Intraventricular Ziconotide Therapy Improves Functional Outcomes Following Lateral Fluid Percussion Traumatic Brain Injury

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Introduction

Traumatic brain injury (TBI) is a major source of morbidity and mortality worldwide.[1,2] Survivors of moderate-to-severe TBI frequently have neurological and cognitive deficits.[3,4] There are no pharmacological therapeutics for neuroprotection following TBI.

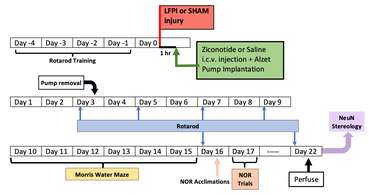
Intravenous ziconotide, (SNX-111, Prialt®), a synthetic ω-conotoxin, is a highly selective N-type voltage-gated calcium channel (VGCC) blocker that has demonstrated potential as a neuroprotective agent in rodent models of TBI[5,6] but failed to translate due to secondary cardiovascular side effects. Similar to TBI, i.v. ziconotide failed to translate for the treatment of chronic pain. However, investigators hypothesized that by providing the drug centrally via an intrathecal catheter, one could achieve a therapeutic dose to reduce pain at significantly lower concentrations while also reducing access to peripheral receptors.[7] Ziconotide is now FDA approved for treatment of pain when administered intrathecally.

Patients with refractory intracranial pressure elevations, often get implanted with an external ventricular drain (EVD), providing an opportunity to deliver therapy centrally (intracerebroventricular, i.c.v).

Using the rat fluid percussion model we tested the following hypotheses:

1) **Ziconotide can be delivered centrally acutely following TBI**
2) **Central delivery will improve motor and cognitive outcomes**
3) **72 hrs of i.c.v. administration will reduce hippocampal cell death**

Methods



Experimental Design:

1. 77 Sprague-Dawley rats (males (M)=36, females (F)=41).
2. Animals either received a sham injury with saline (sham; n=11 M, 11 F), TBI with saline (TBI+Veh; n=11 M, 12 F) or a TBI with Ziconotide (TBI+Zic; n=10 M, 11 F).

Lateral Fluid Percussion Injury (LPFI) & Drug Delivery:

1. Animals were anesthetized, intubated, & mechanically ventilated
2. A craniectomy was made over the right parietal bone (centered at AP = –4.5 mm, ML= +3.0 mm from bregma).
3. Animals received a sham or moderate LFPI.
4. A stereotactic arm was used to administer intraventricular saline or ziconotide ipsilateral to injury one-hour post-injury (AP= -0.8 mm, ML=+1.5, DV=-4.0; 9µl injection; 150 ng in males, 100 ng in females).
5. A cannula was implanted into the contralateral ventricle and attached to a pre-filled micro-osmotic pump filled with saline control or drug (males = 16.67 ng/hr, females =11.11 ng/hr).
6. The pump was removed 72 hours following implantation.

Behavioral Outcomes:

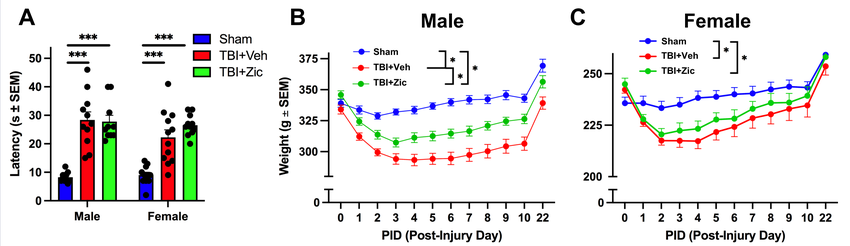
1. *Rotorod (Post-injury days 3, 5, 7, 9, 16 and 22)*: 3 min step-wise accelerating trial (3 trials/day). Analyzed average latency to fall.
2. *Morris water maze (Post-injury days 10-15)*: Four daily trials, one from each cardinal starting points.
3. *Novel Object (Post-injury day 17)*: 5 min training & test with 3 hr inter-trial. Recognition index (time novel/time novel+time original)*100.

Stereology (Post-Injury Day 22):

1. Animals transcardially perfused.
2. Tissue sectioned at 45 µm on cryostat.
3. NeuN (Millipore MAB377) IHC to stain neurons.
4. Stereology to quantify the number of CA3 neurons (every 5th section from -3.30 bregma and ending at 4.52 bregma)

