Intracerebral Hemorrhage as an Axonal Tract Injury Disorder with Inflammatory Reactions

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Intracerebral hemorrhage (ICH) is a neurological disorder frequently accompanied by severe dysfunction. Critical pathogenic events leading to poor prognosis should be identified for the development of novel effective therapies for ICH. Here we focus on the injury of the axonal tract, particularly of the internal capsule, with reference to its contribution to ICH pathology and potential therapeutic interventions in addition to its cellular mechanisms. Studies on human ICH patients and rodent models of ICH suggest that invasion of hematoma into the internal capsule greatly worsens the severity of post-ICH symptoms. A blood-derived protease thrombin may play an important role in the acute phase of axonal tract injury in the internal capsule that includes compromised axonal transport and fragmentation of axonal structures. Several agents such as clioquinol, melatonin and Am80 (a retinoic acid receptor agonist) have been shown to produce therapeutic effects on rodent models of ICH associated with injury of the internal capsule. In the course of examinations on the effect of Am80, we obtained evidence for the involvement of CXCL2, a neutrophil chemotactic factor, in the pathogenesis of ICH. Accordingly, we also refer to the potential roles of infiltrating neutrophils and inflammatory responses in axonal tract injury and resultant neurological dysfunction in ICH.

Key words hemorrhagic stroke; axonal degeneration; motor deficit; chemokine; neutrophil

1. INTRODUCTION

Intracerebral hemorrhage (ICH), triggered by rupture of blood vessels within the brain parenchyma, is a devastating neurological disorder featured by high mortality rate and severe neurological dysfunction. ICH is categorized as a kind of stroke disorders that include ischemic stroke and subarachnoid hemorrhage, and patient populations of ICH are estimated to occupy 10–15% of all stroke cases. Several drugs have been developed for the treatment of stroke disorders other than ICH, such as thrombolytic agents for ischemic stroke and vasodilating agents for subarachnoid hemorrhage-associated vasospasm. In contrast, effective therapeutic drugs for ICH are yet unavailable, which may be a major cause of intractability of this disorder.

Recent advances in preclinical researches have been revealing the pathogenic mechanisms of ICH, and thereby proposing various kinds of drug targets for potential therapeutics. On the other hand, translation of the results from experimental findings in stroke fields into the clinical stages in human stroke patients has experienced extreme difficulties, particularly in the case of ischemic stroke. Similar problems are concerned with ICH, and the critical issues to be considered for preclinical researches on ICH have been discussed in several previous publications. Here we set focus on an overlooked problem of the experimental models of ICH, with regard to axonal tract injury. We discuss why axonal tract injury is important in ICH pathology, how the researchers can perform experiments on ICH with axonal tract injury, and what kinds of interventions have potentials for novel therapies for ICH.

2. AXONAL TRACT INJURY IN ICH

2.1. Human ICH Cases

ICH may occur in all brain regions since vascular architectures are distributed all over the central nervous system. However, because each brain region participates in a specific set of neural functions that regulates cognition, sensation, motor coordination and various others, the severity and the characteristics of the symptoms associated with ICH are strongly dependent on the location of the focal sites affected by the hemorrhage. In humans, several brain regions such as the putamen and the thalamus are particularly susceptible to ICH events. This is at least in part due to the structural characteristics of vascular networks in these brain regions, where artery branches with small diameters are densely distributed. Notably, the severity of the symptoms associated with ICH initiated in the putamen and the thalamus seems to be correlated with the extent of injury of the internal capsule, rather than the damage within the respective brain regions per se. The internal capsule is the white matter region composed of several groups of fiber tracts connecting the cerebral cortex and the lower brain regions such as the spinal cord. These fiber tracts lie between the putamen and the thalamus, and convey descending information such as movement control by the primary motor cortex.

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and ascending information such as sensory inputs initiated by the primary afferents. When ICH occurs in the putamen or in the thalamus, hemorrhage often invades into the nearby internal capsule (Fig. 1). Then, the resultant injury of the axonal tracts may well shut down information processing depending on the communications between the upper brain regions and the lower areas including the peripheral inputs/outputs, thereby induces neurological dysfunction such as sensorimotor hemiparesis.\(^7\)\(^9\) Consistent with this view, a recent study has demonstrated that focal infarcts induced by local injection of endothelin into the internal capsule resulted in long-lasting severe motor deficits in rats.\(^8\) These facts indicate that protection of axonal fiber tracts may serve as a promising strategy to realize effective alleviation of neurological symptoms associated with ICH and other stroke disorders. Therefore, preclinical studies using experimental animal models should incorporate this point of view for their findings to be valid for application to human cases.

