P-glycoprotein (P-gp), a member of the ATP-binding cassette superfamily of transporters, is found in many healthy tissues throughout the body, including the blood-brain barrier (BBB). The activity of this efflux transporter limits central nervous system (CNS) exposure to xenobiotics – exogenous compounds including drugs and environmental toxins. Data suggests P-gp functions as a neuroprotective mechanism in Parkinson’s disease by limiting accumulation of neurotoxins in the brain. However, direct evidence is lacking to confirm that P-gp is involved in the transport of these neurotoxins associated with this disease. The goal of our research is to determine the function of P-gp in the efflux of neurotoxins, initially focusing on paraquat. This research will provide evidence whether P-gp at the BBB functions to prevent accumulation of this neurotoxin. In vitro studies were conducted in two cell lines, MES-SA (with no expression of P-gp) and MES-SA-DXS (with over-expression of P-gp). Intracellular accumulation of paraquat was determined by HPLC. Paraquat-induced cytotoxicity was also measured over a range of concentrations. A significant increase in intracellular accumulation of paraquat was observed in MES-SA cells compared to MES-SA-DXS cells after a five-hour incubation at 500 µM, 161.6 and 33.45 ng/6.0 x 10⁵ cells/5 hours, respectively (p < .0001). Additionally, MES-SA-DXS cells were observed to be significantly more resistant to paraquat-induced toxicity than MES-SA cells. In conclusion, paraquat appears to be a substrate of P-gp, and P-gp may have an important function in preventing toxic exposure of this compound in the brain. Decreased activity or expression of P-gp due to genetic variation may increase susceptibility to Parkinson’s Disease, which will be the focus of future studies.

Our research focuses on the potential role of P-gp in limiting CNS accumulation of the neurotoxin paraquat by active transport at the BBB. This transport may be significantly altered by genetic variation of ABCB1. The research presented here demonstrates that paraquat is a likely substrate of P-gp, which gives significant indication to study the effects of ABCB1 genetic variants on the accumulation of paraquat in the CNS.

For in vitro studies, MES-SA (with no expression of P-gp) and MES-SA-DXS (with over-expression of P-gp) human ovarian cancer cells lines were used. Intracellular accumulation of paraquat was studied in a transport assay of MES-SA and MES-SA-DXS cells plated at a density of 6.0 x 10⁵ cells/well in a six-well format and treated with 500 µM paraquat (PQ) for 1-5 hours. Accumulation at each time point was quantified using high pressure liquid chromatography (HPLC).

Paraquat-induced cytotoxicity was studied using CellTiter-Fluo® Cell Viability Assay (Promega) with cells plated at a density of 2.0 x 10⁵ cells/well in a 96-well format and treated with [PQ] ranging from 0.2-5000 µM for 48 hours. Fluorescence was measured using Molecular Devices Gemini EM microplate fluorescence reader (excitation 380 nm, emission 505 nm).

Future Directions
Our work will continue to examine P-glycoprotein’s (P-gp) role in the development of Parkinson’s disease by investigating the ABCB1 genetic variants and their ability to alter the efflux of paraquat and other neurotoxins. In vitro studies conducted here show direct evidence that there is a role of P-gp in the transport of neurotoxins associated with Parkinson’s disease.

However, additional in vitro studies will be performed to determine the influence of ABCB1 genetic variants on disease susceptibility. Additionally, future work will focus on in vivo studies of ABCB1 knock-out mice and neurotoxin-induced neurodegeneration.

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