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

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Abstract

Formation of brain edema after intracerebral hemorrhage (ICH) is highly associated with its poor outcome, thus it is clinically important to understand the effect brain edema has on 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 μ L) or bacterial collagenase (0.0375 U or 0.075 U). At 24 and 72 hours 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.

Keywords

Neurobehavior; intracerebral hemorrhage; brain edema; experimental models; mice

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

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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 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.

MATERIAL AND METHODS

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 hour 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 μ L arterial blood was collected in a capillary tube. The blood was then quickly transferred into a 250 μ L 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 stereotactically inserted through the cranial burr hole and advanced 3.0 mm below the dura. At this position, 5 μ L of autologous whole blood was injected into the right hemisphere at a

rate of 2 $\mu\text{L}/\text{min}$. The needle was then lowered to the target position at 3.7 mm in depth, and after waiting 5 minutes, 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 $\mu\text{L}/\text{min}$.

After completed injection (of either autologous blood or bacterial collagenase), the needle was left in place for an additional 10 minutes 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 one hundred 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 ($n=26$).

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 hours 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 hours before determining the dry weight. Brain water content (%) was calculated as (wet weight – dry weight)/wet weight $\times$ 100.

Evaluation of hematoma size and volume

Spectrophotometric hemoglobin assays were performed to measure hematoma volumes 24 hours 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 seconds and sonicated for 30 seconds. The homogenates were centrifuged (30 min, 12000 rcf) and 400 μL of Drabkin's reagent (Sigma-Aldrich, St Louis, MO) was added to 100 μL of supernatant. Absorbance was measured 15 minutes 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 hours 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 μ m 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.

Neurofunctional assessments

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

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 evaluation of each sub-test are described in the following paragraph.

- Spontaneous activity (SA): Animals were observed for 5 minutes in their normal environment (cage). Scores indicate the following: 3, mouse approached at least 3 walls of the cage; 2, mouse approached less than 3 walls; 1, mouse barely moved; 0, mouse did not move at all.
- Axial sensation (AS): Tactile stimuli were applied on each side of the animal's trunk using a cotton swap. Scores indicate the following: 3, mouse was equally startled on both sides; 2, asymmetric response; 1, missing response on one side.
- Vibrissae proprioception (VP): A cotton swap was moved from the rear of the animal towards its head, touching the vibrissae gently on each side at a time. Scores indicate the following: 3, mouse equally turned head on both sides; 2, asymmetric response; 1, missing respond on one side.
- Symmetry of limb movement (LS): The mouse was suspended by the tail to assess movement of the limbs. Scores indicate the following: 3, all limbs were extended symmetrically; 2, asymmetric extension; 1, limbs on one side showed minimal movement; 0, hemiplegia (no limb movement).
- Lateral turning (LT): The mouse was suspended by the tail and a blunt stick was moved along each side of the body causing lateral turning towards the stimulus. Scores indicate the following: 3, animal turns at least 45 degrees on both sides; 2, animal turns equally to both sides but less than 45 degrees; 1, unequal turning; 0, no turning at all.
- Forelimb outstretching (FO): The mouse was suspended by its tail allowing both forepaws to touch the edge of a table. Scores indicate the following: 3, forelimbs were equally outstretched and the animal walked symmetrically on forepaws; 2, asymmetric outstretch and impaired forepaw walking; 1, minimal movement of one forelimb; 0, hemiplegia (no limb movement).
- Climbing (CL): The mouse was placed on a rough surface (22 $\times$ 44 cm) at a 45° angle with the table. Scores indicate the following: 3, mouse climbed to the top of the surface; 2, asymmetric or impaired climbing; 1, animal failed to climb or showed tendency of circling.

The final neuroscore given to each animal is the sum of the seven individual test scores. The minimum score is 3 (worst performance) and the maximum is 21 (best performance).

Corner turn test—The corner turn test (CTT) was conducted as previously described [22]. Mice were permitted to approach a 30° corner that was made out of two attached Plexiglas

walls. In order to exit the corner, animals had to turn either to the right or to the left, usually by rearing along the corner wall (Fig. 1). The choice of turning side was recorded for 10 trials per test day, with at least 30 seconds break between the trials. The score was then calculated as number of left turns/all turns $\times$ 100. Only turns involving a full rearing along either wall were recorded.

