Metabolic Investigations of Molecular Mechanisms Associated with Parkinson’s Disease.

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Graphical Abstract

Aging

Genetics

PD

Environment

Lact H+

Glucose

Glucose

Gluc 6-P

NADPH

O2−, ‘NO

ATP

ALDO

AMPK

PPP

PQ

Cell death

Energy depletion

Lact

Pyr

Pyr

PDH

ACO

Mn

PQ

TCA Cycle
Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder characterized by fibrillar cytoplasmic aggregates of α-synuclein (i.e., Lewy bodies [LB]) and the associated loss of dopaminergic cells in the substantia nigra. But, mutations in genes such as α-synuclein (SNCA) account for only 10% of PD occurrences. The exposure to environmental toxicants including pesticides (e.g. paraquat [PQ]) and manganese (Mn), are also recognized as important PD risk factors. Thus, aging, genetic alterations and environmental factors all contribute to the etiology of PD. In fact, both genetic and environmental factors are thought to interact in the promotion of idiopathic PD, but the mechanisms involved are still unclear. In this study, we report a toxic synergistic effect between α-synuclein and either paraquat or Mn treatment. We identified an essential role for central carbon (glucose) metabolism in dopaminergic cell death induced by paraquat or Mn treatment that is enhanced by the overexpression of α-synuclein. PQ “hijacks” the pentose phosphate pathway (PPP) to increase NADPH reducing equivalents and stimulate paraquat redox cycling, oxidative stress, and cell death. PQ also stimulated an increase in glucose uptake, the translocation of glucose transporters to the plasma membrane, and AMPK activation. The overexpression of α-synuclein further stimulated an increase in glucose uptake and AMPK activity, but impaired glucose metabolism. In effect, α-synuclein activity directs additional carbon to the PPP to supply paraquat redox cycling. Alternatively, Mn induces an upregulation in glycolysis and the malate-aspartate shuttle to compensate for energy depletion due to Mn toxicity. Mn treatment causes a decrease in carbon flow through the TCA cycle and a disruption in pyruvate metabolism, which are consistent with a dysfunctional mitochondria and inhibition of pyruvate dehydrogenase. The overexpression of α-synuclein was shown to potentiate Mn toxicity by glycolysis impairment by inhibiting aldolase activity. In effect, α-synuclein overexpression negates the metabolic response to alleviate Mn toxicity that results in an increase in cell death.

Keywords: Parkinson’s Disease; genetics-toxin synergy; molecular mechanisms; NMR & MS
Introduction – Seminar Outline

- Overview of Parkinson’s disease (PD).

- Combining NMR and MS in metabolomics

- Results of Paraquat and Manganese Treatment of Dopaminergic Neuronal Cells.

  - Synergistic Effect of \( \alpha \)-synuclein Overexpression and Paraquat/Manganese Treatment

- Conclusion

<table>
<thead>
<tr>
<th>Nebraska</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60-70</td>
</tr>
<tr>
<td>PD Prevalence (per 100,000)</td>
<td>350</td>
</tr>
</tbody>
</table>

Estimated US Prevalence: 916,348 (\( \geq 40 \) y.o.)
Introduction - Parkinson’s disease (PD)

- Parkinson’s disease (PD) is a chronic progressive neurodegenerative disorder that leads to shaking (tremors) and difficulty with walking, movement, and coordination.

- Loss of dopaminergic neurons from the substantia nigra pars compacta leads to deficiency of dopamine in the caudate and putamen (“striatum”).

- Currently, there is no cure for PD or a treatment to stop PD progression.

https://medlineplus.gov/ency/imagepages/19515.htm

Introduction – Causes of Parkinson’s disease

- The exact cause of PD is unknown.
- Only 10% of PD is Familial (Hereditary).
  - Genetic alterations in \(\alpha\)-synuclein, Parkin, DJ-1, PINK1 and LRRK2 have been associated with PD
- Sporadic (Idiopathic) PD are linked to genetic alterations, *environmental* or occupational factors
- Environmental agents linked to increased incidence/risk to develop Parkinson’s disease
  - *Pesticides (paraquat)*
  - *Heavy Metals (manganese)*
  - Infectious agents
  - Industrialization
  - Dietary factors
Introduction – Paraquat and Manganese are Environmental Risk Factors for PD

