Exploring the Effects of TBI and Alcohol in Inducing Proteinopathy in the Lumbar Spinal Cords of Rodents: Potential Role of ISGylation

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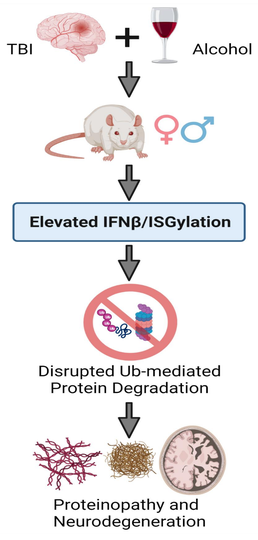
Abstract

Traumatic brain injury (TBI) is a prominent cause of death and disability worldwide, as well as a risk factor for a variety of neurodegenerative disorders including amyotrophic lateral sclerosis (ALS). Military veterans and contact sport players are more susceptible to TBI and neurodegenerative disorders. In addition, alcohol use raises the risk of TBI and neuronal damage, while TBI increases the likelihood for alcohol abuse. Currently, the knowledge on the mechanism(s) underlying TBI-mediated neurodegeneration and whether alcohol modulates these mechanism(s) is not known. There is currently no cure for TBI-induced neurodegeneration, stressing the need to better understand the mechanisms by which TBI triggers neurodegeneration.

Interferon stimulated gene 15 (ISG15), a ubiquitin-like protein, has previously been shown in our lab to antagonize ubiquitin-mediated protein degradation. Studies from our lab have also demonstrated ISG15 is elevated in neurodegenerative diseases, namely in human ALS spinal cords. **Therefore, we tested to see if TBI-induced activation of the IFNβ/ISG15 axis impairs the ubiquitin-mediated turnover of neuronal proteins in the spinal cord. Toxic accumulation of non-degraded proteins leads to neurodegeneration, and alcohol exacerbates this mechanism.**

We used cell culture and rodent models of injury to test our hypothesis. In the cell culture model, we found that TBI induces ISGylation. In rodent models we found that ISGylation of cellular proteins is increased in the lumbar spinal cords (SCLs) of rodents collected 12 weeks post TBI- and TBI-alcohol administered male rats. Interestingly, in the SCLs of 12-week female rats, TBI has no effect on ISGylation induction. To test ISGylation of specific proteins, we chose TDP-43 as a model protein, as its ubiquitin-mediated targeted degradation is affected consequently, non-degraded TDP-43 is accumulated in inclusion bodies. We immunoprecipitated (IP) TDP-43 and analyzed IPs on the Wes assay using anti-ISG15-specific antibody. We found that TBI induced TDP-43 ISGylation in 12-week male rats, yet alcohol reduced it. In the 12-week females, TBI did not induce TDP-43 ISGylation; however, alcohol induced ISGylation of TDP-43 in TBI-exposed female rats. At this point, we conclude that alcohol induces TBI-mediated ISGylation of TDP-43 in females but not in males, suggesting that females may be more vulnerable to TBI.

Hypothesis



Results

HEK293 Cells

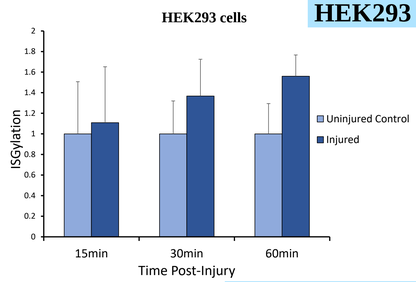


Figure 1. TBI induces ISGylation. HEK293 cells were injured three times over one hour with a Cell Injury Controller II set to apply a pulse of air pressure resulting in a peak pressure of 8psi. Cells were lysed 15, 30, and 60 minutes post-injury and analyzed for ISGylation by western blot. For all groups, n=3.

12 weeks Post-TBI: ISGylation

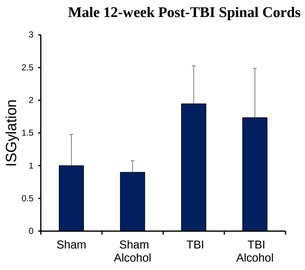


Figure 2. ISGylation of cellular proteins is increased in SCLs. Lumbar spinal cords from male rats that received a mild-moderate TBI and self-administered alcohol were collected 12- weeks post-TBI and lysed for WES analysis. For all groups, n=3.