Forelimb placing test—The ability of a mouse to respond to a vibrissae-elicited excitation by forward moving of its forelimb was evaluated with the forelimb placing test (FPT), as previously described [22]. Briefly, animals held by their trunk, were positioned parallel to a table top and slowly moved up and down, allowing the vibrissae on one side of the head to brush along the table surface (Fig. 2). Refractory placements of the impaired (left) forelimb were evaluated and a score was calculated as number of successful forelimb placements out of 10 consecutive trials.

Wire hang task—To conduct the wire hang task (WHT) as described [24], mice were placed midway on a wire (50×0.15 cm), which was spanned between two platforms, approximately 40 cm above the surface. Presenting an innate tendency to avoid falling, mice moved actively along the wire. The appropriate usage of fore- and hindlimbs, as well as the distance moved along the wire was monitored for 1 minute. A score between 0 (worst performance) and 5 (best performance) was given for each out of three trials (Table 1).

Beam walking—The assessment of beam walking (BW) ability was modified to be used in mice after ICH [21]. To conduct this neurofunctional test, mice were placed midway on a horizontal rod (90×1 cm) that connected two platforms, approximately 40 cm above the surface. Presenting an innate tendency to avoid falling, mice moved along the rod to one of two stable platforms. The distance covered within 1 minute was recorded and an average score (out of three trials) was given: 0 (worst performance) and 5 (best performance) (Table 1).

Statistical analysis

Brain water content and hematoma volume were expressed as mean $\pm$ SD, and analyzed using One-way ANOVA followed by the Tukey's test. All behavioral data were presented as mean $\pm$ SD, and analyzed using Kruskal-Wallis One-way ANOVA on Ranks followed by the Student-Newman-Keuls Method or Dunn's test. Correlation between neurofunctional deficits (dependent variable, presented as percent performance of sham) and brain edema (independent variable, presented as percent brain water) were analyzed with the Spearman's rank correlation coefficient (Rho) and best-fit liner regressions (SigmaPlot 11.0, Systat Software, Inc.). The significance level was set at $p < 0.05$.

RESULTS

Hematoma-induced formation of brain edema after ICH

No mortality was observed in this study. Intrastriatal injections of autologous whole blood (30 μ L), LD collagenase (0.0375 U), and HD collagenase (0.075 U), evoked significant elevation of brain water content (brain edema) in the ipsilateral cortex and ipsilateral basal ganglia, at 24 and 72 hours after surgery ($p < 0.05$ compared to sham; Fig. 3 A, B). Furthermore, autologous whole blood and HD collagenase caused significantly more perihematoma edema formation than LD collagenase ($p < 0.05$), which was paralleled by greater hematoma volumes in both models ($p < 0.05$; Fig. 3 C). Representative hematoxylin-eosin stained cryosections demonstrates hematoma size for each ICH model with the smallest hematoma corresponding to the LD collagenase model and largest hematoma corresponding to the blood and HD collagenase models (Fig. 3 D).

Correlation between neurofunctional deficits and brain edema after ICH

Prior to euthanasia, 24 or 72 hours after surgery, mice were subjected to a series of neurofunctional assessments consisting of the composite Garcia neuroscore (Neuroscore), corner turn test (CTT), forelimb placing test (FPT), wire hang task (WHT), and beam walking (BW), which, with exception of the WHT, reliably detected neurofunctional deficits in mice at both time points after ICH-induction ($p < 0.05$ compared to sham; Fig. 4–8).

The Neuroscore demonstrated significantly worse neurofunctional deficits in animals that received intrastriatal injections of HD collagenase ($p < 0.05$ compared to LD collagenase; Fig. 4 A). The test performances significantly correlated with the magnitude of brain edema 24 hours post-ICH ($p < 0.05$; Fig. 4 B). Apart from spontaneous activity (SA), all sub-tests identified hemorrhage-induced deficits 24 hours post-ICH ($p < 0.05$ compared to sham; Fig. 4 C). Assessments of the Neuroscore showed similar results 72 hours after surgery (Fig. 4 D, E); however, at this time point, SA, lateral turning (LT), and forelimb outstretching (FO) failed to demonstrate significant neurofunctional deficits in ICH animals ($p > 0.05$ compared to sham; Fig. 4 F).

