Research Report

Failure of deferoxamine, an iron chelator, to improve outcome after collagenase-induced intracerebral hemorrhage in rats

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ARTICLE INFO ABSTRACT

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Intracerebral hemorrhage (ICH) is a devastating stroke with no clinically proven treatment. Deferoxamine (DFX), an iron chelator, is a promising therapy that lessens edema, mitigates peri-hematoma cell death, and improves behavioral recovery after whole-blood-induced ICH in rodents. In this model, blood is directly injected into the brain, usually into the striatum. This mimics many but not all clinical features of ICH (e.g., there is no spontaneous bleed). Thus, we tested whether DFX improves outcome after collagenase-induced striatal ICH in rats. In the first experiment, 3- and 7-day DFX regimens (100 mg/kg twice per day starting 6 h after ICH), similar to those shown effective in the whole-blood model, were compared to saline treatment. Functional recovery was evaluated from 3 to 28 days with several behavioral tests. Except for one instance, DFX failed to lessen ICH-induced behavioral impairments and it did not lessen brain injury, which averaged 43.5 mm³ at a 28-day survival. In the second experiment, 3 days of DFX treatment were given starting 0 or 6 h after collagenase infusion. Striatal edema occurred, but it was not affected by either DFX treatment (vs. saline treatment). Therefore, in contrast to studies using the whole-blood model, DFX treatment did not improve outcome in the collagenase model. Our findings, when compared to others, suggest that there are critical differences between these ICH models. Perhaps, the current clinical work with DFX will help identify the more clinically predictive model for future neuroprotection studies.

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1. Introduction

Approximately 10% of strokes result from an intracerebral hemorrhage (ICH). This devastating stroke causes significant mortality and its survivors are often disabled (Sacco et al., 2009). As yet, there are no effective neuroprotective treatments nor is it likely that the rapid mechanical injury that occurs as blood infiltrates the parenchyma is amenable to such treatment. In some, the hematoma continues to grow from on-going bleeding or re-bleeding, and this is associated with poor outcome (Brott et al., 1997; Fujii et al., 1994). Unfortunately, efforts to limit hematoma growth have not convincingly improved outcome (Mayer et al., 2008). Similarly, surgical removal of the hematoma has not been proven to help (Mendelow et al., 2005). Thus, targeting secondary consequences of ICH, including edema, remain leading therapeutic goals.

Erythrocyte rupture and iron release, which catalyzes free radical formation, are key steps in the toxic cascade leading to edema and cell death after ICH (Xi et al., 2006). Indeed, iron

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accumulates and persists for weeks (Hua et al., 2006; Wan et al., 2006, 2009; Wu et al., 2003). Deferoxamine (DFX), a potent iron chelator used to treat iron overload in humans, has been repeatedly evaluated with in vivo models of ICH in rodents (Hua et al., 2006; Huang et al., 2002; Nakamura et al., 2003; Okauchi et al., 2009; Song et al., 2008; Wan et al., 2006, 2009) and piglets (Gu et al., 2009). In these studies, ICH was modeled by injecting blood or its components directly into the brain, and DFX was given starting within a few hours after ICH. It is notable that DFX reduces cerebral iron load, edema, cell death, and atrophy to persistently improve recovery in several species and in young and old rats. This rigorous evaluation is in line with recommendations for evaluating neuroprotectants (Corbett and Nurse, 1998; Dirnagl, 2006; Gladstone et al., 1999).

The use of multiple stroke models is another important consideration in evaluating neuroprotectants as no model perfectly mimics human stroke. As an alternative to directly injecting blood or its components to model ICH (Bullock et al., 1984) and to test treatments, investigators commonly infuse collagenase to cause an ICH (Rosenberg et al., 1990). Neither model reflects all features of human ICH (Andaluz et al., 2002; NINDS ICH Workshop, 2005). For instance, while the whole-blood model mimics a single large bleed found in most patients (Herbstein and Schaumberg, 1974), it does not reproduce the spontaneous or delayed bleeding seen in patients (Brott et al., 1997; Fujii et al., 1994). In contrast, injecting collagenase into the brain might be directly toxic or result in an exagger- ated immune response (Xue and Del Bigio, 2003), although in vitro studies argue against this (Chu et al., 2004; Matsushita et al., 2000). Collagenase-induced ICH also results from the rupture of many vessels, unlike in humans where a single large bleed is the predominant cause (Herbstein and Schaumberg, 1974). Such factors contribute to differences in the pattern and progression of injury observed between models. When matched for hematoma size, the total loss of brain tissue can be twice as much in the collagenase model (vs. blood injection method), and most of this additional injury occurs 1 to 4 weeks after collagenase infusion (MacLellan et al., 2008). Presumably, both the early and late injury may be partly mediated by toxic iron release. Thus, it is surprising that DFX has not been evaluated in the collagenase model.

