Brain Injury After Intracerebral Hemorrhage

The Role of Thrombin and Iron

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Abstract—Intracerebral hemorrhage (ICH) is a subtype of stroke with high morbidity and mortality. The mechanisms underlying ICH-induced brain injury have become better understood during the past decade. Experimental investigations have indicated that thrombin formation, red blood cell lysis, and iron toxicity play a major role in ICH-induced injury and that these mechanisms may provide new therapeutic targets. This article reviews the role of thrombin and iron in ICH-induced injury. (Stroke. 2007;38[part 2]:759-762.)

Key Words: brain edema • cerebral hemorrhage • iron • thrombin

Spontaneous intracerebral hemorrhage (ICH) often causes immediate death. If the patient survives from the ictus, the hematoma can lead to secondary brain injury and sometimes delayed fatality. Recent studies have helped to elucidate the mechanisms that trigger pathophysiological changes in and around the hematoma. In particular, they have focused attention on the role of thrombin and iron, released upon red blood cell (RBC) lysis, as 2 major factors causing brain injury after ICH.

Thrombin and Brain Injury After ICH

Thrombin-Induced Brain Injury

Thrombin is a serine protease and an essential component in the coagulation cascade. It is produced immediately in the brain after an ICH to stop the bleeding. However, direct infusion of large doses (5 U) of thrombin into brain causes inflammatory cell infiltration, brain edema formation, and neuronal death. We have demonstrated that thrombin is responsible for early brain edema formation after ICH and that such edema results partly from a direct opening of the blood-brain barrier. Because thrombin can be detrimental at high concentrations and protective at low concentrations, it is important to know the concentrations of thrombin that may occur in the brain after ICH. The concentration of prothrombin in the plasma is high enough (1 to 5 μmol/L) to produce a substantial amount of thrombin in the brain parenchyma after a hemorrhage. Thus, 1 mL of whole blood can produce 260 to 360 U of thrombin, and a 50-μL clot could be expected to produce up to 15 U of thrombin. In the rat, the brain edema produced by intracerebral hematoma can be significantly inhibited by thrombin inhibitors, and an intracerebral infusion of 5 U of thrombin causes a degree of edema similar to that of a 50-μL clot. Therefore, this dose of thrombin was used in our previous experiments.

To examine whether delayed and systemic administration of a thrombin inhibitor could reduce ICH-induced injury, experiments were performed with argatroban. Intracerebral infusion of blood caused a marked increase in perihematomal water content. Intracerebral injection of argatroban 3 hours after ICH caused a significant reduction in edema measured at 48 hours. The systemic administration of high-dose argatroban (0.9 mg/h) starting 6 hours after ICH also significantly reduced edema.

Advances have been made in understanding the manner in which intracerebral thrombin may have adverse effects on the brain. Thrombin at high concentrations kills neurons and astrocytes in vitro. Thus, to assess the direct toxic effects of thrombin on brain cells, mixed rat neuron/astrocyte cultures were exposed to different doses of thrombin (1, 2, 5, 10, 20, 50, or 100 U/mL) for 24 hours, and media lactate dehydrogenase concentrations were determined as an indicator of cell viability. Low doses of thrombin (1 and 2 U/mL) did not induce cell death. However, doses >5 U/mL resulted in dose-dependent lactate dehydrogenase release.

Thrombin-induced brain injury may be mediated by the complement cascade. Intracerebral infusion of thrombin in rat resulted in a 7-fold increase in complement C9 and deposition of complement C9 on neuronal membranes. Clusterserin, an inhibitor of the membrane attack complex formation, was also upregulated by the thrombin and found in neurons. The effects of coagulation cascade on complement activation are not well studied. However, studies suggest that there is a very close relationship between thrombin and complement. For example, thrombin-cleaved C3a-like fragments are chemotactic for leukocytes and induce enzyme release from neutrophils.
Tumor necrosis factor-alpha (TNF-α) is one of the major proinflammatory cytokines, and it may contribute to brain injury after ICH. TNF-α levels in the brain are increased after intracerebral infusion of thrombin and ICH.11 ICH-induced brain edema was less in TNF-α knockout mice than in wild-type mice (Figure 1). In addition, thrombin activates matrix metalloproteinase–2 in endothelial cells.12 Matrix metalloproteinases are members of a family of zinc-dependent proteases that can degrade extracellular matrix and cause blood-brain barrier disruption.

Intracerebral infusion of thrombin does not cause ischemic brain damage. Thus, after infusion of 10 U of thrombin in the rat, cerebral blood flow declined over the first hour, rose to baseline or above by 2 hours, and returned to baseline by 24 hours. The lowest blood flow, 45 ± 7 mL/100 g per minute, was recorded in the ipsilateral hemisphere at 1 hour, and this is well above the values expected to cause ischemic damage.3

Thrombin-Induced Neuroprotection
Thrombin can also have beneficial effects in ICH. Thus, it is involved in stopping the initial bleed and preventing hematoma enlargement that occurs in a percentage of ICH patients over the first day. This latter observation forms the basis of current factor VIIa trials.13 In addition, many studies indicate that although high concentrations of thrombin produce deleterious effects, brain exposure to low concentrations can induce protective effects. Thrombin preconditioning reduces brain injury in models of ICH and focal cerebral ischemia. Although the precise mechanisms of thrombin-induced brain tolerance to hemorrhagic and ischemic stroke are not known, activation of thrombin receptors, upregulation of iron handling proteins, and heat shock proteins in the brain may be associated with the induced tolerance.1,14

RBC Lysis, Iron, and ICH-Induced Brain Damage

RBC Lysis and Brain Edema Formation
Lysis of RBCs occurs several days after ICH, which results from either depletion of intracellular energy reserves or formation of membrane attack complex after activation of the complement system, or both.15,16 RBC lysis contributes to edema formation after ICH. A clinical study of edema and ICH indicates that delayed brain edema is related to significant midline shift after ICH in humans.17 This delayed brain edema (in the second or third week after onset in humans) is probably attributable to hemoglobin and its degradation products. Another recent study in ICH patients also suggests that delayed brain edema after ICH may result from hematoma lysis.18 A total of 17 spontaneous ICH patients were chosen in that study. All patients had the first CT scan within 5 hours of symptom onset and then underwent second, third, and fourth CT scans at 1, 3, and 10 days later. That study found that hematoma size was reduced significantly at day 10 as a result of clot lysis. However, edema volume increased gradually over that time.