The CTT detected significant differences in neurofunctional deficits for all ICH animals ($p < 0.05$). Furthermore, those that received intrastriatal injections of autologous blood and HD collagenase performed significantly worse than the LD collagenase group ($p < 0.05$; Fig. 5 A, C). These performances significantly correlated with hemorrhage-induced edema formation at 24 and 72 hours post-ICH ($p < 0.05$, Fig. 5 B, D).

Analogously, the FPT showed significantly worse paw placing abilities in ICH mice at 24 and 72 hours after surgery ($p < 0.05$). Furthermore, the animals subjected to intrastriatal injections of autologous blood and HD collagenase performed significantly worse than the LD collagenase group ($p < 0.05$, Fig. 6 A, C). A significant correlation was found between these deficits and the magnitude of brain edema at both time points ($p < 0.05$, Fig. 6 B, D).

Evaluation of the WHT revealed significantly worse performances of animals in the blood and HD collagenase groups compared to sham animals 24 and 72 hours post-ictus ($p < 0.05$). Significant differences in performance were also observed for the HD collagenase group compared to mice subjected to LD collagenase ($p < 0.05$; Fig. 7 A, C); however, no differences were found between the blood and collagenase injection models ($p > 0.05$). Furthermore, no significantly different test results were found between the sham and LD collagenase group ($p > 0.05$). Test performances at 24 and 72 hours after surgery did not significantly correlate with the formation of brain edema in ICH mice ($p > 0.05$; Fig. 7 B, D).

Beam walking ability was significantly impaired in ICH animals ($p < 0.05$ compared to sham; Fig. 8 A, C); however, no differences were observed between the ICH groups at 24 and 72 hours after surgery ($p < 0.05$). No significant correlation was found between beam walking ability and the magnitude of hemorrhage-induced brain edema formation ($p > 0.05$, Fig. 8 B, D).

DISCUSSION

ICH is a dynamic process with three distinct phases consisting of (1) initial hemorrhage, (2) hematoma expansion, and (3) formation of perihematoma brain edema. Following the initial hemorrhage, brain edema develops primarily due to extravascular accumulation of blood components; however, clot retraction and erythrocyte lysis are also implicated in edema progression [6, 9]. As anticipated, the level of brain edema is an accurate predictor for poor outcome in ICH patients [30–32]; thus, developing innovative therapies to effectively reduce or prevent brain edema formation would greatly benefit this patient population.

Two rodent models are widely used to investigate pathophysiological mechanisms and experimental treatments after ICH: the blood injection model [5], which mimics a single intracerebral bleed, and the collagenase injection model [3], which utilizes enzymatic disruption of blood vessels to imitate spontaneous and continuous bleeding into the surrounding brain tissue [33]. Perihematomal brain edema peaks between the first and third day in both models, before it gradually declines within approximately seven days [34]. Our results, which are supported by others [34], show significantly increased brain water content in the ipsilateral cortex and basal ganglia at 24 and 72 hours post-ictus. The magnitude of brain edema was similar following autologous blood and HD collagenase injection, suggesting that both models can induce injuries of similar severity. In contrast, LD collagenase evoked significantly less perihematomal brain edema than intrastriatal injections of autologous blood or HD collagenase. Preclinical studies indicate a 3–5 % increase in perihematomal brain edema during the first 3 days after ICH induction [34]. At first glance, a 4 % increase in brain edema may seem trivial; yet, this translates into a 25 % increase in tissue volume, leading to high intracranial pressure, hypoperfusion of neurons, and cell death [35–37], ultimately culminating in devastating morbidity, coma, and/or death.

Although preclinical ICH modeling may elicit similar cerebrovascular pathophysiological processes as observed in humans, the overall outcome, including mortality, is often not directly comparable. For instance, 35–52 % of ICH patients die during the first month, and half of all deaths occur within the first 48 hours after symptom onset [38]. In contrast, rodents subjected to experimental ICH present with substantially lower mortality rates (0–3 %) [39–41]. Debilitating co-morbidities as well as anatomical differences between the human and mouse brain may contribute to the varied outcomes [12].

