^{1}H-[^{13}C]-NMR Study to Evaluate the Efficacy of Levodopa Treatment in MPTP Mouse Model of Parkinson's Disease

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Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide affecting 1-2% of population above 65 years of age[1]. MPTP mouse model is a well established neurotoxic model for studying the pathophysiology of PD as it mimics the specific degeneration of dopaminergic (DA) neurons in the substantia nigra[2]. The supplementation of DA by Levodopa (L-dopa) is the most widely used treatment for symptomatic relief in PD. The current study is intended to assess the effects of acute L-dopa treatment on glutamatergic and GABAergic functions in the cortical and striatal regions of brain in MPTP mouse model of PD.

Materials and Methods: Male C57BL6 mice (3-month old) were divided into 4 groups: Control (n=6), Control + L-dopa (n=6), MPTP (n=6), and MPTP + L-dopa (n=6). Mice were treated with MPTP (25 mg/kg, i.p.) or normal saline for 7 days, and L-dopa (140 mg/kg, i.p.) or normal saline on day 21. Paw grip strength was measured using grip strength meter on day 0, 8 and 21. For metabolic measurements, overnight fasted mice were anesthetized with urethane and administered [1,6-$^{13}C_2$]glucose (0.225 M, i.v.) for 10 min[3]. At the end of the infusion, head was frozen *in situ* using liq. N_2 and metabolites were extracted from cortical and striatal regions of brain[4]. ^{1}H-[^{13}C]-NMR spectroscopy was carried out on tissue extracts for the measurement of concentration and ^{13}C enrichment of neurometabolites[5].

Results and Discussion: Paw grip strength in MPTP group mice was found to be significantly reduced (MPTP Control 670±140; 590±120 mN; p=0.00001) which recovered after acute L-dopa treatment (MPTP+L-dopa 640±81mN, Control+L-dopa 600±50, p=0.25). Level of glutamate (Control 12±0.6; MPTP 12.6±0.4 μmol/g, p=0.003), GABA (Control 4.7±0.3; MPTP 5.8±0.7 μmol/g, p=0.00002) and inositol (Control 7.0±0.4; MPTP 7.9±0.7 μmol/g, p=0.0003) was found be significantly increased in striatum of MPTP treated mice. The ^{13}C labeling of Glu_{C4} (Control 2.29±0.3; MPTP 1.53±0.17 μmol/g, p=0.002), $GABA_{C2}$ (Control 0.17±0.03; MPTP 0.12±0.004 μmol/g, p=0.03) and Gln_{C4} (Control 0.29±0.04; MPTP 0.15±0.03 μmol/g, p=0.0004) was lower in MPTP treated mice indicating a reduction in glutamatergic and GABAergic activity in cortical region (Fig. 1A). Similar results were found in the striatal region (Fig. 1B). Though acute L-dopa did not normalize the level of neurometabolites, it improved the cerebral activity as measured by ^{13}C labeling of amino acids from glucose in cortex (Fig. 1A) and striatum (Fig. 1B). Since L-dopa is not able to restore the homeostasis of neurometabolites, it may not be a good strategy for the cure of PD. However, L-dopa offers an option for acute recovery of cerebral function.

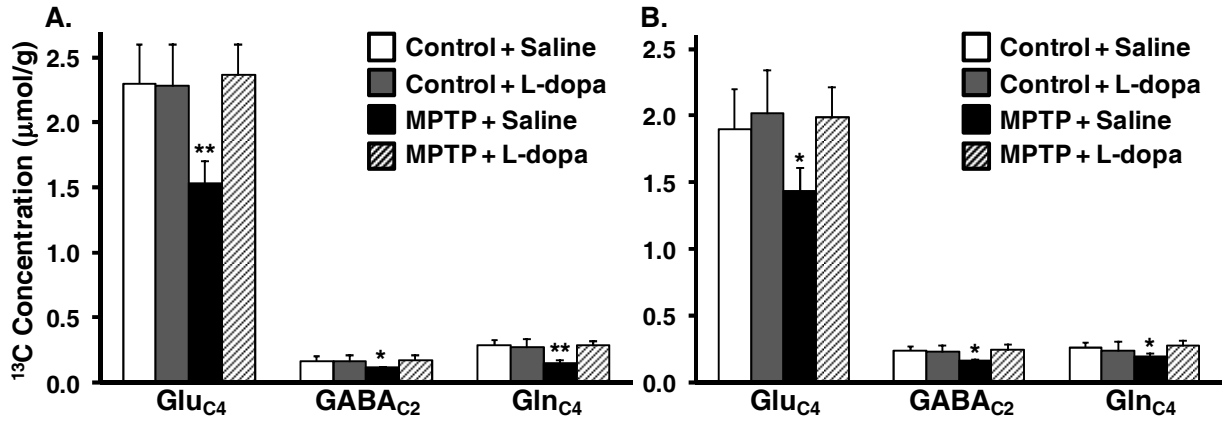

Fig 1. Concentration of ^{13}C labeled amino acids from [1,6-$^{13}C_2$]glucose at 10 min. **A.** Cortex and **B.** Striatum. *p< 0.01, **p<0.001 when compared with control (saline or L-dopa) or MPTP+L-dopa

References: 1. Orth *et al* (2003) *Movt Disord* **18**:729; 2. Bloem *et al* (1990) *J Neurol Sci* **97**:273; 3. Fitzpatrick *et al* (1990) *J Cereb Blood Flow Metab* **10**:170; 4. Patel *et al* (2001) *Brain Res* **919**:207; 5. de Graaf *et al* (2003) *Magn Reson Med* **49**:37.

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