Presently, we investigated the effects of 3 (DFX-3d) and 7 days (DFX-7d) of DFX treatment (vs. saline; SAL) administered starting 6 h after collagenase-induced striatal ICH. The DFX regimen is similar to that used in several whole-blood ICH studies (Gu et al., 2009; Hua et al., 2006; Nakamura et al., 2003; Okauchi et al., 2009). Use of a moderate-sized lesion allowed us to detect either beneficial or harmful treatment effects. Functional recovery, the clinical endpoint of greatest importance, was evaluated with four tests starting from 3 days after ICH until euthanasia at 28 days. These tests were a neurological deficit scale (NDS), the corner turn test to assess movement bias, the horizontal ladder task to measure walking, and the staircase test of skilled reaching. The volume of brain tissue lost was also calculated. We predicted that DFX would improve functional outcome and lessen tissue loss with greater benefit occurring with the longer treatment. Another experiment tested whether DFX starting at 0 (DFX-0h) or 6 h (DFX-6h) after collagenase infusion mitigates edema at 3 days, which was expected.

### 2. Results

#### 2.1. Experiment 1 (long-term outcome)

A timeline of events for this experiment is illustrated in Fig. 1. There was no mortality in this study. A repeated-measures ANOVA of body weight included the following Time factor levels: entry into study, day of ICH, and days 1, 7, and 28 post-ICH. This analysis revealed a significant Time effect ($p < 0.001$), a non-significant Group main effect ($p = 0.893$), and interaction ($p = 0.883$). Thus, the pattern of weight change over time was similar among groups. On average, rats weighed 222 g at the start of the experiment, 363 g at the time of ICH, and 427 g at euthanasia. Rats experienced a slight ($\sim 10$ g) and transient loss of body weight after ICH.

2.1.1. Neurological Deficit Score Scale

Almost all rats received an NDS score of 0 (normal) during baseline testing and there was no significant difference among groups (Kruskal–Wallis test: $p = 0.346$; Fig. 2). Post-ICH scores were significantly elevated at all test times from 3 to 28 days (Wilcoxon test: $p < 0.001$ vs. baseline), although these deficits lessened somewhat over time (e.g., day 3 vs. day 7: $p < 0.001$). However, there were no significant differences...
among groups on any test day ($p \geq 0.154$). Thus, ICH caused significant and persistent neurological deficits that were not attenuated by either DFX treatment.

2.1.2. Corner turn test

Several rats were excluded from the corner turn test due to bias during baseline testing leaving $N$s of 9, 9, and 8 in the SAL, DFX-3d, and DFX-7d groups, respectively (Fig. 3). Baseline scores approached 50% (no asymmetry), as expected, and did not differ among groups (1-way ANOVA: $p = 0.147$). Post-ICH turning bias, towards the side of the ICH, was significantly greater than baseline on all test days ($p < 0.001$). A repeated-measures ANOVA on the post-ICH data showed a significant Time main effect ($p = 0.006$), which was due to lessening bias with time. However, the Group main effect was not significant ($p = 0.888$) nor was the interaction ($p = 0.291$). Thus, the corner turn test was sensitive to ICH, but neither DFX treatment regimen lessened turn bias.

2.1.3. Horizontal ladder walking test

Baseline scores for the forelimbs (Figs. 4A and B) and hind limbs (Figs. 4C and D) were not significantly different among groups (1-way ANOVAs: $p \geq 0.124$) and in most cases animals obtained perfect or near-perfect scores. Six to seven rats in each group (≈ half) would not cross the ladder on day 3 post-ICH and were excluded from analysis for that test day. There were no Group effects with the remaining data on this day.

