Examining the Effects of Environmental Toxins on Amyotrophic Lateral Sclerosis

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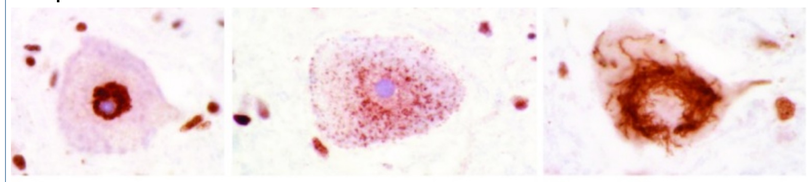
Introduction

- Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that affects neurons which control voluntary muscle movements.
- 97% of ALS cases involve the TAR DNA-binding protein (TDP-43).
- TDP-43 is a protein that is normally located in the nucleus of neurons.
- Under stress conditions such as oxidative stress, TDP-43 delocalizes to the cytoplasm and forms toxic protein aggregates which cause dysfunction of nuclear pore processes.



- Environmental toxins have been associated ALS pathogenesis through reactive oxygen species (ROS).
- ROS can be affected by intracellular levels of zinc, which was found to reduce TDP-43 expression.

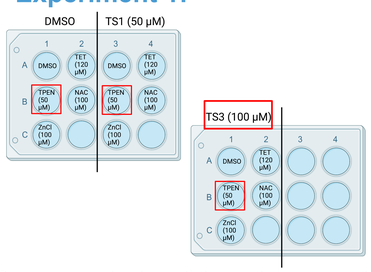
Research Questions:

1. Examine whether two pesticides: TS1 and TS3 induce toxic TDP-43 aggregation
2. Examine whether oxidative stress is involved in response to the pesticides

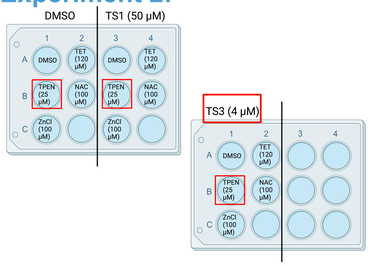
Hypothesis:

TS1 and TS3 induce toxic TDP-43 aggregation leading to increase in intracellular levels of zinc.

Experiment 1:



Experiment 2:



Samples were treated with the pesticides and cotreatments for 3 hours, collected, and split into soluble and insoluble protein fractions. To make sure that each sample had the same amount of protein when loading a western blot gel, we normalized all samples to the loading control by using a BCA Assay. Western blotting was conducted and were analyzed by transferring the bands on the gel onto a nitrocellulose membrane where the following antibodies were used.

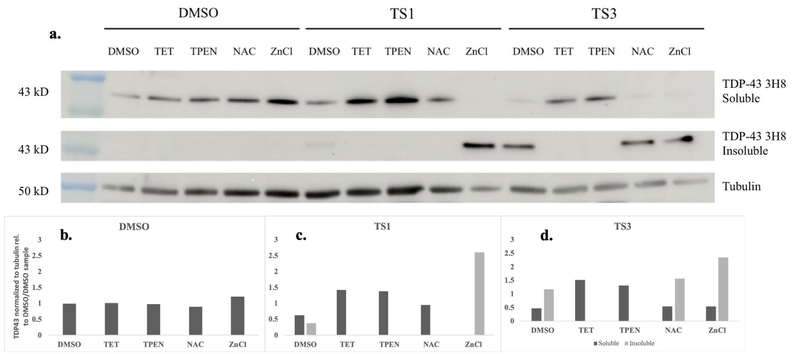
Primary Antibody	Manufacturer	Dilution
Anti-TDP43 clone 3H8	Millipore Corp	1:1000
α Tubulin	Santa Cruz	1:1000

Cotreatments

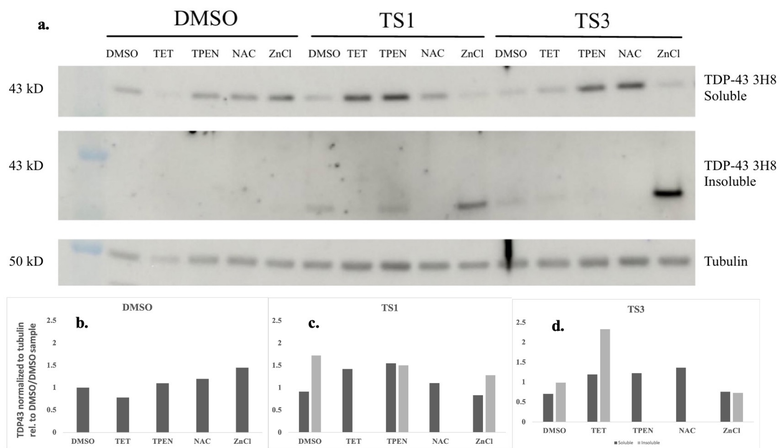
- TET – Copper chelator
- TPEN – Zinc chelator
- NAC – prevents oxidation of cysteines
- ZnCl – used to increase intracellular levels of zinc

WB Results for Experiments 1 and 2

Experiment 1:



Experiment 2:



Conclusions

1. TS1 and TS3 induce toxic TDP-43 aggregation.
2. TS1 and TS3 have an increased response to the ZnCl cotreatment indicating that drugs may increase intracellular levels of zinc. This may cause oxidative stress which leads to toxic aggregation.

Limitations and Future Directions

- Should have more consistent replications maintaining same dosages of treatments and cotreatments
- Potential immunofluorescent imaging studies should examine oxidative stress pathways that lead to toxic TDP-43 aggregation
- Heavy focus on examining the relationship between TS1, TS3, and zinc as a potential causal factor for ALS

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