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Research report

Hemoglobin-induced neuronal degeneration in the hippocampus after neonatal intraventricular hemorrhage

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ABSTRACT

Neuronal degeneration following neonatal intraventricular hemorrhage (IVH) is incompletely understood. Understanding the mechanisms of degeneration and cell loss may point toward specific treatments to limit injury. We evaluated the role of hemoglobin (Hb) in cell death after intraventricular injection in neonatal rats. Hb was injected into the right lateral ventricle of post-natal day 7 rats. Rats exposed to anesthesia were used for controls. The CA-1 region of the hippocampus was analyzed via immunohistochemistry, hematoxylin and eosin (H&E) staining, Fluoro-Jade C staining, Western blots, and double-labeling stains. Compared to controls, intraventricular injection of Hb decreased hippocampal volume (27% decrease; $p<0.05$), induced neuronal loss (31% loss; $p<0.01$), and increased neuronal degeneration (2.7 fold increase; $p<0.01$), which were all significantly reduced with the iron chelator, deferoxamine. Hb upregulated p-JNK (1.8 fold increase; $p<0.05$) and increased expression of the Hb/haptoglobin endocytotic receptor CD163 in neurons in vivo and in vitro (cultured cortical neurons). Hb induced expression of the CD163 receptor, which co-localized with p-JNK in hippocampal neurons, suggesting a potential pathway by which Hb enters the neuron to result in cell death. There were no differences in neuronal loss or degenerating neurons in Hb-injected animals that developed hydrocephalus versus those that did not. Intraventricular injection of Hb causes hippocampal neuronal degeneration and cell loss and increases brain p-JNK levels. p-JNK co-localized with the Hb/haptoglobin receptor CD163, suggesting a novel pathway by which Hb enters the neuron after IVH to result in cell death.

Abbreviations: aCSF, artificial cerebrospinal fluid; DFX, deferoxamine; Fe, iron; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; FJC, Fluoro-Jade C; Hb, hemoglobin; H&E, hematoxylin and eosin; IVH, intraventricular hemorrhage; JNK, c-Jun N-terminal kinase; MRI, magnetic resonance imaging; NSE, neuron-specific enolase; p-JNK, phosphorylated c-Jun N-terminal kinase; T-JNK, total c-Jun N-terminal kinas

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1. Introduction

Intraventricular hemorrhage (IVH) is a significant source of mortality in preterm infants, resulting in infant death in about a quarter of cases (Murphy et al., 2002). IVH is associated with neuronal degeneration and cognitive dysfunction (Georgiadis et al., 2008; Lewis and Bendersky, 1989). Because of its function in learning and memory, the hippocampus has been previously investigated as a target of injury-induced neuronal degeneration in the CA-1 through CA-4 and dentate gyrus regions (Ramani et al., 2013; Song et al., 2007; Zhang et al., 2015; Zlotnik et al., 2012). However, studies performed examining the effects of specific blood components in neonatal IVH-induced brain injury have been limited. Such studies would be beneficial, as they could reveal targets for therapeutic intervention.

One of the most abundant proteins in blood, hemoglobin (Hb), has been found to be toxic to cortical neurons in vitro (Regan and Panter, 1993), and direct injection of Hb into the hippocampus in adult rats induces neuronal death (Song et al., 2007). A recent finding suggests that intraventricular Hb induces cytotoxic markers in astrocytes and can cause significant cell death and inflammatory activation in the choroid plexus (Gram et al., 2013, 2014). It is known that the heme moiety released by Hb interacts with intracellular heme oxygenase (HO) proteins (such as HO-1 in macrophages and HO-2 in neurons), releasing bilirubin, carbon monoxide, and iron (Fe) cations able to participate in Fenton and Haber-Weiss reactions (Lee et al., 2010; Lok et al., 2011; Wagner et al., 2003; Wang and Dore, 2007). However, the mechanism for intracellular transit of the heme moiety into neurons is not fully understood. The main Hb receptor, CD163, has been thought to only be expressed in macrophages and monocytes (Polfliet et al., 2006). For this reason, it is not clear whether this HO-mediated mechanism is a direct actor in neuronal cell death, or if neuronal death is a bystander phenomenon from this process in other cells.

