Deferoxamine Attenuates White Matter Injury in a Piglet Intracerebral Hemorrhage Model

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Background and Purpose—Deferoxamine reduces neuronal death in a piglet model of intracerebral hemorrhage (ICH).

This study examined the effect of deferoxamine on perihematomatous white matter edema in piglets.

Methods—ICH was induced by an injection of autologous blood into the right frontal lobe of piglets. In the first part of study, the time course of edema formation was determined. In the second part, the effects of deferoxamine on ICH-induced white matter edema, tumor necrosis factor α, and receptor-interacting protein kinase 1 were examined.

Results—ICH resulted in marked brain edema and increased tumor necrosis factor α and receptor-interacting protein kinase 1 levels in white matter. Systemic treatment with deferoxamine markedly reduced white matter tumor necrosis factor α and receptor-interacting protein kinase 1 levels and attenuated white matter edema after ICH.

Conclusions—Deferoxamine reduces white matter edema, tumor necrosis factor α, and receptor-interacting protein kinase 1 levels after ICH in piglets, suggesting deferoxamine is a potential effective therapeutic agent for patients with ICH. (Stroke. 2014;45:290-292.)

Key Words: brain edema ■ cerebral hemorrhage ■ deferoxamine ■ receptor-interacting protein serine-threonine kinase 1 ■ tumor necrosis factor-alpha

Intracerebral hemorrhage (ICH)—induced white matter injury is an important factor in patient outcomes. Approximately 50% human brain tissue is white matter. However, white matter injury after ICH has not been well studied.

Iron has a key role in brain edema formation after ICH.1 Perihematomatous brain edema develops immediately after an ICH and peaks several days later. Edema formation after ICH elevates intracranial pressure and may result in herniation.2 Perihematomatous edema is mainly located within white matter. However, the role of iron in ICH-induced white matter edema has not been studied.

ICH causes brain cell death, including necrosis/necroptosis, apoptosis, and autophagy.1 Necroptosis, also called programmed necrosis, is associated with the kinase activities of receptor-interacting protein kinase 1 (RIPK1). Tumor necrosis factor α (TNF-α), via activation of RIPK1, can induce necroptosis.3 Necroptosis contributes to brain injury in both ischemic and hemorrhagic stroke.3,4

In the present study, we examined the time course of white matter edema formation and the effect of deferoxamine on perihematomatous white matter edema in the piglet model of ICH. The effects of deferoxamine on brain TNF-α and RIPK1 were also examined.

Materials and Methods

Animal Preparation and Intracerebral Blood Infusion

The animal protocols for this study were approved by the University of Michigan Committee on the Use and Care of Animals. Male piglets, body weight of 8 to 10 kg, were obtained from Michigan State University (East Lansing, MI). All surgical procedures were performed with the use of aseptic techniques. Pigs were sedated with Telazol (6 mg/kg, IM) and Xylazine (2.2 mg/kg, IM.) for induction of anesthesia and endotracheal intubation. Piglets were inhaled with 2.0% isoflurane delivered via nose cone. The isoflurane concentration was maintained at 1.0% to 1.5% during the surgical procedures. Core temperature was maintained at 37.5±0.5°C by a warm water blanket (Gaymar, Orchard Park, NY). The right femoral artery was inserted with a polyethylene catheter (PE-160) to obtain blood for injection and to monitor arterial blood pressure and arterial blood gases.

A 1.5-mm cranial burr hole was drilled at a point 11 mm right of the sagittal suture and 11 mm anterior to the coronal suture. An 18.5-mm-long 18-gauge sterile plastic catheter was placed stereotaxically into the center of the right frontal cerebral white matter at the level of the caudate nucleus. First, 1 mL of autologous arterial blood was infused for 10 minutes with an infusion pump. Then, a second 1.5-mL blood was infused for 10 minutes after a 5-minute break. Sham-operated pigs had the same procedure except blood injection.3

There were 2 parts to this study using a total of 51 piglets. First, piglets had 2 injections of total 2.5-mL autologous blood injected into the right front lobe and were euthanized at days 1, 3, 7, and 14 (n=4–6) after the brains had been frozen in situ. Control piglets had a needle insertion (n=4) and were euthanized at day 3. Second, piglets had an ICH or a sham operation and were treated with vehicle (saline, n=9 for ICH and n=3 for sham) or deferoxamine (50 mg/kg, IM at 2 hours after ICH and given every 4 hours for 2 doses and then every 12 hours ≤72 hours; n=10 for ICH; n=3 for sham) for 3 days. Brains were used to determine brain water content and Western blot analyses.

Brain In Situ Freezing and Sampling

Brains were frozen in situ by decanting liquid nitrogen into a 12 oz bottomless foam cup adhered to the head with Dow-Corning high
vacuum grease (Dow Corning, Midland, MI), as described previously. The duration time of this process usually took ≈1 hour, and the head was removed after the injection of potassium chloride to stop the heart beating. The frozen head was then cut with a band saw into 5-mm-thick coronal sections. Tissues were sampled from the points of interest.

