Activity of Pentose Phosphate Pathway and Pyruvate Dehydrogenase is decreased in MPTP Model of Parkinson's Disease: A ^{13}C NMR Study

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Target Audience: Clinicians and researchers interested in brain energy metabolism in Parkinson's disease

Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, affecting >3% of population above 65 years of age[1]. Administration of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) leads to specific degeneration of dopaminergic neurons in the substantia nigra (SN)[2]. Although, glycolysis followed by pyruvate dehydrogenase pathway (PDH) is the major pathway of glucose metabolism, the pentose phosphate pathway (PPP) accounts for a significant fraction of glucose metabolism in the matured central nervous system[3]. Reduced glucose metabolism in the basal ganglia and cerebral cortex in PD patients and animal models has been reported by many studies[4,5] . However, the impact of PD on PPP flux is not understood very well. The current study is intended to assess the PPP and PDH flux in cortical and midbrain regions in the MPTP model of PD.

Materials and Methods: Male C57BL6 mice (3-month old) were divided into 2 groups: Control (n=6) and MPTP (n=6). Mice were treated with MPTP (25 mg/kg, i.p.) or normal saline for 7 days. Motor function was assessed by Four limb grip endurance and Forepaw grip strength meter on 9th day. Overnight fasted mice were anesthetized with urethane (1.5 g/kg, i.p.) and infused with [1,2-$^{13}C_2$]glucose for 90 min[6]. Metabolism of [1,2-$^{13}C_2$]glucose via PDH pathway incorporates ^{13}C label into Glu_{45}, Gln_{45} and $GABA_{12}$, while, the PPP labels Glu_4, Gln_4 and $GABA_2$. At the end of the infusion, brain metabolism was arrested in 1s using a Microwave Fixation System (MMW-05 Muromachi, Japan) and the metabolites were extracted from cerebral cortex and midbrain tissues[7]. The percent enrichment of amino acids was measured in ^{13}C-[^{1}H]-NMR and ^{1}H-[^{13}C]-NMR[8] spectra of tissue extracts. The fractional contribution of PPP to glucose metabolism was calculated as $(S_4+D_{34})/(S_4+D_{34}+D_{45}+DD_{345})$ while that of PDH as $(D_{45}+DD_{345})/(S_4+D_{34}+D_{45}+DD_{345})$, where S_i and D_i represent the ^{13}C isotopomers of Glu_4. This information was used to deconvolve the ^{13}C-[^{1}H]-NMR measured Glu_{C4} labeling via PPP and PDH pathway. Student's t-test was used to determine the significance of difference between MPTP and control group.

Results: MPTP treatment led to a significant loss in the motor function measured by the four limb hanging test (Fig. 1A) and the forepaw grip strength test (Fig. 1B) suggesting that MPTP exposure in mice mimics the PD symptoms of humans. The ^{13}C labeling of brain metabolites from [1,2-$^{13}C_2$]glucose is depicted in Figure 2. Well resolved isotopomers of Glu_4 are seen in the inset. The contribution of PPP (Glu_{S4}) to total glucose metabolism was found to be very small (Cortex 5.4±0.6%; Midbrain 5.3±0.6%) as compared to the PDH pathway. The contribution of PPP pathway to total energy metabolism (PPP/PDH) was found to be increased significantly (p=0.041) in midbrain (Control 0.053±0.007, MPTP 0.065±0.010, p=0.041). The total flux through PPP and PDH pathways was estimated using the values of $CMR_{Glc(ox)}$ reported previously[5]. MPTP treated mice showed reduction in PDH flux by 37 and 33% in the cerebral cortex and midbrain region, respectively (Fig. 3A). PPP flux was also found to be decreased in the cerebral cortex (Control: 0.038±0.002; MPTP 0.027±0.001 μmol/g/min, p=0.0015) and midbrain (Control: 0.035±0.002; MPTP 0.029±0.002 μmol/g/min, p=0.0015) in MPTP mice as compared to control but to a lesser extent (17-29% of the control, Fig. 3B).

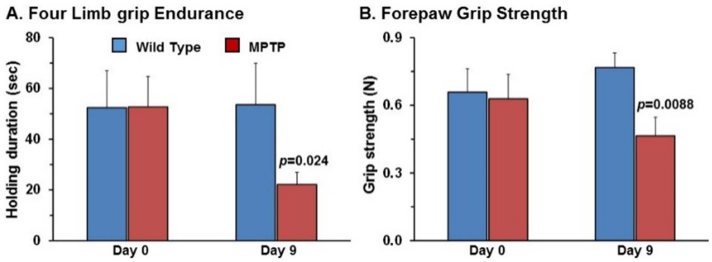

Fig. 1 A. Four limb grip endurance and **B.** Fore paw grip strength in MPTP treated

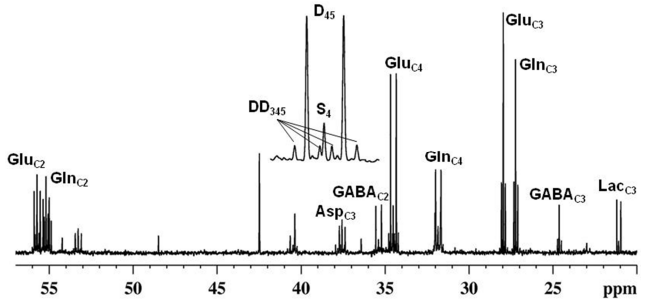

Fig. 2. ^{13}C-[^{1}H]-NMR spectrum of midbrain extract of control mouse infused with [1,2-$^{13}C_2$]glucose for 90 min

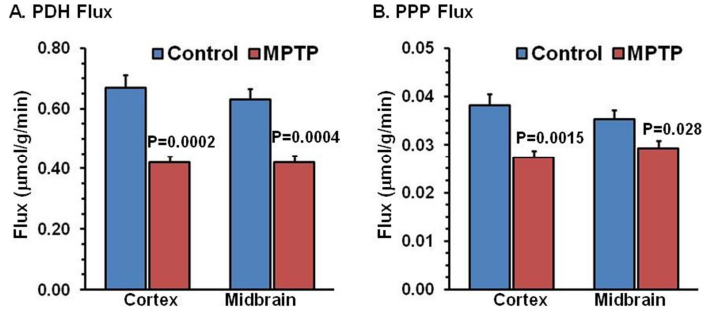

Fig. 3 A. PDH and **B.** PPP fluxes in MPTP treated mice

Discussion: PPP generates NADPH, which acts as a cofactor for glutathione reductase, the enzyme involved in maintaining glutathione in the reduced form. Hence, PPP flux indicates the antioxidant reserve of cells. The finding of reduced PPP flux in cerebral cortex and midbrain regions suggests a compromised antioxidant defense capacity of neural cells in MPTP treated mice that might be responsible for impaired neuronal activity in the PD condition.

References: 1. Orth *et al* (2003) *Movt Disord* **18**:729; 2. Araki *et al* (2001) *Eur J Pharm Sci* **12**:231; 3. Brekke *et al* (2012) *J Cereb Blood Flow* Metab **32**:1788; 4. Borghammer *et al* (2012) *Acta Neurol Scand* **125**:303; 5. Bagga *et al* (2013) *J Neurochem* **127**:365; 6. Fitzpatrick *et al* (1990) *J Cereb Blood Flow* Metab **10**:170; 7. Patel *et al* (2001) *Brain Res* **919**:207; 8. de Graaf *et al* (2003) *Magn Reson Med* **49**:37

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