^{1}H Spectrscopic Imaging Determines Immunotherapeutic Efficacy in a Murine Model of Parkinson's Disease

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Introduction: Neurodegeneration of dopamineric neurons in the substantia nigra pars compacta (SNpc) and nerve termini in the striatum are pathological hallmarks of human Parkinson's disease (PD). Human disease can be mirrored following injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Indeed, MPTP elicits dopaminergic neurodegeneration within SNpc and recapitulates motor, neuroimmunological and pathological features of human disease. Increased interest in Copaxone (Cop-1) an approved treatment for rapidly remitting multiple sclerosis has recently emerged after its success in providing neuroprotection for a number of animal models of degenerative diseases of the brain and spinal cord. Cop-1 induces T- cell immunity against myelin basic protein leading to neuroprotective activities for neural trauma, amyotrophic lateral sclerosis and in Parkinsons' disease (PD). Importantly, we have recently demonstrated that the adoptive transfer of Cop-1 immune cells, splenocytes, into MPTP recipient mice significantly protected dopaminergic neurons. To evaluate whether neuronal injury and protection could be monitored non-invasively in this model, quantitative proton spectroscopic imaging (^{1}H MRSI) was used to assess brain metabolite concentrations. Affected brain regions were detected through immunohistochemical co-registration methods. ^{1}H MRSI revealed that NAA concentrations in the SNpc of MPTP treated mice were significantly diminished after MPTP intoxication compared to control (saline treated) animals. In contrast those animals that received Cop-1 immune cells maintained NAA levels comparable to controls. These results indicate that ^{1}H MRSI may be used to evaluate dopaminergic degeneration within the SNpc following MPTP intoxication and its subsequent protection by Cop-1 immune cells.

Materials and Methods : Spectra were obtained on a Bruker Biospec 7T/21 system operating at 300.41 MHz, using an actively decoupled volume coil transmitter and a 1.25x1.5 cm actively decoupled surface coil receiver. Spectra were obtained using a numerically optimized binomial excitation (1) designed with a water null and a flat frequency response from 300 to 1400 Hz, refocused using three orthogonal slice selective refocusing pulses (BEVR) (2). Spectroscopic images through the substantia nigra were obtained by preselecting an 8 mmx4.2 mmx1.5 mm voxel, 24x24 encoding, 20 mm FOV, TE=33 ms, TR=4s, 2 averages, total acquisition time=80 min. Spectroscopic images through the striatum were acquired with the same parameters except for phase encoding resolution to 16x16. Unsuppressed water spectroscopic images were acquired from each slice with the same resolution as the metabolite SI, but with TR=1s, as internal signal amplitude standards for quantitation. Spectra were fit using AMARES (3) in the jMRUI 1.2 package (4). Male 8-11 week old C57BL/6NCrlBR mice from Charles Rivers Laboratories were administered four ip injections (one every two hours) of 21.8 mg MPTP.HCl (18 mg free base)/10 ml phosphate buffered saline(PBS)/kg body weight. Half of the MPTP treated group was injected with splenocytes harvested from Cop-1 treated mice 12 hrs after the last MPTP injection. Control mice received 10 ml PBS/kg body weight over the same time period. MRI and spectroscopic imaging were performed two and six days after PBS or MPTP administration. Voxels for analysis were selected by 3D coregistration of anti-tyrosine hydroxylase stained (TH) histological sections (to identify dopaminergic neurons) with multislice MRI to select voxels containing SNpc (Fig 1). Voxel shifting of the data in k-space was performed to center the SNpc within the voxel.

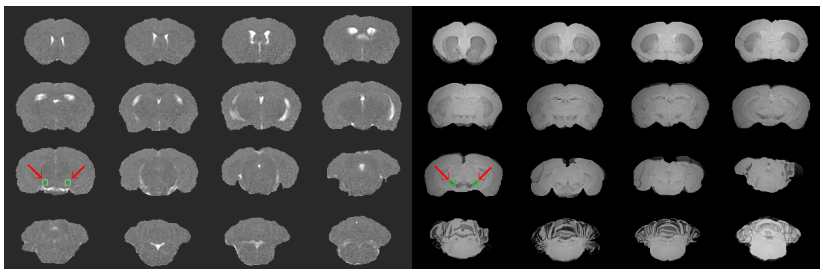

Figure 1. *Coregistration of digitized TH stained histology (right) and subimaged T_2 maps (left) from the same mouse brain. These data were used to determine the ^{1}H MRSI voxel selected for analysis of the SNpc (arrows).*

Results: Results from SNpc are displayed in Table 1. There is siginificant loss of NAA due to MPTP treatment. This loss is prevented by injection of the Cop-1 immunized splenocytes. Similar, but less significant trends were observed in the striatum, likely due to partial volume effects of non-dopaminergic termini in the striatum.

Table 1: *Comparison of quantitative spectroscopic imaging results (mean ± SEM) days two and six after MPTP or PBS treatment. Statistical comparisons were done using Bonferroni multiple comparison test. *p<0.05 compared to PBS treated mice.*

Sustantia Nigra Pars Compata	NAA (mM)		Cre (mM)		Cho (mM)	
	2 day	6 day	2 day	6 day	2 day	6 day
PBS (N=9)	8.5±0.4	10.7±1.1	9.7±0.6	9.9±1.4	2.2±0.2	2.2±0.2
MPTP (N=2)	5.4±0.2*	5.8±0.7*	5.2±1.1*	8.5±0.8	1.2±0.4	1.6±0.2
Cop-1 (N=4)	9.1±0.6	10.0±0.6	12.1±1.7	9.7±0.8	2.2±0.4	2.4±0.4

Conclusions: ^{1}H MRSI can assess dopaminergic neurodegeneration in an MPTP model of human PD. Immune mediated dopaminergic neuroprotection is afforded by adoptive transfer of Cop-1 immune cells as evidenced by retention SNpc NAA levels and numbers of dopaminergic neurons. We propose that Cop-1 vaccination strategy may be useful to slow or halt progression of Parkinson's disease and that this treatment strategy can be adequately assessed through ^{1}H MRSI.

References:

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