Fig. 3 – Average bias (% turn towards lesion side) before and after ICH. There was no turning bias prior to ICH as scores approached 50%. Following ICH, there was a significant and persistent bias, but there were no significant differences among groups.

Fig. 4 – Average % successful steps with the ipsilateral forelimb (A), contralateral forelimb (B), ipsilateral hind limb (C), and contralateral hind limb (D). Significant impairments occurred after ICH, except with the ipsilateral forelimb. The DFX-7d treatment significantly improved success with the contralateral hind limb on last test session, but not at other times or with other limbs. Thus, DFX treatment generally did not lessen missteps.
than the SAL group (p=0.025). Data were collected for every rat on all other test days and thus were initially analyzed with repeated-measures ANOVAs. For the contralateral-to-stroke forelimb, the Time main effect was significant (p=0.025), but the interaction (p=0.767) and Group main effect (p=0.437) were non-significant. Thus, the DFX treatments did not affect the ICH-induced impairment with this limb. The ipsilateral-to-stroke forelimb data were not analyzed because the group means ranged from 99.2% to 100% correct over the test days (i.e., no biologically meaningful impairment at any time). The ipsilateral hind limb data were analyzed by ANOVA, which showed a significant Time main effect (p<0.001) and non-significant Group (p=0.245) and interaction effects (p=0.156). Thus, the DFX treatments did not affect the ICH-induced impairment with this limb. Finally, the contralateral hind limb data showed a significant Time main effect (p=0.029), a non-significant Group effect (p=0.408), but a significant interaction (p=0.038). Owing to a significant interaction, we conducted 1-way ANOVAs on the 7-day (p=0.715), 14-day (p=0.593) and 28-day data (p=0.011). Owing to a significant Group main effect in the last comparison, we follow up with post hoc tests that showed that only the DFX-7d group was significantly better than the SAL group (p=0.012). In summary, all but one comparison with one limb showed that the DFX treatments failed to improve stepping success in the horizontal ladder test.

2.1.4. Montoya Staircase Test

Two rats in the DFX-3d group failed to reach criterion performance in the staircase test and were excluded from further analysis with this test. Baseline scores, prior to ICH, were similar among groups for both the forelimb that would become the ipsilateral-to-stroke limb (Fig. 5A) as well as the contralateral forelimb (1-way ANOVAs: p=0.169; Fig. 5B). For each limb, the scores on every post-ICH test day were significantly lower than baseline (p<0.008). A repeated-measures ANOVA on the contralateral forelimb test data revealed a significant Time effect (p<0.001) with a non-significant Group effect (p=0.727) and interaction (p=0.233). For the ipsilateral limb, the Time effect was significant (p<0.001) as was the interaction (p=0.046). However, the Group main effect was not significant (p=0.461). Owing to the significant interaction, we analyzed each test day with 1-way ANOVAs that showed only non-significant Group main effects (p≥0.144). Thus, ICH impaired reaching success with both limbs, which improved slightly over test days, but these impairments were not attenuated by DFX-3d or DFX-7d treatment.

2.1.5. Histology

Brain injury mainly occurred in the striatum (caudate putamen), as expected, but occasionally it extended to affect the globus pallidus, corpus callosum, and cortex. Lesion volume averaged 43.5 mm³ and did not significantly differ among groups (1-way ANOVA: p=0.375, Fig. 6). Thus, neither DFX treatment lessened lesion volume.

2.2. Experiment 2 (cerebral edema at day 3 post-ICH)

No mortality or exclusions occurred in this experiment. A repeated-measures ANOVA of body weight included the following Time factor levels: day of ICH and 3 days post-ICH. This analysis revealed a significant Time effect (p<0.001) with a non-significant Group main effect (p=0.914) and interaction (p=0.119). On average, rats weighed 358 g at the time of ICH and 337 g at 3 days following ICH. This modest loss of body weight was statistically significant but not different among groups. Moderate edema occurred in the injured striatum (vs. contralateral striatum: p<0.001), but this was not affected by either the DFX-0h or the DFX-6h treatment (1-way ANOVA: p=0.441; Fig. 7).

