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Research report

Correlation between subacute sensorimotor deficits and brain edema in two mouse models of intracerebral hemorrhage

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HIGHLIGHTS

- Brain edema after ICH is associated with clinical outcomes.
- Brain edema and neurological function were tested in animal models.
- Brain edema is correlated with sensorimotor deficits.
- Corner turn test and forelimb placing test are more sensitive to brain edema.

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ABSTRACT

Formation of brain edema after intracerebral hemorrhage (ICH) is highly associated with its poor outcome. However, the relationship between cerebral edema and behavioral deficits has not been thoroughly examined in the preclinical setting. Hence, this study aimed to evaluate the ability of common sensorimotor tests to predict the extent of brain edema in two mouse models of ICH. One hundred male CD-1 mice were subjected to sham surgery or ICH induction via intrastriatal injection of either autologous blood (30 μL) or bacterial collagenase (0.0375 U or 0.075 U). At 24 and 72 h after surgery, animals underwent a battery of behavioral tests, including the modified Garcia neuroscore (Neuroscore), corner turn test (CTT), forelimb placing test (FPT), wire hang task (WHT) and beam walking (BW). Brain edema was evaluated via the wet weight/dry weight method. Intrastriatal injection of autologous blood or bacterial collagenase resulted in a significant increase in brain water content and associated sensorimotor deficits ($p < 0.05$). A significant correlation between brain edema and sensorimotor deficits was observed for all behavioral tests except for WHT and BW. Based on these findings, we recommend implementing the Neuroscore, CTT and/or FPT in preclinical studies of unilateral ICH in mice.

1. Introduction

Spontaneous intracerebral hemorrhage (ICH) accounts for 10–30% of all stroke-related hospitalizations and affects annually approximately two million people worldwide [1]. Many patients who survive the ictus deteriorate progressively due to formation of space-occupying brain edema [2]. The high disease-associated morbidity and mortality has spurred extensive preclinical research, which has led to (1) the development of various ICH animal models [3–5], (2) exploration of injury mechanisms [2,6–9], and (3) the search for innovative treatment strategies for this stroke sub-type [10–14].

While numerous therapies are effective in preclinical ICH studies, their translation from bench to bedside remains unsuccessful [15,16]—a possible consequence of inadequate utilization of behavioral tests in the laboratory setting [17,18]. Unpaired improvement of brain morphology is insufficient in determining the effectiveness of a treatment, and a neuroprotectant ought to primarily and evidently ameliorate functional deficits. Diverse behavioral tests have been developed to qualitatively and quantitatively assess functional outcomes in animal models of neurological diseases [19–22]. The choice of behavior test depends on the ailment studied; intrastriatal ICH greatly impairs sensorimotor function.

Common sensorimotor tests for rodents are the Garcia neuroscore (Neuroscore), wire hang task (WHT) and beam walking (BW). These tests were originally developed to evaluate deficits

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in rodents after ischemic brain injury [19–21], but have also been utilized in murine models of ICH [23–25].

While the WHT and BW assess an animal's overall performance of a specific task (moving along a wire or a beam), the forelimb placement test (FPT) and corner turn test (CTT) compare an animal's deficits on the affected contralateral with the normal ipsilateral body side. The Neuroscore is a composite sensorimotor test that includes side specific evaluations as well as task performances such as forelimb walking and climbing [19].

The purpose of this current study was to evaluate the ability of common sensorimotor behavioral tests to predict the extent of subacute brain edema in mice subjected to ICH induction via intrastriatal injection of either autologous blood or bacterial collagenase.

2. Material and methods

2.1. Experimental animals and surgical procedures

All experiments involving laboratory animals were conducted in compliance with the *NIH Guidelines for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee at Loma Linda University. Eight week old male CD-1 mice were housed in a temperature and humidity controlled environment, with a 12 h light/dark cycle and fed ad libitum.

Two established preclinical ICH models were utilized to generate hemispheric bleeds in mice: the double injection model of autologous whole blood [5], and the collagenase injection model, with minor modifications [3]. Briefly, after achieving general anesthesia by intraperitoneal co-injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), mice were positioned prone and secured onto a stereotactic frame (Kopf Instruments, Tujunga, CA). Next, the scalp was incised and a cranial burr hole (1 mm) was made in the right parietal bone (0.2 mm anterior from bregma, 2.0 mm lateral from the midline).

