

Effects of Experimental Drugs on Traumatic Optic Neuropathy in mice

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Introduction

- In the United States, traumatic brain injuries (TBI) are the number one cause of disability and death, affecting 2.8 million Americans. (CDC 2017)
- TBI is an immediate source of visual deficits and impairments via direct or indirect trauma to the eyes, optic nerve, and/or visual processing areas of the brain, also known as traumatic optic neuropathy (TON). (Sen, 2017)
- Recent research has suggested Endoplasmic Reticulum (ER) Stress, with the associated Unfolding Protein Response (UPR) is involved in apoptosis of retinal cells as a result of TON. (Kroeger et al., 2018)
- Pharmacological manipulations of the PERK pathway (such as with Salubrinal or ISRIB) have displayed promising neuroprotective effects, mediated by the signaling molecule eIF2 α (eukaryotic initiation factor-2 alpha). Salubrinal is a selective phosphatase inhibitor of eIF2 α . Conversely, ISRIB inhibits phosphorylation of eIF2 α (p-eIF2 α). (Rubovitch et al., 2015)

Hypothesis

We hypothesized that following a TBI, treatment with Salubrinal will improve neuroprotection while ISRIB treatment will decrease neuroprotection, compared to vehicle.

Methods

Animals

- 8-week-old adult C57Bl/6 adult male mice (Jackson Laboratories, Bar Harbor, ME, USA).

Traumatic Brain Injury

- 400 gram closed-head weight drop 1.5 cm above scalp around the area of the bregma

Drug Administration

- Mice were injected intraperitoneally once daily for seven days post injury with Salubrinal (1 mg/kg), ISRIB (2.5 mg/kg), or vehicle (1 mg/kg)
- All diluted in 6.25% DMSO

Histology

- Retinas were used for Western Blotting
- Membranes were blocked in 5% milk for 1 hour and incubated in respective primaries (p-eIF2 α 1:500; ATF4 1:1500).

Statistical Analyses

- Western band densities quantified using ImageJ.
- Two-way ANOVA ran using GraphPad Prism.