- Largest epidemiology study of Parkinson’s disease in the US:
  - More common in Midwest and Northeast
  - Areas associated with Agriculture and Metal processing
- Environmental factors are likely common contributors to PD
  - Prolong exposure to herbicides and insecticides used in farming
  - Prolong exposure to metals, such as manganese
- Correlation between Paraquat agricultural usage and PD rates
- Paraquat selectively induces dopaminergic degeneration, one of the pathological hallmarks of PD.
Introduction – Paraquat and Manganese are Environmental Risk Factors for PD

- Urban areas of East and Midwest contain the majority of metal-emitting facilities
  - PD more common in Midwest and Northeast
  - Mn 5th most abundant metal in the earth’s crust
  - Mn essential cofactor for several enzymes (e.g., superoxide dismutase, SOD)

- Mn is environmental factors for idiopathic PD
  - “manganese-induced parkinsonism” or “manganism” similar symptoms with idiopathic PD.
  - Mn reported to specifically target dopaminergic neurons in *C. elegans* to cause neurodegeneration

### Annual Incidence of Parkinson’s Disease in Urban Counties

<table>
<thead>
<tr>
<th>Exposure Category</th>
<th>Incidence</th>
<th>95% Confidence Interval</th>
<th>Relative Risk</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>No reported copper, manganese, or lead(^a)</td>
<td>274.0</td>
<td>226.8, 353.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High reported copper release</td>
<td>304.2</td>
<td>276.0, 336.8</td>
<td>1.11</td>
<td>0.94, 1.31</td>
</tr>
<tr>
<td>High reported manganese release</td>
<td>489.4</td>
<td>368.3, 689.5</td>
<td>1.78</td>
<td>1.54, 2.07</td>
</tr>
<tr>
<td>High reported lead release</td>
<td>283.7</td>
<td>249.3, 337.5</td>
<td>1.04</td>
<td>0.88, 1.23</td>
</tr>
</tbody>
</table>

\(^a\) Less than 100 pounds (45.36 kg) of reported copper, manganese, or lead release.
Introduction – Environmental Toxins & Mitochondrial Dysfunction

- Neurons have very high energy demands and high glucose usage
- Energy metabolism alterations have been reported in early PD
  - Mitochondrial dysfunction in PD
  - Toxins alters redox homeostasis, energy metabolism and central carbon metabolism
- A clear role for metabolomics in investigating PD
Introduction – \( \alpha \)-Synuclein is a Genetic Risk Factor for PD

- Formation of intracellular aggregates (Lewy bodies) is a pathological hallmark of PD
- \( \alpha \)-synuclein is a major component of Lewy Bodies
  - 140 aa soluble protein of unknown function
- Oligomerization of \( \alpha \)-synuclein fibril formation is central to pathogenesis of PD

Mechanisms of \( \alpha \)-synuclein aggregation and propagation

Substantia nigra from patients with PD
Lewy Bodies stained for \( \alpha \)-synuclein


Introduction – Gene-Environment Interactions in PD

- Mitochondrial dysfunction and energy failure induced by environmental toxicants can lead to \( \alpha \)-synuclein misfolding and aggregation by an impairment in protein quality control mechanisms.
Results and discussion – Metabolomes Extracted from Dopaminergic Cells and Brain Tissues

Tissues/Cells

C57BL/6 mice (8–10 weeks old)

Brain dissection

Weighing & snap freeze by liquid N2

Lyse and extract metabolites with Methanol/water 1:1

Normalized by tissue weight

2 ml

MS analysis

NMR analysis

0.2 ml

1.8 ml

Dopaminergic neuronal cells (N27, SK-N-SH)

Lyse and quench with -80°C methanol

Collect cells & lysates

Extract metabolites with 80%/20% Methanol/water and 100% water

Normalized by the total protein

2 ml
Results and discussion – A Combined NMR and MS Metabolomics Protocol was applied to Investigate PD

Grow treated and untreated cells (10x)
• Extract metabolome
• Split for MS and NMR

Collect NMR and MS spectra
• NMR and MS spectra for each cell culture

Process NMR and MS spectra
• using our MVAPACK software

Generate Combined and Individual Model
• MB-PCA and MB-PLS
• using our MVAPACK software

Lei et al. (2014) ACS Chem Biol. 9(9):2032-2048
Results and discussion – NMR and MS Spectral Data Processed with Multiblock-PCA and our MVAPACK Software

Integrate Data From Multiple Analytical Methods


MVAPACK Metabolomics Toolkit
http://bionmr.unl.edu/mvapack.php

Multiblock-PCA

Results and discussion – Parkinson’s Disease and Mitochondrial/Environmental Toxins

- Mitochondrial dysfunction and energy failure: Herbicides, pesticides, and designer drugs induce Parkinson’s-like symptoms
  - Used as Equivalent molecular models for Parkinson’s Disease
  - All result in dopaminergic neuronal cell death

- Our Metabolomics data indicate different molecular mechanisms of action
  - Focused on Paraquat (PQ)

Results and discussion – Paraquat (PQ) Treatment of Dopaminergic Neuronal Cells Leads to Irreversible Cell Death

PQ Induces Irreversible Cell Death after 24 hrs.