This current study implemented intrastriatal blood as well as collagenase injections in order to identify adequate sensorimotor tests across both ICH models. Furthermore, increasing dosages of collagenase were utilized to create a wider range of injury severity, enabling the correlation of perihematomal brain edema and functional performances. Increasing the dosage of collagenase in order to create a more severe intracerebral bleed is feasible to a certain extent. The same is not true for the blood injection model, as larger volumes of autologous whole blood increase the injection time, thus facilitating unwanted clot formation in the glass syringe.

We evaluated a battery of common sensorimotor tests, specifically their ability to predict the magnitude of brain edema following ICH induction. The Neuroscore appropriately differentiated between the severity of LD and HD collagenase injections; and test performances correlated significantly with the magnitude of perihematomal brain edema. Interestingly, when regarding each sub-test individually, spontaneous activity (SA) failed to demonstrate a significantly different test performance of sham-operated and ICH animals at 24 and 72 hours after surgery. This may be partially due to the lengthy test duration; thus, reducing the exploration time for each animal may improve the validity of this sub-test.

The corner turn test (CTT) was originally developed to evaluate neurofunctional deficits in mice subjected to focal cerebral ischemia [42]. This test evaluates an animal's tendency to turn in a particular direction depending on the extent and localization of the hemispheric brain injury. Particularly rodents with unilateral basal ganglia injury demonstrate ipsilateral turn preferences because of impaired weight shifting movements of the limbs contralateral to the side of injury [43]. Mice subjected to right intrastriatal injection of autologous blood or HD collagenase exited the apparatus more often by turning to the right than to the left side. This behavioral test differentiated between severely injured (blood and HD collagenase injection), mildly injured (LD collagenase injection) and uninjured (sham) animals.

Furthermore, a significant correlation between CTT performance and the magnitude of brain edema was found at 24 and 72 hours after ICH induction.

The ability of a mouse to respond to a vibrissae-elicited excitation by extension of its forelimb can be evaluated with the forelimb placement test (FPT) [22]. Unilateral injury to the basal ganglia and/or to the sensorimotor cortex leads to impairment of the contralateral forelimb placing response, which normally follows a tactile stimulus of the vibrissae on the same side [43]. The ipsilateral forelimb; however, exhibits normal forward movement even when the contralateral vibrissae are stimulated. This “crossed-midline placing” indicates that the deficit is primarily of motor etiology and does not necessarily require loss of the rodent’s sensory function [44]. The performance of the FPT was significantly correlated to the degree of brain edema after ICH.

In contrast to the Neuroscore, CTT, and FPT, the wire hang task (WHT) and beam walking (BW) performances did not correlate with the extent of brain edema following ICH induction. The latter two demonstrated limited abilities in predicting the severity of unilateral brain injury since these tests examine the overall performance of a task, and animals can compensate for deficits with the unimpaired body side. When conducting the WHT we observed animal behavior indicating compensation for contralateral forelimb weakness. For example, upon extended time of testing, severely injured mice held on to the wire by pinning it between their proximal forelimb and axilla, instead of clutching it with their paw. Severely injured animals demonstrating this compensatory behavior received similar test scores as mildly injured animals. In order to make the WHT more sensitive, the scoring should be adapted to properly account for this behavior. BW can be improved by using a rectangular beam (such as 90 cm height × 45 cm length × 1 cm thick) instead of a rod in order to evaluate other measures such as missed steps (foot faults) and turning counts.

The correlation of brain water content with the composite Neuroscore, CTT, and FPT is likely due to the tests’ comparison of ipsilateral and contralateral side function. In contrast, the WHT and BW did not correlate with the extent of brain edema. These tests have limited abilities in predicting the outcome of unilateral ICH, as both tests examine overall performances; and ICH animals may compensate for deficits through the use of contralateral brain centers.

Evaluating the relationship between edema and sensorimotor deficits allows for novel therapeutics targeting the progression of edema to be examined using the most appropriate tests. The recommended tests, which are most capable of identifying the subacute functional outcome following ICH based on the correlation of neurological outcome and brain edema, are the composite Neuroscore, CTT, and FLP test. Furthermore, these behavioral tests present the following advantages. They do not: (1) require extensive training of the animals, (2) require food restriction, and (3) need expensive equipment.