### 2.2. Rodent Models of ICH

Rodents such as rats and mice have been conventionally used for preclinical studies on ICH, although a small number of studies deal with other species such as rabbits, dogs and pigs.\(^11\)\(^12\) Injection of autologous blood and injection of collagenase (to disrupt vascular walls) into the brain parenchyma are the representative procedures to produce pathology of ICH in rodent brains. In many studies the striatum (including the putamen) is used as the focal site of experimental ICH because this region is susceptible to ICH incidents in humans and is easily accessible by surgical manipulations. Analyses of histological and biochemical events in experimental ICH models have revealed the involvement of various critical components in the pathogenesis of ICH that are initiated by blood-derived proteases and hemolysis-derived products of heme degradation, which has been reviewed elsewhere.\(^2\) Unfortunately, few studies have paid attention to the injury of axonal tracts such as the internal capsule, probably because rodent brains have much smaller volume of white matter than human brain. Nevertheless, the importance of white matter injury in ICH pathology has been suggested by several key studies. For example, a pioneering work by Masuda et al.\(^13\) reported a rat model of ICH induced in the internal capsule by injection of collagenase. They demonstrated that severe motor dysfunction was induced by a much smaller amount of collagenase injected near the internal capsule than that injected into the striatum, suggesting that the injury of the internal capsule determines the severity of neurological symptoms.\(^15\) We set two sites for injection of collagenase: one was located near the internal capsule, and the other was distant from the internal capsule to minimize the possibility for the hematoma to expand into the axonal tract (Fig. 2). We observed that, when hematoma volume exceeded 5 mm\(^3\), the mortality rate and the severity of sensorimotor dysfunction were independent of the absolute volume of hematoma. Rather, the degree of the resultant dysfunction was clearly affected by the presence/absence of hematoma in the internal capsule. Namely, mice with the internal capsule injured by hematoma exhibited a low rate of survival and impaired per-
3. MECHANISMS AND POTENTIAL THERAPEUTIC INTERVENTIONS

3.1. Mechanisms of Axonal Tract Injury in ICH
Axonal degeneration is recognized as a critical process that determines the severity of the symptoms of various neurological disorders. However, information on the pathogenic mechanisms of axonal tract injury in ICH is limited. We recently addressed the correlation between the histopathological events and neurological dysfunction of mice after ICH. Fragmentation of axonal structures as determined by neurofilament-H immunoreactivity was evident from 6 h after ICH induced by collagenase injection near the internal capsule. In contrast, severe neurological dysfunction was observed already at 3 h after induction of ICH. The early manifestation of neurological dysfunction seemed to be related with the impairment of axonal transport that was revealed by accumulation of amyloid precursor protein (APP) as a substrate for fast axonal transport. Indeed, we observed severe deficits in motor performance at 3 h after injection of an axonal transport inhibitor colchicine into the internal capsule of mice, whereas the destruction of axonal structures was not observed until 72 h after colchicine injection. Notably, thrombin injection into the internal capsule also resulted in concurrent manifestation of neurological dysfunction with impairment of the axonal transport, which preceded fragmentation of axonal structures. Therefore, a blood-derived protease thrombin may play a key role in the acute phase of ICH pathology by triggering deficits in axonal transport. Detailed molecular mechanisms of the action of thrombin remain an open question, and we demonstrated that stimulation of protease-activated receptor-1 was not sufficient for inhibition of axonal transport.

Injury of axonal tracts involves demyelination process as well as the destruction of axonal fibers. In a recent study, Zhuo et al. addressed the mechanisms of demyelination in rats that received autologous blood injection into the area adjacent to the internal capsule. Under their experimental conditions, demyelination was observed progressively between 1 and 7 d after blood injection, as revealed by the ultrastructural changes in myelinated nerve fibers as well as the decrease of myelin basic protein. Concurrently, apoptosis of myelin-forming oligodendrocytes was observed as activation of caspase-3 in this cell population. Caspase-3 activation can be initiated by several pathways, and Zhuo et al. observed both activation of caspase-4 (that may represent the involvement of endoplasmic reticulum stress) and release of cytochrome c (that represents activation of mitochondrial apoptotic pathway).

Overall, investigations on the cellular and molecular mechanisms of axonal tract injury in ICH are just at the beginning, and advancement in this research field may prove novel therapeutic targets for ICH.

3.2. Exploration of Drugs for ICH with Axonal Tract Injury
Provided that axonal tract injury is the major determinant for the resultant neurological dysfunction, research efforts aiming to establish effective therapies for ICH should utilize appropriate experimental models accompanied by the injury of axonal tracts, particularly of the internal capsule. However, there are only a few studies that take this viewpoint into consideration. The earliest work by Masuda et al. demonstrated that clioquinol as a metal-chelating agent attenuated ICH-induced injury of the internal capsule and motor deficits in rats, probably via diminution of oxidative stress. The same group later reported the effect of melatonin on the same experimental model of ICH induced by collagenase injection near the internal capsule. Daily oral administration of melatonin attenuated ICH-induced oxidative stress, preserved neurons in the sensorimotor cortex and diminished motor deficits.

We have made several attempts to find out novel therapeutic targets for ICH, using a conventional mouse model of ICH induced by collagenase injection into the striatum. In the course of these investigations, we found that a retinoic acid receptor (RAR) agonist Am80 produced a beneficial effect. RAR is expressed in neurons, astrocytes and microglia and therefore widely distributed in the central nervous system. Notably, a subsequent study revealed that Am80 inhibited accumulation of APP in the perihematomal region, suggesting that the drug had a protective effect on axonal function. These results prompted us to examine the effect of Am80 on ICH accompanied by the injury of the internal capsule.