Cells Recover when Treated with Other Toxins

Results and discussion – Paraquat (PQ) Induces Dramatic Changes in metabolome of Dopaminergic Neuronal Cells

Integrate Data From Multiple Analytical Methods

NMR (A) and MS (B) paraquat-induced spectral changes

Backscaled multiblock-PLS-DA loadings from NMR (A) and MS (B) data

Results and discussion – Paraquat (PQ) Induces Dramatic Changes in Metabolome of Dopaminergic Neuronal Cells

Pentose phosphate pathway

Glycolysis

Nucleotide biosynthesis

TCA cycle

Glucose metabolism/extracellular media

Results and discussion – Paraquat (PQ) Induces Alterations in Glucose Metabolism and Pentose Phosphate Pathway (PPP)

Results and discussion – Paraquat (PQ) Induces Dramatic Changes in Proteome of Dopaminergic Neuronal Cells

Results and discussion – Integration of Metabolomics and Proteomics Data

- Increase in pentose phosphate pathway (PPP) enzymes
  - G6PD, glucose-6-phosphate dehydrogenase
- Increase in PPP metabolites
  - glucose 6-phosphate, fructose 6-phosphate, glucono-1,5-lactone and erythrose 4-phosphate
- Decrease in glycolysis and TCA cycle

Results and discussion – **G6PD Regulates Paraquat Toxicity**

- Over-expression leads to increase in cell death with paraquat treatment G6PD
  - No change for other Mitochondrial/Environmental Toxins

- Cell death and oxidative stress induced by PQ is alleviated by G6PD inhibitor
  - **6-AN, 6-aminonicotinamide**

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Results and discussion – Paraquat “Hijacks” the Pentose Phosphate Pathway

- Paraquat-induced oxidative stress requires NADPH as an electron donor for its redox recycling
  - increases NADPH reducing equivalents
  - Stimulates paraquat redox cycling, oxidative stress and cell death

Results and discussion – Glucose Metabolism Regulates PQ Toxicity

PQ Induces Glucose Uptake

PQ Toxicity is Diminished with Inhibition of Glucose Transporter

PQ Toxicity is Diminished with Glucose Deprivation

PQ Toxicity is Diminished with Inhibition of production of glucos-6-phosphate

Results and discussion – Glucose Metabolism Regulates PQ Toxicity

Results and discussion – AMPK Protects Against PQ Toxicity

PQ Induces AMPK phosphorylation and activation

<table>
<thead>
<tr>
<th>PQ [μM]</th>
<th>1400W [100 μM]</th>
<th>-</th>
<th>+</th>
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<td>50</td>
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<td>100</td>
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</tbody>
</table>

pAMPK T172

AMPKα1

vs Cont 1 0.5 0.7 12 30 10 102 27

PQ Toxicity Increase due to AMPK inhibition is reversed with glucose deprivation

PQ Toxicity is Increased with Inhibition of AMPK

PQ & AMPK inhibition impairs glycolysis and mitochondrial respiration

Cell survival [%]

Paraquat [μM]

OCR / ECAR

- PQ  + PQ
Results and discussion – *In vivo* Metabolic Dysfunction Induced by PQ

**Midbrain**

- Paraquat-induced toxicity is brain region specific (no noticeable animal response)
- Largest impact on Midbrain – location of *substantia nigra*, where dopaminergic neurons are concentrated
  - Male C57/BL/6 mice (8-10 weeks old)
  - One intraperitoneal injection of 10 mg/kg paraquat or saline control twice a week for 9-weeks

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Results and discussion – Cell Death and Metabolic Dysfunction Induced by Manganese Toxicity

Mn Induced Cell Death Correlated with in vivo [Mn]

Mn Treatment Perturbs the Metabolome of the Midbrain

S. Lei et al. (2016) J. Neurochem, in preparation
Results and discussion – Mn Increases Glucose Metabolism and Malate-Aspartate Shuttle, and Decreases TCA Cycle

