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Full-length Article

Changes in motor function, cognition, and emotion-related behavior after right hemispheric intracerebral hemorrhage in various brain regions of mouse

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ABSTRACT

Intracerebral hemorrhage (ICH) is a detrimental type of stroke. Mouse models of ICH, induced by collagenase or blood infusion, commonly target striatum, but not other brain sites such as ventricular system, cortex, and hippocampus. Few studies have systemically investigated brain damage and neurobehavioral deficits that develop in animal models of ICH in these areas of the right hemisphere. Therefore, we evaluated the brain damage and neurobehavioral dysfunction associated with right hemispheric ICH in ventricle, cortex, hippocampus, and striatum. The ICH model was induced by autologous whole blood or collagenase VII-S (0.075 units in 0.5 μl saline) injection. At different time points after ICH induction, mice were assessed for brain tissue damage and neurobehavioral deficits. Sham control mice were used for comparison. We found that ICH location influenced features of brain damage, microglia/macrophage activation, and behavioral deficits. Furthermore, the 24-point neurologic deficit scoring system was most sensitive for evaluating locomotor abnormalities in all four models, especially on days 1, 3, and 7 post-ICH. The wire-hanging test was useful for evaluating locomotor abnormalities in models of striatal, intraventricular, and cortical ICH. The cylinder test identified locomotor abnormalities only in the striatal ICH model. The novel object recognition test was effective for evaluating recognition memory dysfunction in all models except for striatal ICH. The tail suspension test, forced swim test, and sucrose preference test were effective for evaluating emotional abnormality in all four models but did not correlate with severity of brain damage. These results will help to inform future preclinical studies of ICH outcomes.

1. Introduction

Factors that predict short-term mortality after intracerebral hemorrhage (ICH) are fairly well known, but little is understood about long-term functional outcomes (Moulin and Cordonnier, 2015). Patients with ICH experience not only locomotor and memory impairment (Brainin et al., 2015; Langhorne et al., 2009) but frequently depression as well (Christensen et al., 2009; Hacket and Pickles, 2014; Koivunen et al., 2015). For example, ICH in the frontal cortex can lead to cognitive dysfunction and emotional changes (Lee et al., 2012; Moulin et al., 2016; Tang et al., 2011). Additionally, intraventricular hemorrhage (IVH), which occurs as an extension of intraparenchymal hemorrhage or subarachnoid hemorrhage into the ventricular system (Hallevi et al., 2008; Hwang et al., 2012), is associated with increased cognitive deficits and mortality (Brand et al., 2014; Hinson et al., 2010). Although the

Abbreviations: c-ICH, cortical intracerebral hemorrhage; h-ICH, hippocampal intracerebral hemorrhage; ICH, intracerebral hemorrhage; IVH, intraventricular hemorrhage; PSD, post-stroke depression; s-ICH, striatal intracerebral hemorrhage.

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hippocampus is not a common site of bleeding, damage to it can cause deficits in memory and social interaction (Rubin et al., 2014).

Most animal models that have been developed to mimic the clinical progression of ICH target the striatum (Keep et al., 2012; MacLellan et al., 2011; Wang, 2010; Wang and Dore, 2007). Few studies have modeled ICH in the ventricular system, cortex, and hippocampus, despite their clinical prevalence. Moreover, the behavioral tests used in rodent models of ICH focus primarily on functional deficits and recovery after striatal ICH in the left hemisphere. Very few studies have evaluated the cognitive and emotional changes associated with ICH in the right hemisphere.

Many tests exist to assess locomotor function, grip and forelimb strength, memory, and emotion-related behavior in rodents subjected to ICH models. These include the 24-point neurologic deficit score (Han et al., 2016; Wang et al., 2003), wire-hanging test and cylinder test (Auriat and Colbourne, 2009; Lan et al., 2017a; Manaenko et al., 2009), novel object recognition test (Perez-Urrutia et al., 2017; Yang et al., 2017b), tail suspension test, forced swim test, and sucrose preference test (Perez-Urrutia et al., 2017; Zhu et al., 2017). However, these tests have been used primarily to evaluate cognitive and emotion-related behavioral changes after striatal ICH and rarely applied to ICH models of the right lateral ventricle, cortex, and hippocampus. Therefore, our goal was to characterize brain damage and functional outcomes in ICH models that affect right-hemispheric structures using histology, immunofluorescence, and a battery of behavioral tests to evaluate motor, cognitive, and emotion-related behavior. We hypothesized that brain damage and behavioral deficits after ICH differ by brain region affected.

2. Materials and methods

2.1. Mice

This study was conducted in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health. Animal use protocols were approved by the Johns Hopkins University Animal Care and Use Committee (Approved protocol number: MO15M83). Measures were taken to minimize the number of laboratory mice used and to ensure minimal pain and discomfort. Adult male C57BL/6 mice (20–25 g, total n = 433) were placed into a clean induction chamber and anesthetized by isoflurane (3–4% initially and 1–2% for maintenance, Baxter healthcare Co.) in an oxygen-air mixture (20%:80%). Rectal temperature was monitored with a thermometer and maintained at 37 ± 0.5 °C by an electronic thermostat-controlled warming blanket (Stoelting Co.) throughout the surgical procedure.

