The effects of deferoxamine(DFO) on murine traumatic brain injury

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VETERINARY MEDICINE
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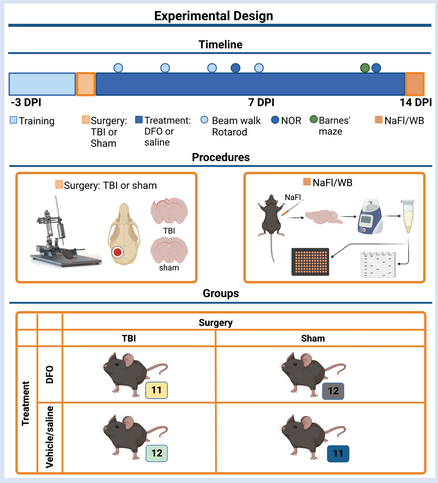
Background and Aims

- A traumatic brain injury (TBI) is a major cause of death and disability in humans and impacts animals around us as well. TBI can often lead to disturbance of the blood-brain barrier (BBB) and its normal function, which can trigger secondary neuroinflammation.
- Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor regulating oxygen homeostasis. It has been proven to have a protective effect on the neurovascular unit in animal models of stroke, retinopathy, and glaucoma. HIF-1α exert a dual effect promoting cell proliferation and survival, and inhibiting inflammation and apoptosis.
- Deferoxamine (DFO) is an FDA-approved iron chelator and can stabilize HIF-1α by inhibiting the activity of HIF-prolyl hydroxylase. DFO has been considered as a therapeutic agent for many neurodegenerative conditions.

We hypothesize that daily DFO administration facilitates faster recoveries and better outcomes after TBI by prolonging the presence of HIF-1α.

Methods

Ten-week-old C57BL/6J mice, male and female, were submitted to TBI or sham surgeries. Mice were anesthetized and secured to a stereotaxic frame for a 5 mm unilateral craniotomy, followed by a controlled cortical impact (CCI), stimulating real TBI, at a rate of 4.0 m/s, dwell time of 200 ms and 0.4° angle. The sham surgery involved an incision but no craniotomy or impact. Mice received 100mg/kg BW DFO or sterile saline via intraperitoneal injection for 14 days post-injury (DPI).



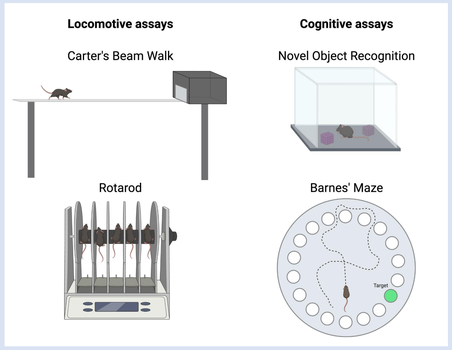
Mice underwent 4 behavioral assays:

1. **Rotarod**: Mice were placed on a rod in stalls, which accelerated over 5 min to a maximum rotational velocity of 40 rpm, and latency to fall was recorded.

2. **Carter's Beam Walk**: Mice were trained to transverse a 1m long beam towards a black shelter box located at the end of the beam. 3 different beam widths were used: 28 mm, 12 mm, and 6 mm. Time and number of hindlimb paw slips were recorded.

3. **Novel Object Recognition**: Each mouse was exposed to 2 identical objects for 5 mins. 90 min later, mice were placed in the same field with one familiar object from the previous exposure and one novel object. Interaction with objects was recorded. A discrimination index (DI) was calculated based on the difference in interaction time between the novel object and the familiar object.

4. **Barnes' Maze**: Mice were trained to associate visual cues with the target hole in the Barnes' maze for 4 consecutive days. On the fifth day, mice were given 5 min to escape from the maze.



We humanly euthanized 2 cohorts of mice at 3- and 7-DPI via intracardiac perfusion with PBS and tissues were harvest for molecular assays.

1. **Sodium fluorescein (NaFl) permeability assay**: mice were injected with 10 mg of NaFl dye in 100 ul of PBS 45 min before perfusion. Blood, brain cortex, other central nervous system tissues (brainstem, cerebellum, etc), liver, and gastrocnemius muscle were harvest. Tissues were homogenized in PBS supplement with proteinase and phosphatase inhibitors, and serum separated from blood. Fluorescence intensity of 1:100 serum samples and tissues lysate were read at 450-540 nm. We quantified the BBB permeability by measuring the fluorescence intensity per gram of wet tissue relative to serum fluorescence per mL. The liver and gastrocnemius served as positive controls.

2. **Western blot**: an addition aliquot of the tissue lysates described above were used for Western blot analysis to quantify the amount of HIF-1α protein in the cortex followed by densitometric analysis.

Results

We performed two-way ANOVA tests for all data sets, except for the cortical HIF-1α level, which we used one-way ANOVA.

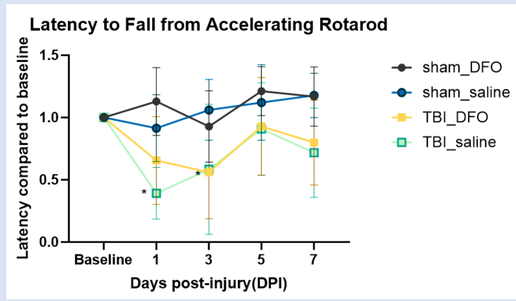


Figure 1. Latency to fall from accelerating rotarod. There was a statistically significant interaction between the effects of treatment groups and performance (p = 0.0023). On 1-DPI, difference in TBI_saline group latency to fall was statistically significant while TBI_DFO performance was similar to sham_saline. However at 3-DPI, TBI_DFO group performance was similar to TBI_saline group.