In animal studies, an intracerebral infusion of lysed RBCs but not packed RBCs resulted in marked brain edema formation and DNA injury within 24 hours.19,20 However, the infusion of packed RBCs caused edema and neurological deficits several days later, suggesting that RBCs are associated with delayed brain edema formation.6,19

Whether or not ischemia contributes to perihematomal edema development is still controversial. An intracerebral infusion of RBC hemolysate induced marked edema, but cerebral blood flow in the vicinity remained near normal. It appears that the edema formation is related to marked blood-brain barrier disruption from the toxic effect of lysed RBCs rather than from ischemia.21

Heme is degraded by heme oxygenase (HO) in the brain into iron, carbon monoxide, and biliverdin. An intracerebral infusion of hemoglobin or its degradation products caused brain edema within 24 hours.22 Our recent studies have shown that HO-1 protein levels are increased after ICH.23 The biological significance of HO-1 upregulation is still uncertain, but tin-mesoporphyrin, a HO inhibitor, attenuates perihematomal edema in a pig ICH model and reduces neuronal loss in a rabbit ICH model.23,24

Intracerebral infusion of iron causes brain edema, and iron can exacerbate thrombin-induced brain edema.25,26 When RBCs start to lyse, hemoglobin degradation can result in iron release. The
Iron concentration could reach as high as 10 mmol/L, which causes marked brain edema.25 Our recent study found that ferrous iron at low concentrations (for example, 0.2 mmol/L) can also induce brain damage.27 Deferoxamine, an iron chelator, reduces hematoma- and hemoglobin-induced edema, suggesting that iron plays an important role in edema formation after ICH.28

Iron Overload, Brain Atrophy, and Neurological Deficits After ICH
After erythrocyte lysis, iron concentrations in the brain reach very high levels. We found a 3-fold increase of brain nonheme iron after ICH in rats, and this level remained high for at least 1 month.22 Iron-induced brain damage may result from oxidative stress.29 Antioxidants block neuronal toxicity induced by hemoglobin.30,31

Clinical and experimental studies have demonstrated that brain atrophy occurs after ICH.32,33 The underlying cause(s) of this atrophy is, however, unknown. In a recent study, we demonstrated that brain atrophy developed gradually and peaked between 1 and 2 months after ICH in the rat33 (Figure 2). Brain atrophy was associated with prolonged neurological deficits. Deferoxamine, an iron chelator, reduced brain atrophy (Figure 3), reduced brain ferritin immunoreactivity, and improved neurological deficits after rat ICH.

The Relationship Among Brain Edema, Cell Death, and Neurological Deficits
A rat ICH model involving infusion of autologous blood into the caudate has been used extensively to study mechanisms of brain edema formation.16,19,34–36 It has, however, been difficult to obtain other quantifiable markers of brain injury because neuronal injury appears to be diffuse (ie, there is no defined infarct), and only a small cavity is found after the clot is absorbed. However, our recent study showed that there is a correlation between ICH-induced neurological deficits and brain edema.6 Although it is not clear whether edema exaggerates cell death, we have demonstrated that deferoxamine reduces brain edema, brain atrophy, and neurological deficits in a rat model of ICH.28,33

Figure 2. Brain atrophy after ICH in the rat. Coronal gross sections (hematoxylin-eosin staining) 4 weeks after intracerebral insertion of needle (sham) (A) or intracerebral injection of 100-μL autologous blood (B). *Site of blood injection. C, Caudate volume was measured at 2, 4, 8, and 12 weeks after either needle insertion or 100-μL autologous blood infusion into the right caudate. Values are mean±SD; n=4 to 5. *P<0.05, #P<0.01 vs saline control. D, Cortex volume was measured at 2, 4, 8, and 12 weeks after either needle insertion or 100-μL autologous blood infusion into the right caudate. Values are mean±SD; n=4 to 5. From J Neurosurg. 2006;104:305–312, with permission.

Figure 3. Deferoxamine (DFX) attenuated caudate atrophy (ipsilateral as a percentage of the contralateral caudate) (A) and lateral ventricle enlargement (ipsilateral as a percentage of the contralateral lateral ventricle) (B) 4 weeks after ICH in the rat. Deferoxamine (100 mg/kg IP) was given every 12 hours for 7 days starting at 2 hours after ICH. Values are mean±SD; n=6. *P<0.05, #P<0.01 vs vehicle. From J Neurosurg. 2006;104:305–312, with permission.
Thrombin causes brain damage at high concentrations and induces neuroprotection at low concentrations. Modulating brain thrombin activity may establish novel therapeutic strategies for ICH, particularly if methods can be devised to elicit only beneficial effects. Iron is a major factor causing ICH-induced brain injury. Deferoxamine, an iron chelator, reduces ICH-induced brain edema, behavioral deficits, and atrophy, suggesting that it may be a useful therapy for ICH patients.

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Disclosures
None.

References
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