Following that, mice subjected to intrastriatal blood injection, were released from the head frame and positioned supine to access the animal's central tail artery. After disinfecting the tail with 70% ethanol, the artery was punctured and a minimum of 30 μL arterial blood was collected in a capillary tube. The blood was then quickly transferred into a 250 μL glass syringe (26 Gauge; Hamilton Company, Reno, NV), and fixed on a microinjection pump (Harvard Apparatus, Holliston, MA). After remounting the animal, the needle was stereotaxically inserted through the cranial burr hole and advanced 3.0 mm below the dura. At this position, 5 μL of autologous whole blood was injected into the right hemisphere at a rate of 2 μL/min. The needle was then lowered to the target position at 3.7 mm in depth, and after waiting 5 min, 25 μL of blood was injected into the striatum.

To perform the collagenase-based ICH model, a 10 μL glass syringe (26 Gauge; Hamilton Company, Reno, NV) was filled with bacterial collagenase (VII-S, Sigma, St. Louis, MO) and lowered 3.7 mm ventrally through the cranial burr hole. Bacterial collagenase (0.0375 U (low dose, LD) or 0.075 U (high dose, HD) dissolved in 0.5 μL PBS) was injected into the right striatum at a rate of 0.25 μL/min.

After completed injection (of either autologous blood or bacterial collagenase), the needle was left in place for an additional 10 min to prevent backflow of blood or collagenase along the needle tract. After withdrawing the syringe at a rate of 1 mm/min, the burr hole was sealed with bone wax and the scalp suture closed. Mice were allowed to recover under observation. Sham operations consisted of needle insertion only.

The present study utilized a total of 100 mice that were randomly assigned to either intrastriatal injection of autologous whole blood ($n = 24$), low dose (LD) collagenase ($n = 24$), high dose (HD) collagenase ($n = 26$), or sham operation ($n = 26$).

2.2. Evaluation of brain water content

Brain water content (brain edema) was measured via the wet weight/dry weight method, as previously described [26]. Briefly, mice under deep isoflurane anesthesia were decapitated at 24 and 72 h after surgery, and brain specimens were quickly removed. Coronal sections were separated 2 mm anterior and posterior of the needle tract. These sections were further divided into the ipsi- and contralateral cortex, and ipsi- and contralateral basal ganglia. The cerebellum was additionally collected as an internal control. All tissue samples were weighed using an analytical microbalance (APX-60, Denver Instrument, Bohemia, NY) in order to obtain the wet weight. The samples were then dried at 100 °C for 24 h before determining the dry weight. Brain water content (%) was calculated as (wet weight–dry weight)/wet weight × 100.

2.3. Evaluation of hematoma size and volume

Spectrophotometric hemoglobin assays were performed to measure hematoma volumes 24 h after ICH-induction [27]. For this purpose, following transcardiac perfusion with PBS, ipsilateral brain hemispheres were collected, placed in glass test tubes with 3 mL of distilled water, and then homogenized for 60 s and sonicated for 30 s. The homogenates were centrifuged (30 min, 12,000 rcf) and 400 μL of Drabkin's reagent (Sigma-Aldrich, St Louis, MO) was added to 100 μL of supernatant. Absorbance was measured 15 min thereafter, using a spectrophotometer (540 nm; Genesis 10uv, Thermo Fisher Scientific Inc.), and hematoma volumes were calculated based on a standard curve and expressed as μL of blood. The standard curve was generated as previously reported [28]. Briefly, following transcardiac perfusion with PBS, hemispheric brain samples were collected from naïve mice. Incremental volumes of autologous whole blood (0, 2, 4, 8, 16, 32, or 64 μL) were added to the naïve brain tissues. Following that, sample preparation and spectrophotometric analysis was conducted as described above. This procedure yielded a linear relationship between measured hemoglobin concentrations and the known blood volumes.

Hematoma size was evaluated by means of hematoxylin-eosin stained cryosections that were obtained at 24 h after surgery, as previously described [29]. Briefly, mice under deep isoflurane anesthesia were transcardially perfused with ice-cold PBS followed by 4% paraformaldehyde. Following that, brains were removed and postfixed in 10% paraformaldehyde (at 4 °C for 2 days), then dehydrated with 30% sucrose in PBS (at 4 °C for 2 days). Frozen coronal brain section of 10 μm thickness were cut on a cryostat (CM3050S; Leica Microsystems), mounted onto poly-lysine coated glass slides (Richard Allen Scientific, Kalamazoo, MI), and hematoxylin–eosin stained.

2.4. Neurofunctional assessments

Neurofunctional tests were conducted in a blinded fashion prior to euthanasia, 24 or 72 h after surgery.

2.4.1. Composite Garcia neuroscore

The neurofunctional assessment, first reported by Garcia et al. [19] has been modified for the use in mice after experimental ICH. This composite assessment consists of seven independent sub-tests evaluating spontaneous activity (I), axial sensation (II), vibrissae proprioception (III), symmetry of limb movement (IV), lateral turning (V), forelimb walking (VI) and climbing (VII). Performance and