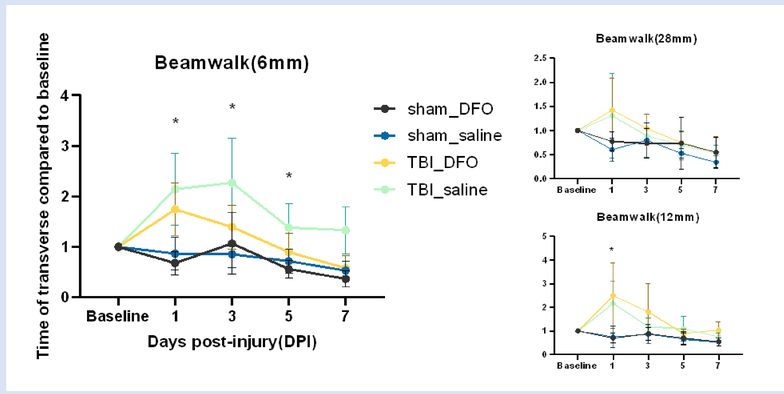


Figure 2. Carter's Beam Walk. There was a statistically significant interaction between the effects of treatment groups and DPI in all 3 beam widths (p = 0.038 for 28 mm, p = 0.0051 for 12 mm, p = 0.0006 for 6 mm). For beam width of 6 mm, the TBI groups performed significantly worse than the sham groups on 1-DPI. However, on 3- and 5-DPI, TBI_saline group performed significantly worse than sham groups, while TBI_DFO group performance was similar to the sham groups.

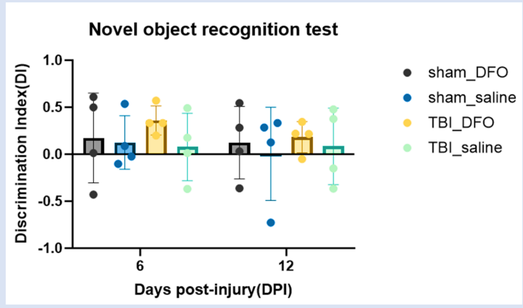


Figure 3. NOR test. There was no statistically significant interaction between the effects of treatment groups and DI for both trials (p = 0.48). TBI_DFO group had the highest DI, suggesting a trend on better short-term memory acquisition when compared to the groups in both trials.

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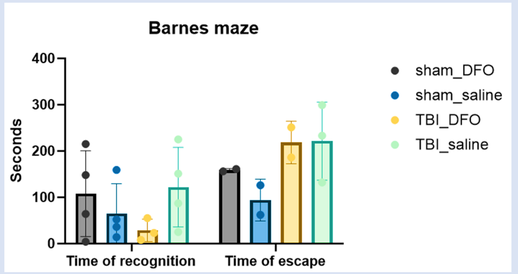
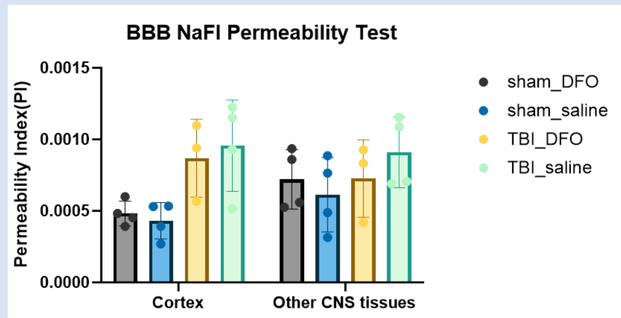


Figure 4. Barnes' maze. There was no statistically significant interaction between the effects of treatment groups and performance for both trials (p = 0.25). On average, TBI_DFO group had the shortest time of recognition among all groups suggesting a trend on better long-term memory acquisition.



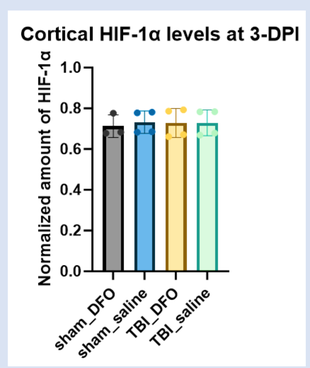


Figure 5. BBB NaFl permeability assay. There was no statistically significant interaction between the effects of treatment groups and permeability index for the cortex and other CNS tissues (p = 0.086). TBI_saline group did have a significantly high PI than the sham groups, while TBI_DFO group similar to both sham groups.

Figure 6. Cortical HIF-1α levels at DPI3. There was not a significant interaction between the effects of treatment groups and cortical HIF-1α levels (p = 0.98). The amount of HIF-1α remained similar between groups.

Conclusion

- Carter's beam walk and rotarod show promising results that suggest DFO may have an impact on the improvement of locomotive functions during the first days post-injury.
- The insignificant data of Barnes' maze and NOR may be due to a smaller sample size (n=16) compared to the locomotive tests. More mice may be needed to make a conclusive claim on the effects of DFO on cognitive function after TBI.
- BBB NaFl permeability test shows that DFO may play a role in the repair of BBB after TBI.

Future studies

Our goal is to complete the molecular assays with the tissue samples harvested from the 7-DPI cohort. Additionally, we will follow up with the long-term cohort and potentially assess if there are any long-term effects of DFO on locomotive and cognitive functions. We also want to test drugs that directly inhibits HIF-PHD activity such as Roxadustat (FD-4592).

Acknowledgements

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