Role of the Drug Transporter, P-glycoprotein, in the Efflux of Paraquat

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Abstract

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-DX5 (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 of intracellular accumulation of paraquat was observed in MES-SA cells compared to MES-SA-DX5 cells after a five-hour incubation at 500 µM, 161.60 and 33.45 ng/6.0 x 10^6 cells/5 hours, respectively ($p < .0001$). Additionally, MES-SA-DX5 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.

Introduction

Parkinson's disease is a common neurodegenerative disease that affects motor control, speech, and other various functions. Though prevalent in the population, there are many aspects of the disease that are not well understood; however, one common association with the disease has been rural, agricultural living that primarily utilizes well water. Paraquat (N,N'-dimethyl-4,4'-bipyridiniun) is one of the most broadly used herbicides worldwide and has been associated with Parkinson's disease in epidemiological studies due to it utilization in agricultural practices[1]. Though these studies show that exposure to paraquat significantly increases the likelihood of developing Parkinson's disease, the mechanism for transport across the blood-brain barrier (BBB) has not been defined.

P-glycoprotein (P-gp) is a transmembrane efflux pump and a member of the ATP-binding cassette (ABC) superfamily of transporters. P-gp was originally discovered as a mechanism for the development of multidrug resistance to chemotherapeutic agents in cancer[2]. This efflux pump is also found throughout the body in healthy tissues important in xenobiotic disposition, particularly the intestine, liver, kidneys, and the BBB. Expression of P-gp at the BBB facilitates the efflux of both pharmaceutical drugs and environmental toxins out of the brain in order to prevent toxic levels of accumulation in the central nervous system (CNS). Though data exists that suggest P-gp functions as a neuroprotective mechanism in Parkinson's disease[3], perhaps by limiting accumulation of neurotoxins, there is a lack of direct evidence to confirm that P-gp transports the neurotoxins associated with the development of the disease (i.e. paraquat, MPTP, rotenone, and maneb).

P-gp is encoded by the *ABCB1* gene (also known as *MDR1*), which has a high degree of genetic variability. Single-nucleotide polymorphisms (SNPs) are present throughout the gene with more than 60 SNPs identified in the coding region alone[2]. Two synonymous SNPs are found at nucleotides 1236 C>T and 3435 C>T and two non-synonymous SNPs are found at nucleotides 1199 G>A and 2677 G>T/A, which code for a Ser400Asn and Ala893Ser/Ala893Thr amino acid transitions in the protein, respectively[2]. These SNPs have been associated with altered susceptibility to the development of Parkinson's disease[4].

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 likely a substrate of P-gp, which gives significant indication to study the effects of *ABCB1* genetic variants on the accumulation of paraquat in the CNS.

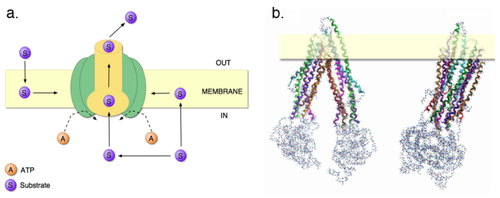

Figure 1. a.) P-gp functions as an ATP-binding efflux transporter for a variety of substrates[5]. b.) Human P-gp homology model based on proposed mouse crystal structure[6].

Methods

For *in vitro* studies, MES-SA (with no expression of P-gp) and MES-SA-DX5 (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-DX5 cells plated at a density of 6.0 x 10^6 cells/well in a six-well format and treated with 500 µM paraquat ([PQ]) for 1-5 hours. Accumulation at each hour was quantified using high pressure liquid chromatography (HPLC).

Paraquat-induced cytotoxicity was studied using CellTiter-Fluor™ Cell Viability Assay (Promega) with cells plated at a density of 2.0 x 10^5 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).

Data

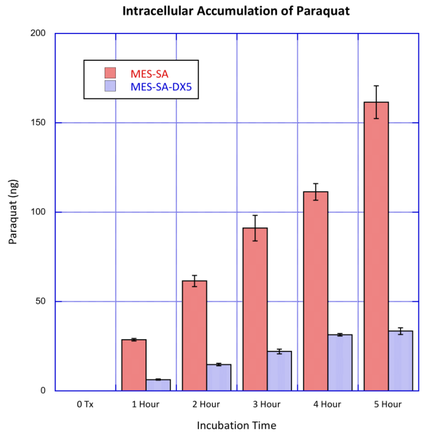

Figure 2. Intracellular accumulation of paraquat at a density of 6.0 x 10^6 cells/well collected hourly from 1 – 5 hours of incubation with 500 µM paraquat and quantified using HPLC.

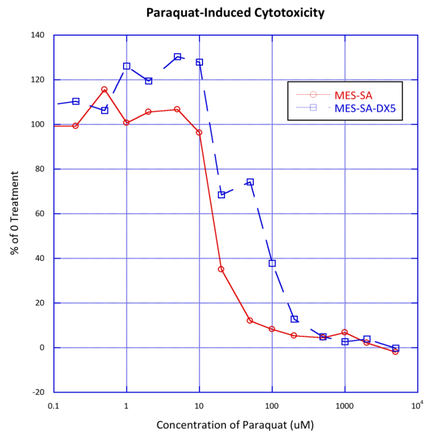

Figure 3. Cytotoxicity of paraquat at a density of 2.0 x 10^5 cells/well at various concentrations, ranging from 0.2 – 5000 µM, following a 48 hour incubation period.

Results

- MES-SA cells accumulated a significantly higher amount of paraquat than MES-SA-DX5 cells at all time points over the 5-hour incubation at 500 µM paraquat.
- After 5 hours of incubation at 500 µM paraquat, the values were 161.60 and 33.45 ng/6.0 x 10^6 cells/5 hours for MES-SA and MES-SA-DX5 cells, respectively ($p < .0001$).
- MES-SA-DX cells were significantly more resistant to paraquat-induced cytotoxicity after a 48 hour exposure, an approximate 4.5-fold increase in the EC_{50} value compared to MES-SA cells.

Conclusions

- Paraquat appears to be a substrate of the drug transporter, P-glycoprotein (P-gp), in the *in vitro* cell system we examined.
- P-gp plays a role in the intracellular accumulation of the neurotoxin, paraquat.
- P-gp has the potential to be a key factor in preventing accumulation of paraquat in the central nervous system due to its prominent expression at the blood-brain barrier.

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. The *in vitro* studies conducted here show direct evidence that there is a role of P-gp in the transport of neurotoxins associated with Parkinsons' 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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