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Deferoxamine reduces intracerebral hemorrhage-induced white matter damage in aged rats

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ABSTRACT

Iron contributes to c-Jun N-terminal kinases (JNK) activation in young rats and white matter injury in piglets after intracerebral hemorrhage (ICH). In the present study, we examined the effect of deferoxamine on ICH-induced white matter injury and JNK activation and in aged rats. Male Fischer 344 rats (18 months old) had either an intracaudate injection of 100 μl of autologous blood or a needle insertion (sham). The rats were treated with deferoxamine or vehicle with different regimen (dosage, duration and time window). White matter injury and activation of JNK were examined. We found that a dose of DFX should be at more than 10 mg/kg for a therapeutic duration more than 2 days with a therapeutic time window of 12 h to reduce ICH-induced white matter loss at 2 months. ICH-induced white matter injury was associated with JNK activation. The protein levels of phosphorylated-JNK (P-JNK) were upregulated at day-1 after ICH and then gradually decreased. P-JNK immunoreactivity was mostly located in white matter bundles. ICH-induced JNK activation was reduced by DFX treatment. This study demonstrated that DFX can reduce ICH-induced JNK activation and white matter damage.

Introduction

Intracerebral hemorrhage (ICH) is a common and often fatal subtype of stroke (Sacco et al., 2009). Survivors often experience progressive deterioration in their neurological conditions due to secondary brain injury via toxic blood components, such as iron (Xi et al., 2006, 2014; Keep et al., 2012; Pandey & Xi, 2014; Zhou et al., 2014).

White matter injury is common in ICH patients. Clinical data based on volumetric magnetic images showed more extensive white matter damage detected in patients with ICH than those with ischemic stroke or other cerebral small vessel pathologies (Rost et al., 2010). ICH-induced white matter injury reflects the vulnerability of brain to further insults and predicts poor outcome after ICH (Lee et al., 2010). Iron also plays a role in white matter injury after ICH. Our recent study showed systemic treatment with deferoxamine (DFX), an iron chelator, attenuated white matter edema after ICH in piglets (Xie et al., 2014).

c-Jun N-terminal kinases (JNK) are important stress-responsive kinases that are activated in response to various forms of brain insults, such as cerebral ischemia and subarachnoid hemorrhage(Okuno et al., 2004; Yatsushige et al., 2005; Chen et al., 2014. Activated JNK phosphorylates serine on a variety of cellular targets and leads to cell death (Varfolomeev & Ashkenazi, 2004; Guan et al., 2006). Our previous study has demonstrated that both ICH and intracerebral infusion of ferrous iron activated JNK signaling pathway and resulted in neurological deficits. DFX suppressed ICH induced JNK activation and improved neurological outcomes in young rats (Wan et al., 2009).

ICH is mostly a disease of the elderly. Age is considered as a significant factor in determining brain injury. In this study, we examined optimal dose, optimal duration and therapeutic time windows of DFX for ICH-induced white matter damage in aged rats. We also investigated the effects of DFX on JNK activation in the aged rat model of ICH.

Materials and methods

Animal preparation and intracerebral injection

All animal procedures were approved by the University Committee on Use and Care of Animals, University of Michigan, and all studies were conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals. A total of 230 of male Fisher 344 rats at age of 18-month (body weight at 380–450 g from National Institutes of Health, Bethesda) were used for study. Rats were anesthetized with pentobarbital (45 mg/kg, i.p.) and the right femoral artery was catheterized to sample blood and monitor arterial blood pressure, blood pH, PaO_2, $PaCO_2$, hematocrit, and glucose levels. Body temperature was maintained at 37.5 °C by a feedback-controlled heating pad. Rats were then positioned in a stereotaxic frame (Kopf Instruments, Tujunga, CA, U.S.A.) and a cranial burr

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hole (1 mm) was drilled near the right coronal suture 3.5 mm lateral to the midline. A 26-gauge needle was inserted into the right basal ganglia (coordinates: 0.2 mm anterior, 5.5 mm ventral, and 3.5 mm lateral to the bregma). Autologous arterial blood (100 μL) was infused at the rate of 10 μl/min using a microinfusion pump (World Precision Instruments). After that, the needle remained in position for 10 min for the blood clotting and then gently removed. The burr hole was filled with bone wax, and the skin incision was closed with suture after infusion.

Experimental groups

There were 5 parts of experiments in this study.

Part 1: ICH rats were treated with DFX (10, 50 or 100 mg/kg administered intramuscularly; n = 9 for each dose) or vehicle (n = 12) at 2 and 6 h after ICH and then every 12 h for 7 days. Sham rats were treated with 100 mg/kg DFX (n = 5) or vehicle (n = 5). Rats were euthanized for histologic examination at 56 days after surgery.