This study uses a neonatal rat IVH model to examine whether Hb can induce neuronal cell death in the

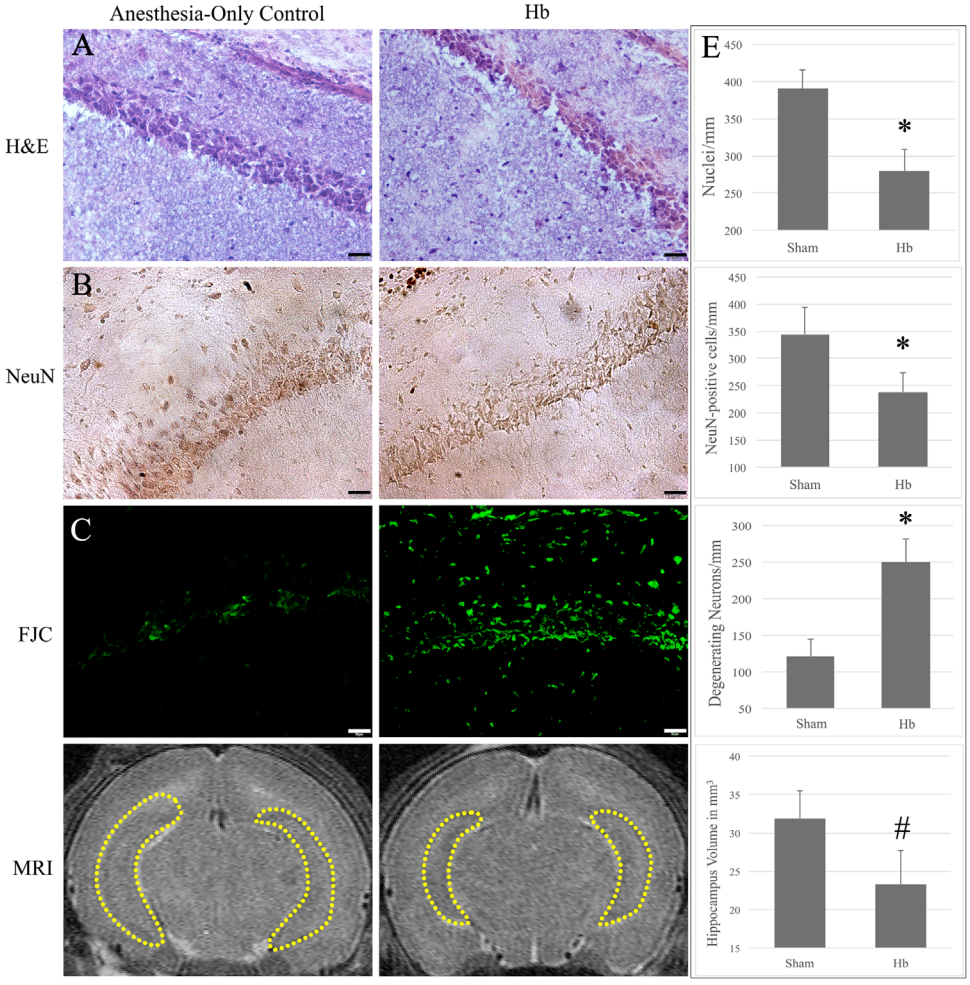

Fig. 1 – Representative images showing that intraventricular injection of Hb caused neuronal loss in the ipsilateral hippocampal CA-1 region at 72 h post-injection. (A) H&E staining (40 × magnification; scale bars = 20 μm), (B) immunohistochemistry for NeuN (40 × magnification; scale bars = 20 μm), (C) FJC staining of neuronal degeneration (40 × magnification; scale bars = 20 μm), and (D) MRI assessment of total hippocampal volume. Areas measured are outlined in yellow. (E) Quantification of the respective staining and hippocampal volume (*$p < 0.01$ vs. control group; #$p < 0.05$ vs. control group).