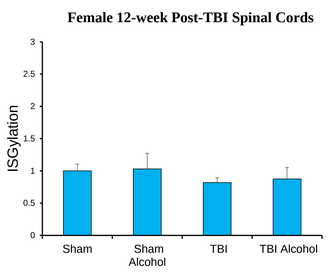


Figure 3. TBI has no effect on ISGylation induction in SCLs. Lumbar spinal cords from female rats that received a mild-moderate TBI and self-administered alcohol were collected 12- weeks post-TBI and lysed for WES analysis. For all groups, n=3 except female Sham and female TBI, both of which have n=2.

12 weeks Post-TBI: ISGylated TDP-43

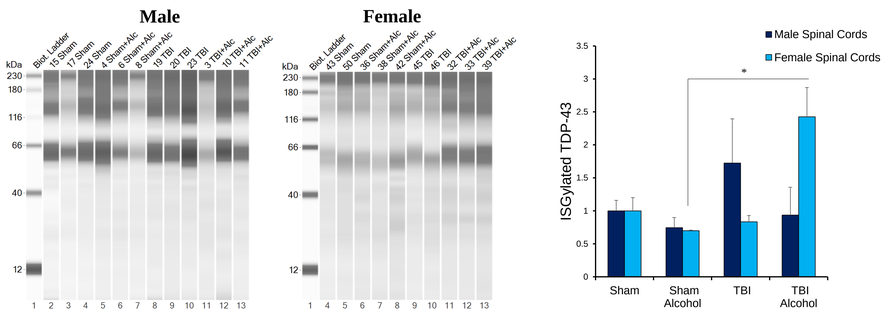
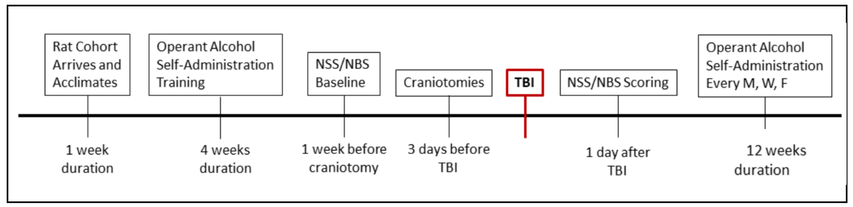
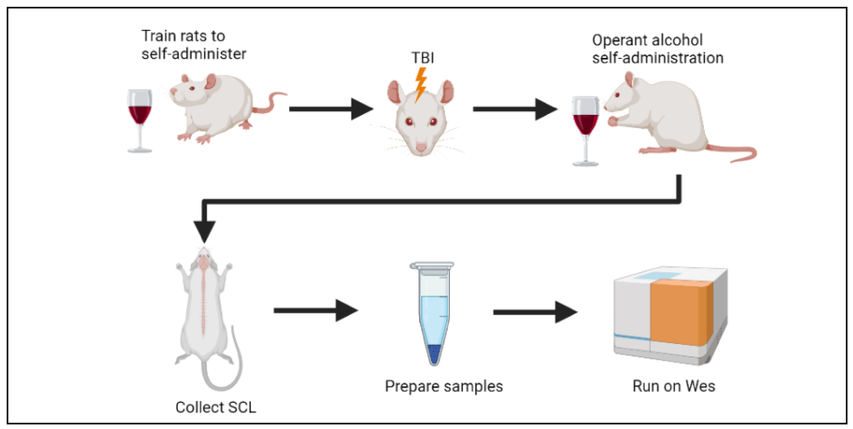


Figure 4. TBI induced TDP-43 ISGylation in 12-week male rats, yet alcohol reduced it. TBI did not induce TDP-43 ISGylation in 12-week female SCL; however, alcohol induced ISGylation of TDP-43 in TBI-exposed female rats. Lumbar spinal cords from male and female rats that received a mild-moderate TBI and self-administered alcohol were collected 12-weeks post TBI. These samples were immunoprecipitated (IP) using a TDP-43 antibody, analyzed by WES with an anti-ISG15-specific antibody. * p = 0.0146. For all groups, n = 3 except female Sham and female TBI, both of which have n = 2.

Timeline



Experimental Design



Conclusions and Future Studies

Conclusions

- ISGylation is elevated post-TBI.
- 12-weeks post-TBI females are at increased risk for pathological TDP-43 accumulation, particularly with alcohol use.

Future Studies

- For future studies, we would like to investigate what mechanisms responsible for making females more vulnerable to TBI.
- We would like to continue this study by investigating shorter or longer time points after TBI in the lumbar spinal cords of both male and female rats.
- Lastly, we are interested in exploring markers of neuroinflammation, such as GFAP.

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