Results

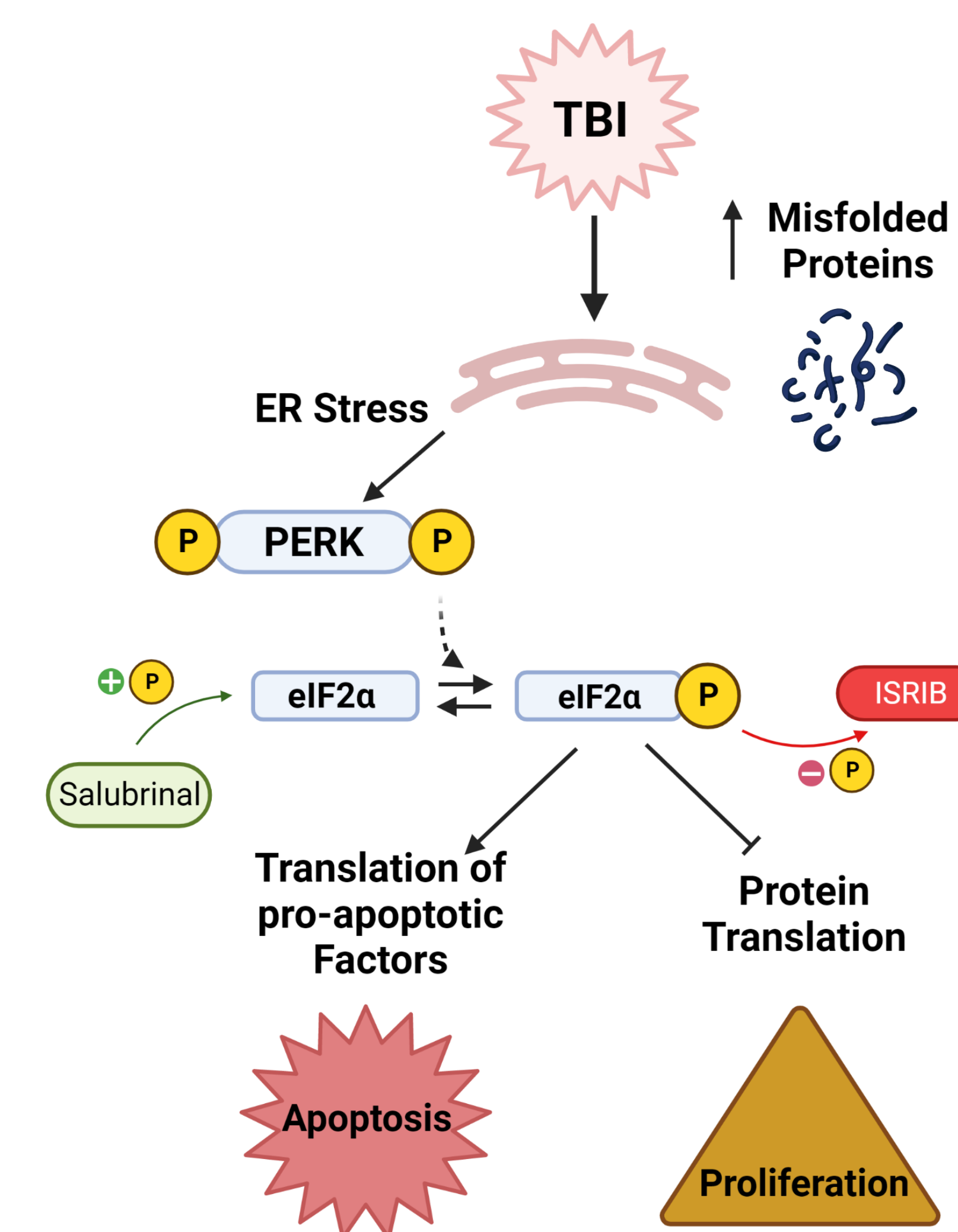


Figure 1. PERK Pathway of ER Stress Response and Drug Interaction. Simplified graphic displaying eIF2 α signaling within the PERK pathway of the cellular ER Stress Response and intracellular interactions of Salubrinal and ISRIB Created on Biorender.com