2.2. Experimental groups and surgical procedures

2.2.1. Striatal intracerebral hemorrhage (s-ICH)

Mice were placed in a stereotaxic frame (RWD Life Science) and infused in the right striatum with either 10 μl (low-dose group, n = 12) or 30 μl (high-dose group, n = 10) of autologous whole blood. Based on a published protocol with minor modifications (Krafft et al., 2012; Rynkowski et al., 2008; Wang et al., 2008a), we made a midline scalp incision and drilled a hole into the right side of the skull (for infusion of 10 μl blood: 0.6 mm anterior and 2.0 mm lateral of the bregma; for infusion of 30 μl blood: 0.2 mm anterior and 2.0 mm lateral of the bregma). The mouse's tail was immersed in warm water (40 °C) for 2 min, and the tail skin cleaned with 70% alcohol. Blood was quickly drawn from the central tail artery with a sterile needle (25-gauge) (Zhu et al., 2014) into a sterile 50-μl Hamilton syringe without anticoagulant. After blood collection, the Hamilton syringe was secured onto a motorized micro-injector (Stoelting Co.), and the blood was infused at a rate of 1 μl/min. For the low-dose group, 4 μl of blood was infused at 3.0 mm below the surface of the skull, and then after a 5-min pause, the remaining 6 μl of blood was infused at a depth of 3.8 mm. The needle was withdrawn at a rate of 1 mm/min beginning 10 min after the second injection. For the high-dose group, mice were similarly infused with 5 μl and 25 μl of blood. The burr hole was filled with bone wax (Ethicon, Somerville, NJ), and the scalp incision was closed with Super Glue (Henkel Consumer Adhesive Inc. Scottsdale, Arizona). Mice in the sham group (n = 8) received only needle insertion into the right striatum.

2.2.2. Intraventricular hemorrhage (IVH)

Either 25 μl (low-dose group, n = 12) or 40 μl (high-dose group, n = 10) of autologous whole blood was infused into the right lateral brain ventricle. A 26-gauge needle attached to a Hamilton syringe was stereotaxically inserted into the right ventricle 2.5 mm below the surface of the skull (coordinates: 0.5 mm posterior and 1.0 mm lateral of the bregma) and autologous blood was infused at a rate of 5 μl/min. After the infusion, the needle was left in place for 10 min and then removed at a rate of 1 mm/min. Mice in the sham group (n = 8) were injected with an equal amount of saline.

2.2.3. Cortical intracerebral hemorrhage (c-ICH)

The c-ICH model was produced according to previous studies (Masuda et al., 2010; Zhu et al., 2014) with minor modifications. Each mouse (n = 10) was stereotaxically injected with collagenase (Type VII-S, 150 U/ml, sterile-filtered, high purity, purified by chromatography, Sigma-Aldrich Co.) at two sites of the right cortex at the following stereotactic coordinates: site 1: 0.0 mm anterior and 1.5 mm lateral of the bregma, 1.6 mm in depth; site 2: 1.0 mm anterior and 2.0 mm lateral of the bregma, 1.6 mm in depth. The low-dose group received 0.3 μl per site, and the high-dose group received 0.4 μl per site at a rate of 0.1 μl/min. The needle was withdrawn slowly 20 min after each injection to minimize backflow. Mice in the sham group (n = 8) received an injection of the same amount of saline into each site. We chose these volumes because larger volumes did not produce well-defined hematomas.

2.2.4. Hippocampal intracerebral hemorrhage (h-ICH)

Mice (n = 10) were injected with 0.2 μl of collagenase at a rate of 0.1 μl/min in the right hippocampus at 2.5 mm posterior and 1.7 mm lateral of the bregma, 1.8 mm in depth (Rogove et al., 2002). The needle was withdrawn slowly 10 min after injection. Mice in the sham group (n = 8) were injected with 0.2 μl of saline. We chose this volume because in preliminary experiments, 0.3 μl and 0.1 μl of collagenase failed to produce a well-defined hematoma.

2.3. Behavioral tests

Mice were housed in a temperature- and humidity-controlled room that was maintained on a 12-h light/dark cycle, with food available ad libitum. All behavioral tests were conducted during the light cycle phase in enclosed behavior rooms. The same mice were used for all behavioral tests (Supplementary Fig. 1). All behavioral tests were evaluated and analyzed by an observer blinded to the study. Data were mapped with Graphpad Prism 5 software (GraphPad Software, Inc. USA).

2.3.1. Assessment of neurologic deficits

Neurologic function of each mouse was tested on days 1, 3, 7, 14, and 21 post-ICH with assessments of body symmetry, gait, climbing, circling behavior, front limb symmetry, and compulsory

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