	Yes		No	Yes	Yes					72, 73
Simvastatin	rat/B	p.o.	24 h post, then daily	Yes		Yes	Yes						73
Rosiglitazone	mouse/B	i.p.	24 h post, then daily	Yes		Yes					Yes	Yes	80
Dexamethasone	rat/C	i.p.	1 and 24 h post	Yes		Yes		Yes		Yes			81
Dexamethasone	rat/C	i.p.	2, 4, or 6 h post, then 24 h post	Yes * ⁴		Yes		Yes * ⁴		Yes			82
Am80	mouse/C	p.o.	2 or 6 h post, then every 24 h	Yes	No	No		Yes	Yes			Yes	88
TUDCA	rat/C	i.a.	1 or 3 h post	Yes		Yes		Yes					89
Valproic acid	rat/C	i.p.	2 h post, then twice daily	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes		90
Cystamine	rat/B	i.p.	2 h post, then daily	Yes	Yes			Yes					91
Bortezomib	rat/C	i.v.	2 h post		Yes	Yes		Yes	Yes	Yes	Yes		92
Telmisartan	rat/C, B	p.o.	2 h post	Yes	Yes	Yes		Yes	Yes	Yes	Yes	Yes	93

*¹ See text and figures for details of the actions of each drug. Actions of argatroban, BB-1101, GM6001, deferoxamine, clioquinol, C3aRA, and C5aRA are illustrated in Fig. 1 and mentioned in section 2. Actions of other drugs are mentioned in section 3. The mechanisms of actions of rosiglitazone and Am80 are also illustrated in Fig. 2. *² 'C' represents the collagenase-injection model, and 'B' represents the autologous blood-injection model. *³ 'Yes' means that the pathological change was alleviated by drug treatment, and 'No' means that the drug showed no beneficial effect on the respective parameter. *⁴ Effective when administered up to 2 h post-induction of ICH, *⁵ up to 12 h post, *⁶ up to 24 h post. Abbreviations: C3aRA, complement C3a-receptor antagonist; C5aRA, complement C5a-receptor antagonist; PBN, α -phenyl-*N*-tert-butyl nitron; G-CSF, granulocyte colony-stimulating factor; TUDCA, tauroursodeoxycholic acid.

genesis of ICH, several of which may have potentials to be targeted by drug therapies. However, detailed mechanisms of ICH pathogenesis still remain largely unexplored. Microarray analyses have indicated that hundreds of genes, including those involved in inflammation, metabolism, and neuronal signal transduction, are up-regulated or downregulated in the rat ICH model and human ICH patients (94, 95). Clarification of the roles of these gene products in ICH pathogenesis might reveal novel therapeutic targets.

On the experimental basis, the number of drugs effective for improving pathological and neurological outcomes of ICH is increasing. However, whether these drugs also show efficacy in human patients remain unproven. A problem is that many studies on drug effects in animal models focused mainly on the acute phase of neuropathology, and data of the long-term outcome of drug treatments over months are still scarce. During the extended time course after ICH, repair processes may take place in the damaged brain, which may also be affected by drug treatments. For example, inhibition of the protein kinase Src can improve the acute outcome, such as neurodegeneration and abnormal behavior, after autologous blood injection in rats (96). On the other hand, prolonged inhibition of Src in the ICH model disturbs repair processes in the neurovascular niche that forms the blood–brain barrier (97), which might worsen long-term outcome. Notably, several drugs including G-CSF (64) and statins (73) have been reported to stimulate neurogenesis in hemorrhagic brain, but contribution of neurogenesis to the effects of these drugs in improving pathological and neurological parameters is unclear at present.

Another issue to be considered is that the severity of neurological symptoms after ICH may be closely associated with the extent of white matter injury (38, 98), whereas the majority of experimental studies is concerned about loss of neuronal cell bodies. We should keep in mind that from the clinical point of view, the most important issues in ICH therapies are reduction of mortality and alleviation of neurological deficits. In this context, research on ICH animal models may require some kinds of refinement for the experimental results to be extrapolated into clinical situations. Without these considerations, we might be trapped into pitfalls reminiscent of those experienced in research on ischemic stroke, where a wide variety of treatments were proven to be effective in animal models but the vast majority of them failed in clinical trials (99).

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