Mn Toxicity Increases Glycolysis

Mn Toxicity Increased Due to Glucose Deprivation

Mn Toxicity Increases MAS and Decreases TCA Cycle

Mn Toxicity Leads to Impairment in Pyruvate Metabolism

S. Lei et al. (2016) J. Neurochem, in preparation
Results and discussion – Mn Increases Glucose Metabolism and Malate-Aspartate Shuttle, and Decreases TCA Cycle

- Mn toxicity produces an energy depletion
  - Inhibition of glycolysis enhances loss of ATP

- Mn appears to inhibit pyruvate dehydrogenase (PDH) activity

S. Lei et al. (2016) J. Neurochem, in preparation
Results and discussion – Impaired Glycolysis Enhances Mn Toxicity

Mn Toxicity Increased Due to Replacing Glucose as Carbon Source

Mn Toxicity Increased Due to Glucose Deprivation

Mn Toxicity Increased Due to Decrease in Glucose Uptake

S. Lei et al. (2016) J. Neurochem, in preparation
Results and discussion – **Upregulated Glycolysis is the Metabolic Response to Energy Depletion Induced by Mn**

![Diagram showing the metabolic pathway from glucose to ATP with upregulated glycolysis and inhibition by Mn at the PDH step.](image-url)
Results and discussion – AMPK Protects Against Mn Toxicity

Mn Induces AMPK phosphorylation and activation

Energy stress

AMPK

GLT4

PKF2

Increased glycolysis flux

Mn Toxicity Increased Due AMPK Inactivation

S. Lei et al. (2016) J. Neurochem, in preparation
Results and discussion – \( \alpha \)-synuclein Potentiates PQ or Mn Toxicity and Metabolic Dysfunction

\( \alpha \)-synuclein over-expression leads to high-MW aggregates

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\( \alpha \)-synuclein increase cell death

\( \alpha \)-synuclein increase cell death

\( \alpha \)-synuclein increases metabolome changes

\( \alpha \)-synuclein increases metabolome changes

S. Lei et al. (2016) J. Neurochem, in preparation
Results and discussion – Glucose Metabolism Contributes to Synergistic Toxicity Between Paraquat and α-Synuclein

- Overexpression in α-synuclein and exposure to paraquat:
  - increase glucose uptake, glycolysis
  - translocation of glucose transporters to plasma membrane
  - upregulation of the pentose phosphate pathway
  - stimulated the activation of adenosine monophosphate-activated protein kinase (AMPK)
    - master regulator of metabolism in response to energy deficiency

Results and discussion – Glucose Metabolism Contributes to Synergistic Toxicity Between Mn and α-Synuclein

α-synuclein Impairs Glycolysis and Negates Cell Response to Mn Toxicity

S. Lei et al. (2016) J. Neurochem, in preparation
Results and discussion – Glucose Metabolism Contributes to Synergistic Toxicity Between PQ and α-Synuclein

Glucose deprivation prevents paraquat induced cell death

AMPK activation prevents paraquat induced cell death
(significant metabolic changes only observed with a dominant-negative form of AMPK)

Ascorbic acid (AA) enhances AMPK activation prevents paraquat induced cell death

Results and discussion – α-synuclein Impairs Aldolase Activity Through a Protein-Protein Interaction

S. Lei et al. (2016) J. Neurochem, in preparation
Results and discussion – α-synuclein Potentiates PQ or Mn Toxicity and Metabolic Dysfunction

S. Lei et al. (2016) J. Neurochem, in preparation
Conclusions

- **Paraquat**
  - Hijacks the NADPH from the PPP to redox cycle, induce oxidative damage and impair antioxidant defenses
  - Increases glucose transport and carbon flux to the PPP.
  - Impairs TCA cycle leading to increased citrate accumulation, which leads to an impairment in glycolysis

- **Manganese**
  - Toxicity results in energy depletion
  - Inhibits pyruvate dehydrogenase
  - Induces an increase in glycolysis

- **α-synuclein**
  - Inhibits Aldolase activity
  - Impairs glycolysis and upregulates glucose transport
    - Channels carbon flux to the PPP to increase PQ’s redox cycling and ROS formation
  - Potentiates environmental toxicity (Manganese and Paraquat)
    - Facilitates ATP depletion induced by Mn exposure

- **Glucose metabolism regulates α-synuclein + PQ toxicity**
  - Paraquat increases glucose transport and translocation of glucose transporters.
  - Inhibition of GLUT-like transporters prevents α-synuclein + PQ toxicity.
  - Inhibition of PPP protects against α-synuclein + PQ

- **AMPK signaling exerts a protective effect**
  - Activation of AMPK can be mediated by both ROS and ATP depletion.
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