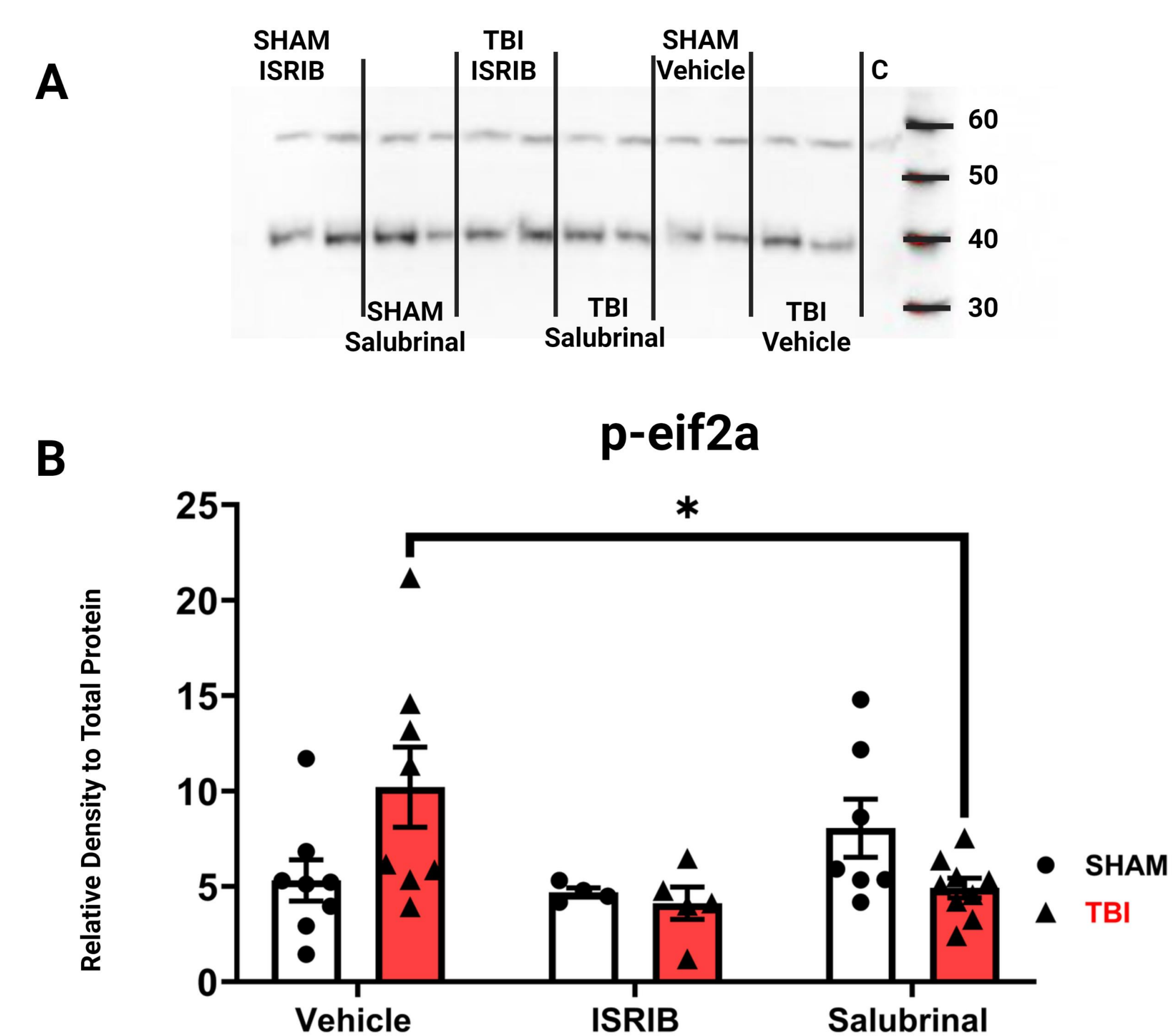


Figure 3. Western Blot Analysis and Quantification of Relative p-eIF2 α Levels. (A) Western Blots stained for p-eIF2 α showing relative band densities between SHAM and TBI mice and drug treatment. (B) Graph measuring relative density of p-eIF2 α to total protein in both SHAM and TBI mice between drug treatment groups. Statistical significance indicated by asterisks ($p < 0.05$). Created on Biorender.com. Analyzed and created using GraphPad Prism.