3. Discussion

Our first experiment showed that DFX failed to lessen behavioral impairment or lesion volume whereas the second experiment showed no effect on edema of DFX after collagenase-induced ICH. These findings contrast with numerous studies that show DFX improves outcome after ICH in rats (Hua et al., 2006; Huang et al., 2002; Nakamura et al., 2003; Okauchi et al., 2009; Song et al., 2008; Wan et al., 2006) and piglets (Gu et al., 2009). While there are several differences (e.g., endpoints, lesion size) among studies that could partly

![Fig. 5 – Average number of pellets consumed with the ipsilateral (A) and contralateral-to-stroke forelimb (B). The ICH significantly lessened reaching success, but this was not mitigated by DFX treatment.](image-url)
account for the discordant findings, one likely explanation is that we used the collagenase model whereas others directly injected blood or its components to mimic ICH.

Based upon the aforementioned studies, we expected DFX to mitigate tissue loss because the collagenase model results in early neuronal injury (Del Bigio et al., 1996; Wasserman and Schlichter, 2007), including peri-hematoma dendritic atrophy (Nguyen et al., 2008), that should be amenable to DFX treatment. Furthermore, the more delayed cell death and atrophy occurring in this model (MacLellan et al., 2008; Nguyen et al., 2008) should also be mitigated, although additional treatments may be needed for full benefit. Nonetheless, a discernable neuroprotective effect was not obtained, possibly because collagenase-induced cell death (vs. whole-blood model) is less dependent upon iron toxicity and therefore less amenable to DFX. Instead, other factors, such as inflammation (Xue and Del Bigio, 2003), may play a comparatively greater role in the collagenase model. Perhaps too the collagenase insult was too severe. In this study, tissue lost averaged 43.5 mm³ at a 28-day survival. While this a moderate-sized lesion relative to our previous studies (DeBow et al., 2003; MacLellan et al., 2004, 2008; Nguyen et al., 2008), the damage appears larger than in DFX studies using the whole-blood model (e.g., Okauchi et al., 2009). However, as the volume of tissue loss has not been reported in those studies, a direct comparison is not possible. Assuming that our insult caused greater injury than in other DFX studies, it might account for the failure to improve outcome in our study. However, the collagenase dose we used results in at most a comparable intraparenchymal hematoma volume to that of the 100-μL blood injection model (MacLellan et al., 2008). Thus, the lack of benefit in this study seems not to be due to causing a larger bleed than that injected in the whole-blood DFX studies. Nonetheless, evaluating the efficacy of DFX against a range in insult severities (hematoma volumes) in each model would resolve this issue.

We selected a 100-mg/kg dose of DFX given twice daily for 3 or 7 days because others report that similar doses reduce cerebral iron accumulation and provide benefit after whole-blood-induced ICH (Gu et al., 2009; Hua et al., 2006; Nakamura et al., 2003; Okauchi et al., 2009). For instance, DFX reduced edema when given at a dose of 100 mg/kg twice per day starting 6 h after a 100-μL blood injection (Nakamura et al., 2003). This is the same dosing regimen that failed to improve outcome in our experiments. While dosing does not appear to explain our failure to improve outcome with DFX, our study could have been improved by determining the effectiveness of DFX in mitigating cerebral iron levels in this model. Furthermore, it remains possible that some behavioral or histological benefit might have been obtained with, for instance, earlier intervention or more protracted treatment. Note that a 6-h delay after onset of bleeding was chosen as it is clinically relevant. As well, we wished to avoid attenuating or aggravating the hematoma volume since bleeding has ceased by this time (at ∼4 h) in the collagenase model (MacLellan et al., 2008). It follows then, that our DFX treatment, even though it was delayed for 6 h after collagenase infusion, was administered within ∼2–3 h of active hemorrhaging. Such a delay is in line with DFX studies in the whole-blood model (Gu et al., 2009; Hua et al., 2006; Nakamura et al., 2003; Wan et al., 2006).

Several DFX studies have relied upon edema as a primary endpoint, and they show that it is lessened by DFX after ICH (Huang et al., 2002; Nakamura et al., 2003; Okauchi et al., 2009; ...
Song et al., 2008), which we did not observe in the collagenase model. Furthermore, while edema can be life-threatening after stroke, its significance after ICH is not always clear (NINDS ICH Workshop, 2005; Zazulia et al., 1999). Similarly, the impact of ICH-induced edema in rat models is not obvious. For instance, severe edema after striatal ICH, including some in surrounding structures (e.g., overlying cortex), rarely causes mortality in rats even when there is near-total striatal destruction (MacLellan et al., 2005). Furthermore, reductions in edema and inflammation do not always correspond to improved recovery or lessened injury as demonstrated with hypothermia treatment after whole-blood-induced ICH in rats (MacLellan et al., 2006b). Thus, while DFX certainly reduces edema in the blood injection model, this finding alone should not lead one to assume that a comparable improvement in histological and functional outlook would occur in this or other models, or in humans.