Long-term neurological deficits remain a major consequence of ICH patients surviving the initial bleed [45]. Therefore, it is of interest to examine the effects of ICH on long-term functional outcome. The clinical predictor of poor outcome in patients may be ICH-induced edema, hematoma size, or the combined deleterious effects of edema and hematoma. Preclinical studies have demonstrated that pharmacological attenuation of subacute brain edema results in improved long-term sensorimotor and cognitive function in rodents subjected to experimental ICH [14, 46].

MacLellan *et al.* provided an extensive analysis of the correlation between lesion volume (quantified 30 days post-ictus) and functional outcome after ICH in rats [47]. Three doses of bacterial collagenase were used to create mild, moderate, and severe injuries. Sensorimotor

deficits were examined over 30 days using the horizontal-ladder walking test, forelimb use asymmetry (cylinder) test, adhesive tape removal test, beam walking test, neurologic deficit scale, staircase test, single pellet test, and forelimb inhibition (swimming) test. Rats were trained pre-ictus for all tests. The authors found that the horizontal-ladder walking test, adhesive tape removal test, neurologic deficit scale, staircase test, and swimming test were capable of distinguishing between ICH severities (e.g. lesion volumes). Furthermore, combining all of the behavioral tests into a composite behavioral score lead to a clear correlation with lesion volume. MacLellan *et al.* attributed the lack of functional difference at later time points in other functional tests to possible learning and/or rehabilitation [47]; MacLellan 2010.

In addition to sensorimotor tests, learning/memory tests (Morris water maze [29, 48], T-maze [48], radial arm maze [48]) and anxiety tests (open field test [29, 48], elevated plus maze [48]) have been tested for long-term functional outcome after experimental ICH [29]. Studies by MacLellan *et al.* and Hartman *et al.* examined long-term functional deficits in sham and ICH rats and found that the majority of learning/memory and anxiety tests showed no significant differences between injured and uninjured animals [29, 48].

Further studies are needed to investigate the effects of ICH on acute and long-term functional outcome. These studies can utilize imaging, such as MRI, to uncouple the effects edema, hematoma size, and lesion volume has on neurofunctional outcome. We suggest the implementation of MRI for edema analysis. MRI has several advantages to the wet weight/dry weight method for quantifying edema: (1) MRI allows for acute edema to be correlated with long-term functional deficits since animals do not need to be sacrificed for wet weight/dry weight measurements, and (2) MRI is the gold standard for quantifying edema and is widely used clinically. Implementation of MRI will aid in understanding the true impact of edema and hematoma on functional outcome which will determine which preclinical studies of ICH treatments are best suited for translation from bench to bedside.

Conclusion

Herein, a correlation between cerebral edema and functional outcome was assessed for five common sensorimotor tests. The results of this study can aid in determining relevant behavior tests when new therapeutics are being developed for reduction of brain edema. The most appropriate functional tests for unilateral injuries, such as ICH, are those that score neurological outcome based on ipsilateral versus contralateral performance.

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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.

Figure 1. Corner turn test: Naïve mouse rearing up the right corner wall (arrow), while turning. Dashed lines display the Plexiglas apparatus.

Figure 2. Forelimb placing test: **(A)** Unilateral vibrissae stimulation (arrow head) on table top followed by ipsilateral paw placement (arrow) in a naïve mouse. **(B)** Vibrissae stimulation (arrow head) without consecutive paw placement in a mouse at 24 hours after right hemispheric intracerebral hemorrhage (ICH), involving the basal ganglia.

Figure 3.

Morphological changes after ICH: (A) Brain edema at 24 and (B) 72 hours after surgery. (C) Hematoma volume at 24 hours post-ICH. (D) Representative hematoxylin-eosin stained cryosections illustrating hematoma size following intracerebral injections of low dose (LD) collagenase (0.0375 U), blood (30 µL), and high dose (HD) collagenase (0.075 U) at 24 hours. Values are expressed as the mean $\pm$ SD ($n=12-14$ per group [brain edema evaluation] and $n=6$ [hematoma volume]). * $p<0.05$ compared to sham, † $p<0.05$ compared to LD collagenase injection. Dashed lines encircle the hematoma (scale bar = 1 mm).