Part 2: Rats were treated with 50 mg/kg DFX 2 and 6 h after ICH, then every 12 h for either 2, 5, 7, or 14 days (n = 9 for each duration) or treated with vehicle (n = 3) for 14 days. Sham rats were treated with DFX (n = 3) or vehicle (n = 3) for 14 days. Rats were then euthanized for histologic examination at 56 days after surgery.

Part 3: ICH rats were treated with DFX starting at 2, 4, 12, 24, or 48 h (n = 9 for each) or administered vehicle at 48 h (n = 3), and then a second injection was administered 4 h after the first injection, then followed by an injection every 12 h for 7 days. Rats were killed for histologic examination at 56 days after ICH.

Part 4: ICH or sham rats were treated with DFX (100 mg/kg administered intramuscularly; 2 h after blood infusion and then at 12 h intervals for up to 7 days) or vehicle (the same amount of saline) treatment. The ICH rats were euthanized at 1, 3 and 7 days later (n = 5 each group, each time points), and the sham control rats were killed at 7 days (n = 3 each group), for histological examination.

Part 5: Rats received an intracerebral infusion of 100 μL autologous blood, and then received either DFX (100 mg/kg administered intramuscularly; 2 h after blood infusion and then at 12 h intervals for up to 7 days) or vehicle (the same amount of saline) treatment. Rats were euthanized at days 1, 3 and 7 (n = 4 each group) for Western blot analysis.

Immunohistochemistry staining

Immunohistochemistry staining was performed as described previously (Jin et al., 2013). Briefly, Rats were euthanized (pentobarbital,

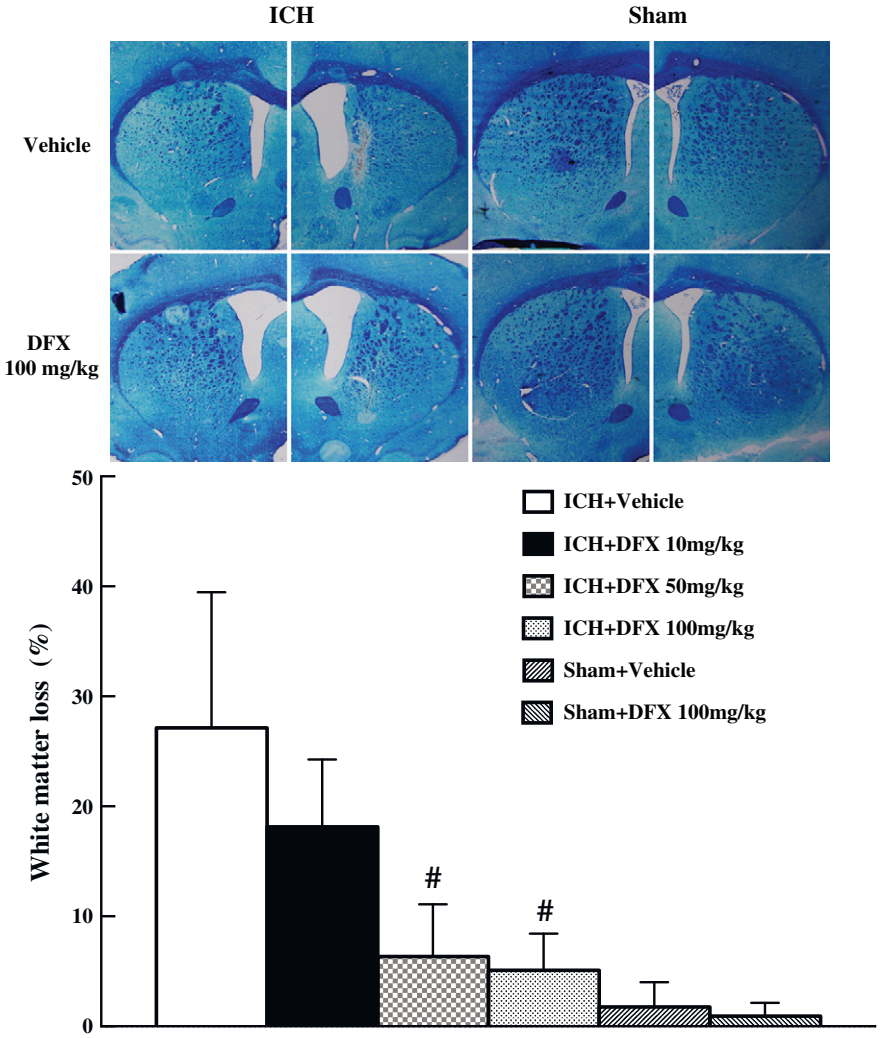

Fig. 1. Luxol fast blue stained white matter in the ipsilateral and contralateral basal ganglia and percentage of ipsilateral white matter reduction of aged rats treated with different doses of DFX (10 mg/kg, 50 mg/kg and 100 mg/kg, started at 2 h after ICH and lasted for 7 days) at 2 months after ICH or sham operation. Values are mean ± SD, #p < 0.01 vs. with vehicle group.