Of the numerous DFX studies, only a few (Gu et al., 2009; Hua et al., 2006; Okauchi et al., 2009) provide histological evidence of neuroprotection. The two studies in rats measured caudate atrophy in one coronal section per animal (Hua et al., 2006; Okauchi et al., 2009), but they did not measure the volume of tissue lost as presently done. Without knowing the relationship between these methods, to determine the validity of using only an area measurement from one coronal section, one might speculate that DFX’s neuroprotective effect has been overestimated in the whole-blood ICH studies. Conversely, we did not quantify peri-hematoma cell death as little would be expected at 28 days. As well, counting the remaining neurons in defined peri-hematoma regions (grids) is also not a valid technique at long survival times owing to potentially substantial tissue volume changes due to the loss of cells, the gain of inflammatory cells, and the cellular hypo- or hypertrophy. Thus, DFX may have attenuated cell death early after ICH, but in an amount that was not sufficient to affect 3-day edema, or subsequent functional recovery and long-term lesion volume.

Behavioral testing is an essential step in evaluating neuroprotectants because a reduction in cell death or edema does not necessarily translate into improved recovery, which is the clinical endpoint of greatest importance (Corbett and Nurse, 1998; Stroke Therapy Academic Industry Roundtable (STAIR), 1999). Accordingly, it is an important finding that DFX improves behavioral recovery after whole-blood-induced ICH (e.g., corner turn test; Okauchi et al., 2009), although benefit was sometimes inconsistent and not observed on all tests (Hua et al., 2006). In this study, we used 4 tests that were sensitive to striatal ICH over the test times used (3–28 days). Despite this, only the DFX-7d treatment statistically improved outcome, and then only with the contralateral hind limb on day 28. Given the lack of benefit at other test times, with the other limbs, and on the other tests, it seems that this one positive finding is probably due to chance. Note that post hoc tests, including the Tukey test, reduce the risk of Type 1 errors within a data set (e.g., hind limb data), but the risk of an error goes up with the number of tests conducted (e.g., for each behavioral test). Finally, it should be noted that moderate deficits occurred on all tests with room for treatment-induced improvement. Thus, there were no floor or ceiling effects masking a beneficial effect of DFX. The failure to find benefit in this study does not appear to be due to improper test selection but, instead, by the failure of DFX to reduce edema and lesion volume.

In summary, DFX did not improve behavioral or histological outcome or reduce edema after collagenase-induced ICH. While other DFX regimens may improve outcome, especially after milder insults, our findings suggest that this drug is not markedly neuroprotective in the collagenase model. Interestingly, DFX has already advanced to clinical trial (Selim, 2009) based upon the work done primarily in the whole-blood model and largely from one group and their collaborators. We feel, and as recommended by the STAIR report (Stroke Therapy Academic Industry Roundtable (STAIR), 1999) and others, that evidence should come from multiple stroke models across several labs prior to advancing a therapy to clinical trial. Unfortunately, neither the collagenase nor the whole-blood model fully reproduces the clinical course of ICH, nor is it known which model better predicts clinical efficacy. Our negative findings, therefore, are important in that they suggest that DFX may not work clinically for ICH. Such negative findings should not be ignored in our evaluation of putative neuroprotectants (Diguet et al., 2004). Indeed, we must consider of all the evidence, including both positive and negative findings, while taking into account model differences and effect sizes.

4. Experimental procedures

4.1. Animals

Seventy-two young-adult (~11 weeks old at the time of ICH), male, Sprague–Dawley rats were used in this study and were obtained from the Biosciences breeding colony at the University of Alberta. They were weighed upon entry into the study and intermittently thereafter. Rats were given free access to water and food, unless under food deprivation for behavior testing, which is described below. All procedures were approved by the Biosciences Animal Care and Use Committee at the University of Alberta and were in accordance with the guidelines of the Canadian Council on Animal Care.