**Brain Water Content**

White matter tissue adjacent to the hematoma, including superficial gyral white matter and deep white matter, was sampled. Sampled brain tissue was placed on foils and weighed on an electronic analytic balance (model AE 100; Mettler Instrument Co) to obtain the wet weight (WW). Samples were then dried in a gravity oven at 100°C for 24 hours to obtain the dry weight (DW). Brain water content (%) was calculated as (WW−DW)/WW×100%.

**Western Blotting**

White matter tissue adjacent to the hematoma was sampled for Western blot analysis that was performed as previously described. The primary antibodies were mouse anti–TNF-α (1:1000 dilution; AbD serotec), mouse anti-RIPK1 (1:1000 dilution; R&D), or mouse anti–β-actin (1:10,000 dilution; Sigma). The secondary antibody was antimouse IgG HRP conjugated (Bio-Rad).

**Statistics**

All data in this study are presented as mean±SD. Data were analyzed by ANOVA followed by Scheffe post hoc test or Student t test. Significant difference was set at P<0.05 levels.

**Results**

In the time course study, we found that marked brain edema was observed on the first day after whole blood injection. Perihematomal edema was still severe at day 3, declined at day 7, and was resolved at day 14 (Figure 1). Thus, we tested deferoxamine effects on brain edema at day 3 because severe brain edema at day 1 after ICH results from clot retraction and thrombin formation.

Intracerebral infusion of 2.5-mL blood caused a marked increase in perihematomal white matter water content (83.2±2.3 versus 73.5±0.8% in the contralateral side; P<0.05).

Systemic administration of 50-mg/kg deferoxamine (IM) significantly reduced edema (80.7±2.7 versus 83.2±2.3% in vehicle-treated group; P<0.05; Figure 2). In addition, deferoxamine treatment (50 mg/kg, IM) did not change brain water

**Figure 1.** Hematoma resolution (A) and brain water content in white matter (B) at different times after intracerebral hemorrhage. Values are mean±SD. *P<0.05 vs the contralateral side.

**Figure 2.** A, Brain water content in white matter 3 days after intracerebral hemorrhage (ICH) with deferoxamine (DFX) or vehicle (Veh) treatment. B, Tumor necrosis factor α (TNF-α) levels in white matter 3 days after ICH with DFX or Veh treatment. Values are mean±SD. *P<0.05 vs vehicle treatment.

**Figure 3.** A, Time course of receptor-interacting protein kinase 1 (RIPK1) in white matter after intracerebral hemorrhage (ICH). Values are mean±SD. #P<0.05 versus sham. B, White matter RIPK1 levels after ICH with deferoxamine (DFX) or vehicle treatment. Values are mean±SD. *P<0.05 vs the other groups.
content in sham-operated piglets (white matter, 74.2±1.8 versus 73.9±0.4% in vehicle-treated group; n=4; P>0.05).

Little TNF-α expression was found in the white matter after needle insertion. We measured TNF-α in the white matter at days 1, 3, and 7 after ICH and found that TNF-α levels increased significantly at day 1, peaked at day 3 (a 3-fold increase; P<0.05 versus sham), and then decreased at day 7 after ICH. At day 3, deferoxamine reduced white matter TNF-α levels after ICH (560±279 versus 6364±3156 pixels in vehicle; *P<0.05; Figure 2). ICH also resulted in RIPK1 upregulation in white matter, which peaked at day 3 after hemorrhage. Systemic treatment of deferoxamine reduced white matter RIPK1 levels after ICH (Figure 3).

Discussion
The major findings of present study are (1) deferoxamine attenuated ICH-induced white matter edema; (2) deferoxamine reduced white matter TNF-α levels after ICH; and (3) RIPK1 levels in white matter were upregulated after ICH and reduced by deferoxamine treatment.

Iron has a major role in brain damage after ICH. Brain iron overload causes brain edema in the acute phase. We have demonstrated that the iron chelator, deferoxamine, reduces ICH-induced brain edema and neurological deficits in young and aged rats. The dosage and therapeutic time point used in this study for deferoxamine were based on an earlier report in aged rats. Clinical data also suggest a role of iron in ICH-induced brain injury. For example, clot lysis is associated with perihematomal edema development. Recent studies show that high levels of serum ferritin, an iron storage protein, are independently associated with poor outcome and severe brain edema in patients with ICH. Our recent study found that deferoxamine reduces neuronal death in the piglet model of ICH. Here, we demonstrated that deferoxamine reduces ICH-induced white matter edema.

Both TNF-α and RIPK1 levels were increased in white matter after ICH. TNF-α is a major proinflammatory cytokine. ICH causes significant increases of brain TNF-α in rats, mice, and pigs. Our unpublished data showed that iron can upregulate TNF-α levels in the brain. TNF-α can also induce necroptosis through RIPK1. We have found that deferoxamine reduces TNF-α and RIPK1 levels in white matter after ICH. Necroptosis has been found in ICH, and future studies should determine whether deferoxamine can reduce ICH-induced necroptosis.

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Disclosures
None.

References
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