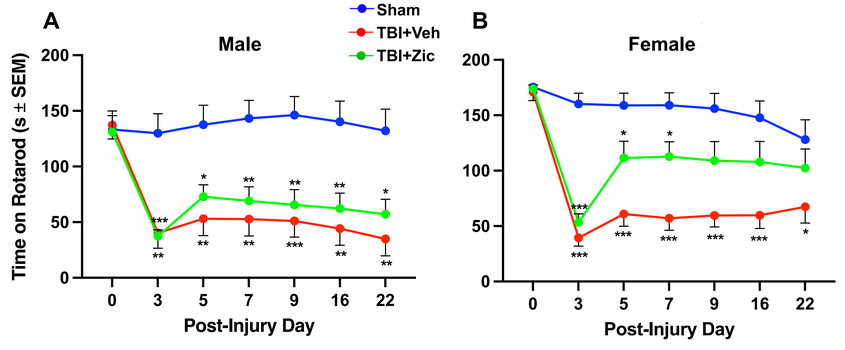
Results

Fig 1: Biological Data



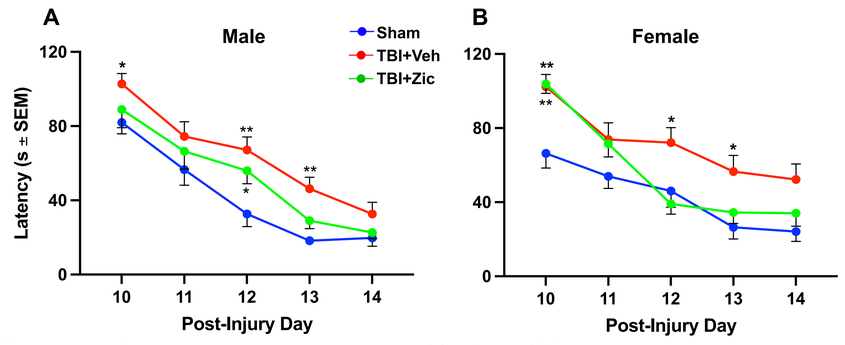
In both male and female rats TBI resulted in significant increases in righting times **(A)**. There was no difference between TBI treatment groups. Both male **(B)** and female **(C)** rats lost weight following TBI. In males, treatment of TBI rats with ziconotide resulted in significantly faster weight gain as compared to vehicle, but there was no difference between TBI treatment groups in females. Male and female rats were analyzed with separate ANOVA and rmANOVA with a Bonferroni post-hoc for righting and weight respectively. *p<0.05

Fig 2: Rotarod



While all rats were able to walk at the slowest speeds, male rats with TBI performed significantly worse than sham, regardless of treatment as the rod accelerated **(A)**. In females, ziconotide treated rats recovered to sham levels by post-injury day 9 **(B)**. Separate rmANOVA with Dunnett post-hoc for males and females, each comparing injured rats to sham. *p<0.05, **p<0.01, ***p<0.001

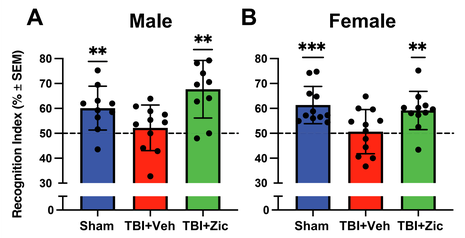
Fig 3: Water Maze



TBI resulted in significant impairment in spatial learning in both males **(A)** and females **(B)**. Treatment with ziconotide led to improved performance in both male and female rats. Separate rmANOVA with Dunnett post-hoc for males and females, each comparing injured rats to sham. *p<0.05, **p<0.01

Results

Fig 4: Novel Object



While sham controls significantly preferred the novel object, both male **(A)** and female **(B)** TBI rats performed at chance. Ziconotide-treated male and female rats both demonstrated significant preference for the novel object. A Wilcoxon signed-rank test compared recognition index to 50%. **p<0.01, ***p<0.001

Conclusions

1. Biological Data

- **There was no difference in ATM (*not shown*), apnea (*not shown*) or righting times between TBI+Veh and TBI+Zic rats**
- **All TBI rats lost weight compared to shams. In males, ziconotide-treated rats had an improved phenotype as compared to vehicle**
- **All ziconotide-treated rats had a mild tremor phenotype during treatment that did not interfere with eating, grooming, etc.**

2. Behavioral Data

- **Rotarod**
  - **All rats could perform the rotarod at slow speeds. TBI rats were impaired at higher speeds**
  - **Ziconotide-treated female rats demonstrated significant recovery on the rotarod**
- **Water Maze**
  - **TBI had similar swim speeds *(not shown)*, but longer latencies as compared to shams**
  - **Both male and female ziconotide-treated rats had improved daily latency on the water maze, likely due to improved search efficiency (*data not shown*)**
- **Novel Object**
  - **Sham rats preferred the novel object, while TBI rats performed at chance**
  - **Both male and female ziconotide-treated rats preferred the novel object**

3. Stereology

- **No significant difference in CA3 neurons were detected (*data not shown*)**

4. Future Directions

- **Evaluation of central ziconotide on mean arterial pressure as well as intracranial pressure**
- **Evaluation of dosing including 1) dose-response, 2) duration of treatment and 3) therapeutic window**

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