Morphological and Metabolic Changes in the Brain of 6-Hydroxydopamine Treated Rats Observed by MRI and In Vivo ^{1}H MRS

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Introduction 6-Hydroxydopamine (6-OHDA) is a catecholaminergic neurotoxin that is capable of inducing progressive degeneration of dopaminergic and noradrenergic neurons [1]. Intracerebral injection of 6-OHDA into substantia nigra (SN) is a commonly used animal model for Parkinson's disease (PD). The animals with intracerebral 6-OHDA injection are often characterized by significant loss of dopaminergic neurons in the SN and striatum [2]. Morphological and metabolic changes in the brain of clinical PD patients, such as decreased volumes of putamen, globus pallidus and prefrontal cortex and decreased NAA/Cr ratio in motor cortex and striatum, can be monitored by anatomical MRI and in vivo ^{1}H MRS [3]. In this study, T_2- and T_2^*-weighted MRI and in vivo ^{1}H MRS were used to monitor cerebral changes induced by unilateral injection of 6-OHDA into the SN of rat, and to see whether the changes, if there are any, resemble those found in clinical PD.

Materials and Methods Fifteen Sprague-Dawley rats were divided into two groups: the control group (Group C, n = 5) and the 6-OHDA treated group (Group M, n= 10). The rats in Group M received stereotaxic injection of 6-OHDA into the left SN. Between two and three months after 6-OHDA injection, motor disturbances in the treated rats were evaluated by an apomorphine-induced (s.c. injection, 1 mg/kg) rotation test [2]. The number of unilateral full-body turns per min was counted. Within 2 days after the test, MRI/MRS experiments were performed under chloral hydrate anesthesia (i.p., 10% solution, 5 ml/kg) on a Bruker Biospec 4.7 T/30 cm spectrometer equipped with actively-shielded gradients. Multi-slice T_2-weighted images were acquired with field of view 3.5 cm×3.5 cm, matrix size 128×128, slice thickness 0.8 mm, TR 3 s and 6 echoes with TE ranging from 20 to 120 ms in order to calculate quantitative T_2 maps. T_2*-weighted imaging on the same slices was performed with TR 500 ms and TE 30 ms. In vivo ^{1}H MRS was performed on bilateral striatum (2.5×2.5×2.5 mm^3 cube, Fig. 1) using PRESS localization, TR 1000 ms and TE 136 ms. Statistical analysis was carried out using two-tailed student's *t*-test.

Results Apomorphine injection induced unilateral full-body rotation in the 6-OHDA treated rats (2-7 turns/min, average 4±2 turns/min). Compared to its contralateral counterpart, the ipsilateral SN to 6-OHDA injection exhibited decreased signal intensity on the T_2*-weighted images and significantly lower absolute T_2 value (Fig. 1A and Table 1). No significant T_2 changes were found in the ipsilateral hippocampus (HC), striatum (Str) and motor cortex (MC) (Fig. 1 and Table 1). The ipsilateral striatum, however, showed significantly decreased NAA/Cr (7.3±0.1%) and NAA/Cho (10.2±0.3%) signal intensity ratios and unchanged Cho/Cr (Table 1 and Fig. 2), compared to the contralateral striatum.

Discussion Decreases of T_2^* and T_2 observed for the ipsilateral SN of the 6-OHDA treated rats are likely due to iron accumulation in this region [4]. NAA is a putative marker of viable neurons, and Cr is a possible indicator of energy metabolism and gliosis. Reduced NAA/Cr and NAA/Cho ratios in the ipsilateral striatum is probably a manifestation of neuronal degeneration in this region resulted from 6-OHDA induced dopaminergic neuron loss in the SN and its effects on striatal dopaminergic neurons via nigrostriatal pathway [5]. The anatomical and metabolic changes in the brain of 6-OHDA treated rats, as observed by MRI and in vivo ^{1}H MRS, resemble those found for clinical PD patients [3].

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Table 1. The results of quantitative T_2 measurements and MRS measurements.

		6-OHDA treated rats		Control rats	
		ipsilateral (L)	contralateral (R)	left (L)	right (R)
T_2 (ms)	SN	58.9±2.4	60.9±3.3[a]	62.6±1.4[a]	62.0±0.9[a]
	HC	62.8±2.1	63.3±2.0	63.4±1.3	63.8±1.3
	Str	60.0±2.2	59.8±2.0	60.1±1.1	60.4±1.2
	MC	60.4±2.1	60.2±1.6	60.6±1.3	60.2±0.9
Signal intensity ratio	NAA/Cr	0.92±0.08	1.05±0.11[a]	1.05±0.12[a]	1.06±0.06[a]
	Cho/Cr	0.64±0.05	0.69±0.11	0.63±0.07	0.68±0.04
	NAA/Cho	1.46±0.15	1.52±0.12[a]	1.65±0.07[a]	1.60±0.15[a]

[a]p<0.05, compared to ipsilateral values in the 6-OHDA treated rats. SN: substantia nigra, HC: hippocampus, Str: striatum, MC: motor cortex.

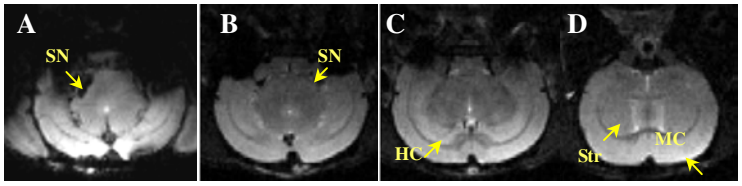

Figure 1. Coronal T_2*- (A) and T_2-weighted (B-D) images of the brain of a 6-OHDA treated rat. SN: substantia nigra, HC: hippocampus, Str: striatum, MC: motor cortex.

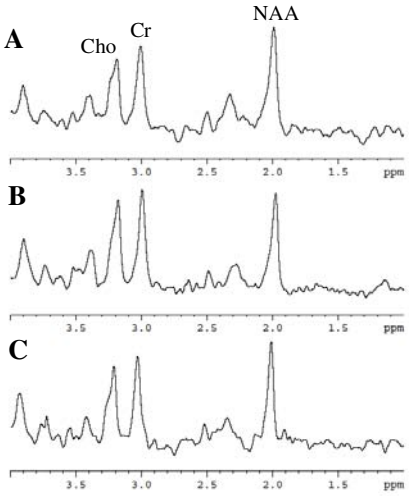

Figure 2. PRESS-localized in vivo ^{1}H MR spectra acquired from left striatum of a control rat (A), and ipsilateral (B) and contralateral (C) striatum of a 6-OHDA treated rat. TR/TE=1000/136 ms.

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