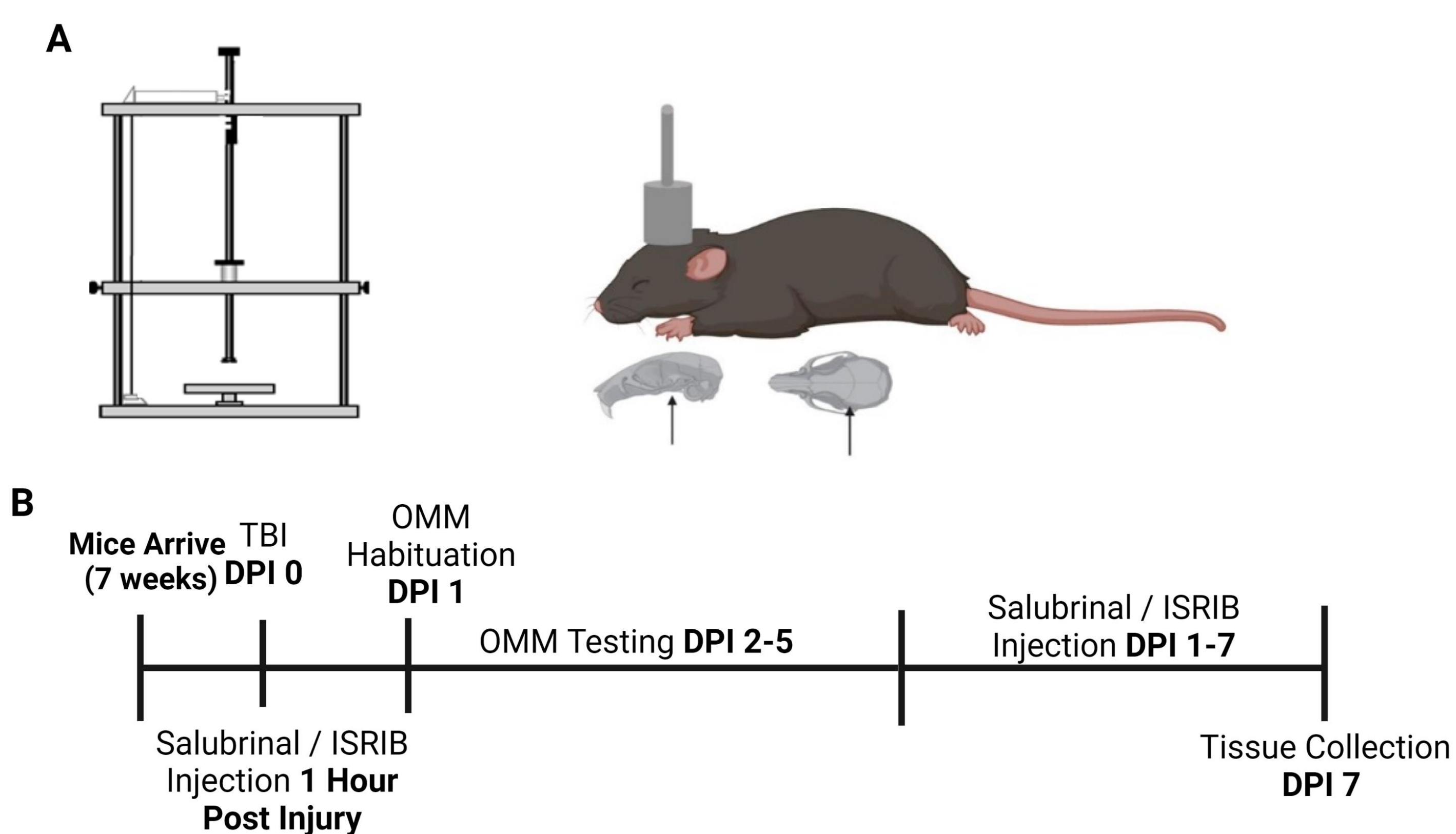


Figure 2. Timeline of Experiment and representative image of TBI device. (A) Model of weight drop device and location of traumatic brain injury (arrows). (B) Experimental timeline. Created on Biorender.com