As expected, Am80 produced a significant ameliorative effect on motor performance in mice after ICH in the internal capsule. At the same time, we found that Am80 inhibited ICH-induced increase in the expression of mRNAs encoding several cytokines and chemokines, which include interleukin (IL)-1β, IL-6 and CXCL2. On the other hand, a glucocorticoid dexamethasone inhibited expression of IL-1β and IL-6 but had no effect of CXCL2 expression, while it did not ameliorate ICH-induced impairment of motor performance. Interestingly, brains of mice after ICH with injury in the internal capsule showed a higher expression level of CXCL2 mRNA than those without injury in the internal capsule. Moreover, when we administered reparixin, an antagonist at chemokine receptors CXCR1/CXCR2, we observed a significant recovery of motor performance of mice after ICH in the internal capsule. These results suggest that CXCL2 plays an important role in the pathogenic events in ICH with axonal tract injury.

3.3. Neutrophils in Axonal Tract Injury
Because CXCL2 is a chemotactic factor for neutrophils (polymorphonuclear leukocytes), the findings mentioned above also imply possible involvement of neutrophils in axonal tract injury in ICH. Neutrophils are the first responders in inflammatory reactions in various tissues and organs. In the case of ICH, infiltration of leukocytes into the brain may occur as a cell population distinct from that resulting from hemorrhage itself. A study by Moxon-Emre and Schlichter provided evidence for a critical role of neutrophils in the pathogenesis of ICH. They examined the effect of neutrophil depletion that was achieved by intravenous pre-administration of anti-polymorphonuclear neutrophil antibody to rats. As a result of neutrophil depletion, ICH induced in the striatum by collagenase injection was accompanied by preserved integrity of the blood–brain barrier, reduced activation of astrocytes and reduced accumu-
lation of microglia/macrophages in the perihematomal region. Importantly, neutrophil depletion also attenuated early axon damage in the striatum as assessed by APP accumulation and fragmentation of myelin basic protein-immunopositive signals, which seemed to correlate with the activation levels of microglia/macrophages. Neutrophil infiltration into tissues can be promoted by several kinds of chemotactic factors including CXCL2. Although we have demonstrated a therapeutic effect of a CXCR antagonist reparixin on the basis of motor performance, the precise modes of action of this drug such as those on neutrophil infiltration and axonal tract injury should be elucidated by further investigations. We should also note here that ICH may be accompanied by increases in several other factors that stimulate neutrophil chemotaxis into the brain. One of the candidates is leukotriene B4 (LTB4), because brain tissues from human ICH patients express increased levels of mRNAs encoding 5-lipoxygenase and its activating protein that are involved in LTB4 biosynthesis. Indeed, we recently obtained evidence for the involvement of LTB4 in the pathogenesis of ICH in the internal capsule, such that blockade of LTB4 receptor attenuated neutrophil infiltration, axonal tract damage and neurological dysfunction in mice.

4. CONCLUSION AND FUTURE PERSPECTIVES

The injury of axonal tracts that connect the upper and the lower regions in the central nervous system may be a critical determinant of the severity of the consequences of ICH, particularly in the cases with putaminal and thalamic ICH. Although few studies have aimed to reveal the mechanisms of axonal tract injury in ICH, key findings have begun to emerge. Prominent examples include several lines of evidence for the involvement of neutrophil-related events as discussed above. As neutrophils are one of the initial players of inflammatory responses, axonal tract injury induced by ICH may be viewed in the context of inflammatory disorders. Consistent with this idea, Moxon-Emre and Schlüchter demonstrated close association of axonal damage in the white matter of the striatum with activation of microglia/macrophages. In addition, recruitment of blood monocytes (that should turn into tissue macrophages) into the brain has been suggested to exacerbate inflammation and outcomes of ICH. Moreover, activation of nuclear factor-κB, a transcriptional regulator of inflammatory responses, was shown to have close relation with the clinical outcome of human ICH patients. On the other hand, it should be noted that both neutrophils and microglia/macrophages can be either destructive or protective to injured tissues, presumably depending on their polarized phenotypes. These cell populations may act in distinct temporal and spatial patterns to affect the integrity of axonal tracts after ICH insults, and our current working hypothesis is depicted in Fig. 3. Overall, further investigations on the cellular and molecular mechanisms of axonal tract injury will be required to identify plausible drug targets for ICH that can alleviate axonal injury and dysfunction.

Finally, we must note again that the clinical consequences of ICH depend on the brain regions affected, and therefore, different approaches from those reviewed here may be explored for ICH that is not accompanied by the injury of the internal capsule. Indeed, ICH in the cerebral cortex (lobar hemorrhage) recently becomes the major type of ICH, probably as a consequence of population aging, advancement in management of blood pressure, and widespread use of anti-thrombotic agents. In this context, we are also engaged in experimental studies addressing the mechanisms and potential therapeutic interventions of ICH in the cerebral cortex. Di verse sets of experimental approaches would aid the discovery of truly effective therapies for ICH.

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