Investigating Toxicant Induced Intracellular Zinc Modulation and Resultant TDP-43 Dysfunction Linked to Amyotrophic Lateral Sclerosis

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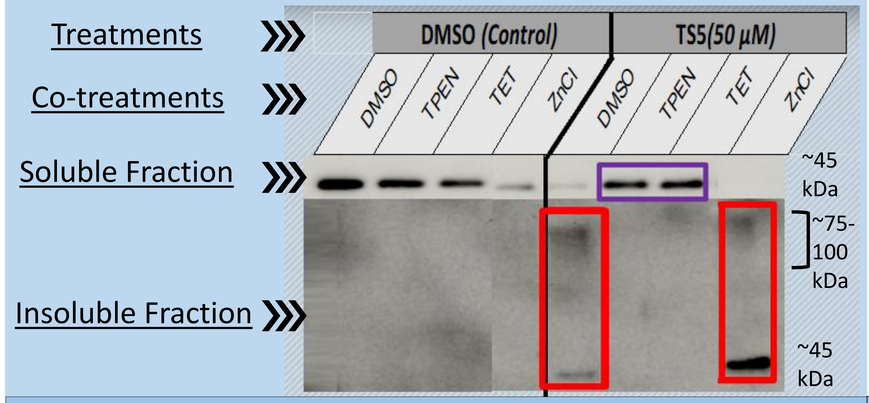
Abstract

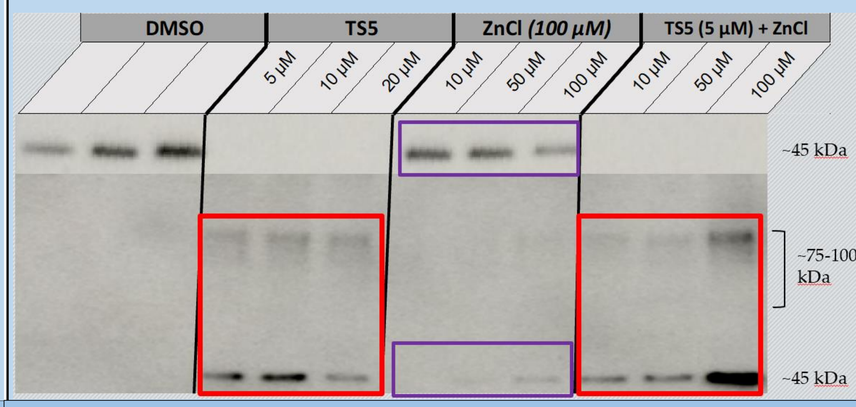
The majority of Amyotrophic Lateral Sclerosis (ALS) cases are sporadic, meaning they originate from unknown causes. This exploratory study proposes that mouse cortical neuron exposure to an environmental toxicant and ionophore, here labeled TS5, induces modulation of zinc concentrations and produces dysfunctional, insoluble aggregates of TDP-43, a protein implicated in ALS pathology. Imaging assays using the fluorescent dye NewPort Green, used to stain cortical neurons for cytoplasmic zinc ions, on control, TS5 alone, and TS5 and ZnCl exposure conditions were conducted. Images taken after exposure to TS5, TS5 and zinc, and zinc alone suggest that TS5 may modulate the intake of zinc into the cytoplasm by increasing its uptake. Subsequent western blot analysis of conditioned neuronal plates indicated that exposure of cortical neurons in-vitro to TS5 alone resulted in the depletion of TDP-43 from the soluble fraction and visible bands in the insoluble fraction. Additionally, exposure to both ZnCl and TS5 resulted in higher intensity bands in the insoluble fraction and total depletion of TDP-43 from the soluble fraction, suggesting that exposure to the ionophore TS5 under higher extra-cellular zinc ion concentrations has a more pronounced effect in soluble TDP-43 depletion and aggregation. The introduction of bivalent metal chelators to exposed cortical neurons resulted in the rescue of pathological TDP-43 into the soluble fraction, serving as potential therapeutic avenues.

Acute Exposure Analysis (3 hrs)



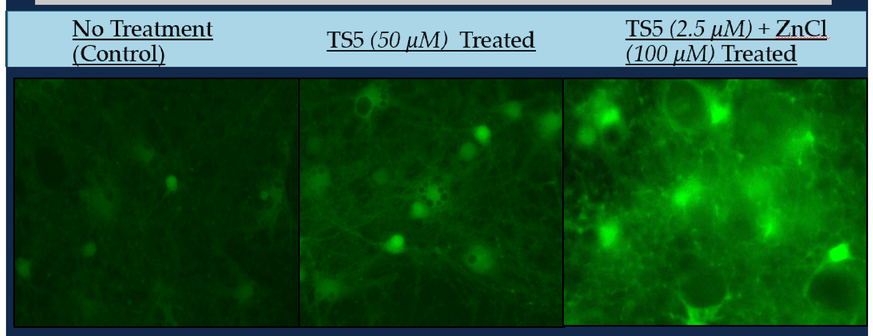
- Soluble presence of TDP-43 diminishes greatly with exposure to TS5. Insoluble aggregates form. Addition of ZnCl to TS5 treatment appear to exacerbate the progression into insoluble TDP-43 compared to the TS5/DMSO control.
- Addition of the co-treatments **TPEN and TET**, metal chelators with affinities for both Zn^{2+} and Cu^{2+} ions, **appear to rescue TDP-43** into the soluble fraction.

Overnight Exposure Analysis



- Soluble presence of TDP-43 diminishes greatly with exposure to TS5. Insoluble aggregates form. A synergistic relationship appears with TS5 and ZnCl.
- With **increased ZnCl concentrations**, soluble TDP-43 is reduced and **insoluble bands form**. This also suggests natural zinc ion permeability to cortical neurons or a cascading effect of insoluble TDP-43 production from increased extracellular zinc concentrations.

Fluorescent Imaging



Images taken after exposure to TS5, TS5 and zinc, and zinc alone suggest that TS5 may modulate the intracellular concentration of zinc ions. We speculate the ionophore TS5 increases zinc ion uptake or releases zinc ions from intracellular compartments. Images of ZnCl Treatment alone did not yield any noticeable change in image intensity.