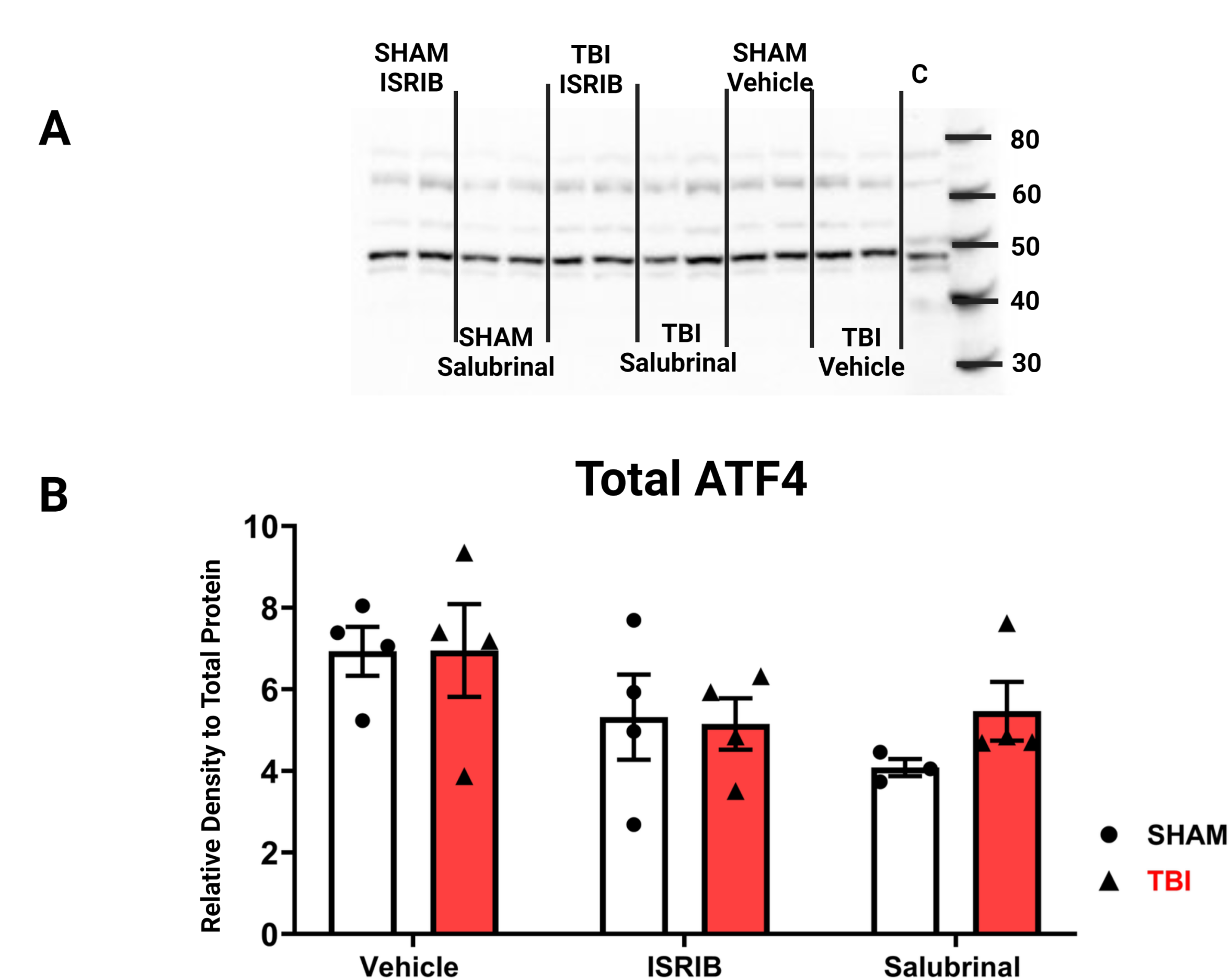


Figure 4. Western Blot Analysis and Quantification of Relative ATF4 Levels. (A) Western Blots stained for ATF4 displaying relative band densities between SHAM and TBI mice and drug treatment. (B) Graph measuring relative density of ATF4 to total protein in both SHAM and TBI mice between drug treatment groups. No statistical significance between SHAM and TBI or drug treatment groups. Created on Biorender.com. Analyzed and created using GraphPad Prism.

Discussion/Conclusions

- In contrast to our predictions, TBI mice treated with Salubrinal had significantly lower levels of p-eIF2 α compared to vehicle.
- In many biological pathways, negative feedback systems play a significant role in regulating levels of vital molecules/proteins. Since tissue was collected around 24 hours after the last injection, it's possible that these negative feedback controls decreased p-eIF2 α levels down significant levels.
- Also, in contradiction to our results, total ATF4 levels remained unchanged across all treatments.
- According to literature, ATF4 is known to increase in response to higher levels of p-eIF2 α . It's likely that over the course of seven days, homeostatic mechanisms decreased ATF4 to insignificant levels.

Future Directions / Limitations

- Limitations**
 - Only male mice were used.
 - ATF4 analyzed from tissue seven days DPI.
 - Tissue for Salubrinal analysis harvested about 24 hours after last injection.
 - Planned, but no completed analysis of CHOP (for apoptotic effects) and RBPMS (for retinal ganglion cell loss).
- Future Directions**
 - Analyze other downstream molecules (such as CHOP).
 - Harvest tissue immediately following final injection of drug.
 - Establish a baseline for ER Stress levels.

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References

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