Figure 4.

Composite Neuroscore: **(A)** Test performances at 24 hours post-ICH. **(B)** Linear regression curve of 24 hours test performance and brain edema (% brain water). **(C)** Sub-test scores at 24 hours after surgery. **(D)** Test performances at 72 hours post-ICH. **(E)** Linear regression curve of 72 hours test performance and brain edema (% brain water). **(F)** Sub-test scores at 72 hours after surgery. Bar graphs: Values are expressed as the mean $\pm$ SD (n=11–14 per group). * p<0.05 compared to sham, † p<0.05 compared to low dose (LD) collagenase injection. Scatter diagrams: Spearman's rank correlation coefficient (Rho) and significance (p-value) as indicated. SA = spontaneous activity, AS = axial sensation, VP = vibrissae proprioception, LS = limb symmetry, LT = lateral turning, FO = forelimb outstretching, and CL = climbing.

Figure 5.

Corner Turn Test: (A) Test performances at 24 hours post-ICH. (B) Linear regression curve of 24 hours test performance and brain edema (% brain water). (C) Test performances at 72 hours post-ICH. (D) Linear regression curve of 72 hours test performance and brain edema (% brain water). Bar graphs: Values are expressed as the mean $\pm$ SD (n=6–14 per group). * p<0.05 compared to sham, † p<0.05 compared to low dose (LD) collagenase injection. Scatter diagrams: Spearman's rank correlation coefficient (Rho) and significance (p-value) as indicated.

Figure 6.

Forelimb Placing Test: (A) Test performances at 24 hours post-ICH. (B) Linear regression curve of 24 hours test performance and brain edema (% brain water). (C) Test performances at 72 hours post-ICH. (D) Linear regression curve of 72 hours test performance and brain edema (% brain water). Bar graphs: Values are expressed as the mean $\pm$ SD (n=6–14 per group). * p<0.05 compared to sham, † p<0.05 compared to low dose (LD) collagenase injection. Scatter diagrams: Spearman's rank correlation coefficient (Rho) and significance (p-value) as indicated.

Figure 7.

Wire Hang Task: (A) Test performances at 24 hours post-ICH. (B) Linear regression curve of 24 hours test performance and brain edema (% brain water). (C) Test performances at 72 hours post-ICH. (D) Linear regression curve of 72 hours test performance and brain edema (% brain water). Bar graphs: Values are expressed as the mean $\pm$ SD (n=10–14 per group). * p<0.05 compared to sham, † p<0.05 compared to low dose (LD) collagenase injection. Scatter diagrams: Spearman's rank correlation coefficient (Rho) and significance (p-value) as indicated.

Figure 8.

Beam Walking: (A) Test performances at 24 hours post-ICH. (B) Linear regression curve of 24 hours test performance and brain edema (% brain water). (C) Test performances at 72 hours post-ICH. (D) Linear regression curve of 72 hours test performance and brain edema (% brain water). Bar graphs: Values are expressed as the mean $\pm$ SD (n=10–14 per group). * p<0.05 compared to sham, † p<0.05 compared to low dose (LD) collagenase injection. Scatter diagrams: Spearman's rank correlation coefficient (Rho) and significance (p-value) as indicated.

Table 1

Wire Hang Task and Beam Walking scoring

Wire Hang Task	Score	Beam Walking Test
Reaches the platform or moves at least 25 cm in 60 seconds	5	Reaches the platform or moves at least 45 cm in 30 seconds
Moves less than 25 cm along the wire but uses forelimbs together with either hindlimbs or tail for more than 30 seconds	4	Reaches the platform or moves at least 45 cm in 60 seconds
Moves less than 25 cm along the wire but uses both paws in a symmetrical manner for more than 30 seconds	3	Moves at least half the distance to the platform (22.5 cm) in 60 seconds
Holds onto the wire for more than 30 seconds (in any manner)	2	Moves less than half the distance to the platform (22.5 cm) in 60 seconds
Holds onto the wire for more than 15 but less than 30 seconds	1	Does not move but stays on the rod for more than 30 seconds (in any manner)
Falls off the wire within 15 seconds	0	Does not move and falls off the rod within 30 seconds