4.2. Intracerebral hemorrhage surgery

Rats were anesthetized with isoflurane (4% induction, 2% maintenance in 60% N2O, and 40% O2) and aseptic surgical technique was used. The scalp was shaved and cleaned with alcohol and betadine, and then 0.2 mL of Marcaine (Sanofi Canada, Markham, OT, Canada) was injected subcutaneously on the skull. Rats also received 5.0 mL of saline subcutaneously on the back to replace any fluids lost during and following the surgical procedure. Rats were then placed in a stereotaxic frame while body temperature was measured with a rectal thermocouple probe and maintained with a heating pad and surgical drapes at 36.5–37.5 °C. A midline scalp incision was made and a hole was drilled 3.5 mm lateral and 1.0 mm anterior to the bregma (Paxinos and Watson, 1998). In Experiment 1, this hole was on the side contralateral to the preferred paw, defined as the paw with the highest average number of pellets retrieved over the last week of staircase training (discussed below). In Experiment 2, the ICH was...
induced in the left striatum. A beveled 26-gauge needle (Hamilton syringe; Hamilton, Reno, NV, USA) was lowered to 7.0 mm below the surface of the skull. After 2 min, 1.0 μL of sterile saline containing 0.2 U of bacterial collagenase (Type IV –S. Sigma-Aldrich, Oakville, ON, Canada) was infused into the striatum over 5 min (MacLellan et al., 2006a; Rosenberg et al., 1990). The needle was left in place for 5 min and then slowly removed. The hole was sealed with a metal screw (model MX-080-2; Small parts, Miami Lakes, Fl, USA), and the scalp was stapled closed. Anesthetic was then discontinued and rats recovered quickly in their home cage.

4.3. Experimental groups

There were three groups in the first experiment (N=12 each): two DFX-treated (Sigma-Aldrich, Oakville, ON, Canada) and one saline (SAL)-treated group. Animals were randomly assigned to these groups. All injections were administered i.p. beginning 6 h after collagenase infusion and then repeated every 12 h for 7 days. Deferoxamine was made fresh before each injection. The SAL group received 7 days of saline injections (14 injections, 0.5 mL/kg). One of the drug-treated groups (DFX-3d) received DFX for 3 days (7 injections, 100 mg/kg at a concentration of 50 mg/mL of saline) with 4 subsequent days of saline injections (7 injections, 0.5 mL/kg). The other drug-treated group (DFX-7d) received injections of DFX for 7 days (14 injections, 100 mg/kg each). Thus, all groups had the same number of injections. The dosing regimens were chosen based upon work done in the whole-blood model of ICH showing DFX to reduce iron load, cerebral edema, and to promote functional recovery (Hua et al., 2006; Nakamura et al., 2003; Okauchi et al., 2009).

There were also three groups in the second experiment (N=12 each): two DFX-treated and one SAL group. For the DFX groups, injections started immediately after collagenase infusion (DFX-0h) and while still anesthetized or after a 6-h delay (DFX-6h). The SAL group received injections starting at 6 h after collagenase infusion. Rats were euthanized on days 3 and 7 after surgery.

4.4. Behavior training and testing (Experiment 1 only)

4.4.1. Neurological Deficit Score (NDS) Scale

An NDS scale was used to evaluate outcome before and at 3, 7, 14, and 28 days after ICH. Briefly, rats were rated on the presence of spontaneous circling, contralateral hind limb retraction after lateral movement, bilateral forepaw grasp, bear walking ability, and contralateral forelimb flexion. The scale, which in total ranged from 0 (normal) to 14 (maximal impairment), is sensitive to striatal ICH in rats (MacLellan et al., 2006a). As with the other behavioral tests, animals were assessed by an experimenter blinded to group identity.

4.4.2. Corner turn test

The corner turn test, which is sensitive to striatal ICH, assesses an animal’s turn bias upon exiting a corner (Hua et al., 2002; Schallert, 2006). Rats were allowed to proceed into the corner, rear either left or right, and exit the corner without hindrance. The corner was constructed of two walls (41 cm tall and 31.5 cm long) that were placed at a 30° angle with a 1-cm gap in between the abutting ends. An alley (45 cm long) leading to these walls guided the rats to explore the corner. The direction of turning over 10 trials was recorded on the day before ICH surgery and at 3, 7, 14, and 28 days after ICH. Rats that had a baseline turning bias of <30% or >70% were excluded from statistical analysis for this test only (i.e., they remained in the study) (Schallert, 2006).

