Poster #4
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Title of Research: Cellular Consequence of Fungicide Exposure and Reactive Dopamine Metabolites on Dopaminergic Neurons
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Introduction/Purpose:
Parkinsons disease is a slow-progressive neurodegenerative disorder affecting 5-6 million people around the globe. The disease is manifested by the rapid deterioration of doparminergic cells in the substantia nigra portion of the brain; however, the pathological mechanism of selective dopaminergic neuronal death is unknown. Dopamine is oxidatively deaminated and catalyzed by monamine oxidase to form the endogenous neurotoxin 3,4-dihydroxyphenylacetaldehye (DOPAL). A reduction in levels of DOPAL is biologically critical as this aldehyde has been shown to be toxic to dopaminergic cells and is a highly reactive electrophile. Investigating neuronal protein targets is essential in determining the cause of toxicity. An essential protein-GAPDH (e.g., glyceraldehyde-3-phosphate dehydrogenase) is an abundantly expressed enzyme known for its glycolytic activity and recent research has implicated its role in oxidative stress-mediated neuronal death. GAPDH has been shown to be highly susceptible to covalent modification and inactivation by DOPAL. Exposure to environmental toxins such as pesticides and fungicides has been recently linked to DOPAL. The fungicide, benomyl is a potent ALDH inhibitor which is postulated to increase DOPAL levels and is positively associated with PD risk.

Experimental Design:
The enzymatic activity of purified GAPDH was spectrophotometrically measured by NAD+ oxidation to NADH and hence an increase in absorbance at 340 nm. All protein modification and analyses have undergone SDS-PAGE and Western blotting. Specific DOPAL-protein adducts are determined by ESI-MS (Electron spray ionization mass spectrometry) The toxicity and risk caused by benomyl was assessed by treating N27 and SH-SY5Y cell lines for 4 hours with benomyl and dopamine. Toxicity was measured by cell incubation with MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).

Results:
Upon treatment of DOPAL (5-25 μM), enzyme activity was significantly inhibited compared to control. Extensive protein cross linking is observed in the presence of DOPAL, but not DA or DOPAC. No cellular toxicity was observed when treatment of just benomyl, or dopamine. Toxicity was observed only with both benomyl and dopamine.

Conclusions:
Given GAPDH's intracellular abundance and its pivotal role in multiple metabolic/apoptotic pathways, compromise on enzymatic activity may have devastating effects on cellular homeostasis. Thus, GAPDH is a viable and possible target of modification by DOPAL. DOPAL can serve as a mechanistic link between benomyl/fungicide exposure and Parkinson's disease.