4.4.3. Horizontal ladder walking test

The horizontal ladder test consists of having rats walk across a 1-m long horizontal series of parallel rungs (each 3 mm in diameter) interspersed 1-4 cm apart. Rats with a damaged motor system (Metz and Whishaw, 2000), including striatal ICH (MacLellan et al., 2006a), are impaired on this task (i.e., greater number of slips made traversing the ladder). Rats were trained for 3 days (4 trials per day) to cross the ladder, which was situated 23 cm above a table and videotaped from below. The last day of training was analyzed for baseline walking ability. Testing was conducted on days 3, 7, 14, and 28 after ICH (4 trials per day). We excluded rats, from this analysis only, that were unable to cross the ladder at least twice. Performance on this test is expressed as: % success=sucessful steps/total steps × 100.

4.4.4. Montoya Staircase Test

The Montoya Staircase Test (Montoya et al., 1991) was used to evaluate skilled reaching ability because this test is among the most sensitive to striatal ICH in rats (MacLellan et al., 2006a). The staircase apparatus (30 cm long, 6.8 cm wide, 12 cm high) has a central platform, which supports the rat’s body, with descending steps on each side beveled to hold 3 food pellets per step (21 pellets per side). Following mild food deprivation (90% of normal body weight), the rats were trained to reach for these pellets (45 mg sugar pellets; Bio-Serv, French-town, NJ, USA) over 3 weeks prior to ICH surgery (two 15-min trials per day for 5 days a week). Rats were excluded from this data analysis if they did not obtain an average of 8 pellets per session over the final 3 days of training. After training, the rats were returned to ad lib feeding. Food deprivation was reinstated for the test sessions that took place on days 24 to 28 after ICH.

4.5. Histology (Experiment 1)

At 28 days after ICH, the rats were injected with sodium pentobarbital (80 mg/kg, i.p.) and then euthanized by systemic perfusion with 0.9% saline followed by 10% formalin. Brains were extracted and later sectioned on a cryostat. Forty-micrometer sections were taken every 200 μm through the entire brain and stained with cresyl violet. Tissue loss was quantified using Image J 4.0 (Scion Corporation, Fredrick, MD, USA) and calculated by subtracting the volume of injured hemisphere from the volume of normal hemisphere. The volume of each hemisphere is calculated as: (average area of complete coronal section of the hemisphere–area of lesion–area of ventricle)×intervals between sections × number of sections (MacLellan et al., 2006a). Histological analysis was done by an experimenter blinded to treatment condition.
4.6. **Cerebral water content (Experiment 2 only)**

Rats in Experiment 2 were anesthetized (isoflurane) at 3 days after surgery and then euthanized by decapitation. After promptly removing the brain, the cerebellum was removed to serve as a control (Fingas et al., 2007). The cerebrum was then blocked into a 4-mm-wide chunk from 2 mm anterior to 2 mm posterior to needle tract. This tissue was then separated into cortex and striatum for each hemisphere. The tissue samples were weighed (wet weight), baked at 100°C for 24 h, and then re-weighed (dry weight). Brain water content (%) was calculated from: \( ((\text{wet weight} - \text{dry weight})/\text{wet weight}) \times 100 \). A 3-day survival time was chosen as it has been used in DFX work (Nakamura et al., 2003; Okauchi et al., 2009) and it is a time when significant edema occurs after ICH in rats (Fingas et al., 2007; Tanaka et al., 2009).

4.7. **Statistical analysis**

Most data were assessed using ANOVA, followed by Tukey HSD post hoc testing or within-subjects contrasts if necessary. For nonparametric data (e.g. NDS), the Kruskal–Wallis test was used to compare between groups and the Wilcoxon sign ranks test was used to compare across time.

**Conflict of interest**

The authors have no conflicting interests with regard to the use of deferoxamine or any product used in this study.

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**References**


Mayer, S.A., Brun, N.C., Begtrup, K., Broderick, J., Davis, S., Diringer, M.N., Skolnick, B.E., Steiner, T., 2008. Efficacy and safety of...


