A magnetization transfer study of mild and advanced Parkinson's disease

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Background and purpose: Magnetization transfer ratio (MTR) technique has identified brain changes in grey and white matter in Parkinson's disease (PD), even in the early phase. However, how these tissue changes differ along the course of the illness is still unclear. This study was aimed at investigating how MTR values change from mild PD (PD1) to patients with advanced PD (PD2).

Methods: We measured MTR values by region of interest, in 11 PD1, 11 PD2 and 10 healthy age-matched subjects.

Results: Compared with controls, patients with PD1 exhibited a significant MTR reduction in substantia nigra pars compacta, substantia nigra pars reticulata, putamen, periventricular white matter and parietal white matter. In addition to the changes observed in PD1, the PD2 group exhibited a significant MTR reduction in caudate, pons, frontal white matter and lateral thalamus.

Conclusion: These results suggest that MTR might reflect morphological changes induced by the disease in distinct brain areas at different stages.

Introduction

Conventional neuroimaging (cMRI) is not able to reveal characteristic aspects of the ongoing degeneration in Parkinson's disease (PD), as the most profound changes are neurochemical and hence better revealed by functional studies such as PET and SPECT [1,2]. An objective evaluation of progressive degeneration in PD has important implications with respect to pathophysiology, prognosis and long-term management. Although cMRI is simple, relatively inexpensive and widely available, its overall ability to differentiate PD from healthy controls is poor [3]. In fact, magnetic resonance imaging (MRI) is commonly normal in patients with PD and only few slight changes can be observed [4]. However, cMRI can be better utilized to differentiate PD from other Parkinsonian conditions [3]. MRI techniques as spectroscopic, volumetric MRI and diffusion-weighted imaging (DWI) have been evaluated in PD [5–7]. Magnetization transfer (MT) imaging presents some advantages: (i) it is a rapid and

low cost technique, (ii) only few minutes are needed in addition to routine MRI sequences, and (iii) overall brain regions can be easily and widely investigated. MT is based on the transfer of energy between highly bound protons within structures and the very mobile protons of free water and has been proven to detect subtle brain tissue abnormalities undetectable with cMRI [8]. A decrease in MT ratio (MTR) values can result from an increase in the mobile proton pool, occurring as a result of inflammation and oedema, or a decrease in the semi-solid proton pool, associated with damage to myelin and other cellular components [9]. In fact, abnormal MTR have been measured in a variety of white matter diseases with no visible abnormality on T2-weighted images [10].

In patients with PD, data deriving from MTR studies showed that in specific brain areas, namely substantia nigra (SN), red nucleus (RN), pons, globus pallidus and periventricular white matter (PWM), MTR values were reduced, even in early phase [11–13]. However, how these tissue changes differ along the course of the illness is still unclear. To test the hypothesis that MTR alterations could involve different brain regions, depending on clinical stage, we carried out a cross-sectional MTR study in mild (PD1) and patients with advanced PD (PD2).

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Methods

Subject population

Twenty-two patients fulfilling the UKPDS Brain Bank criteria [14] for the diagnosis of PD were enrolled and tested using Hoehn and Yahr (H&Y) scale and Unified Parkinson's Disease Rating Scale (UPDRS). Pharmacologic tests (apomorphine, L-Dopa) for dopaminergic responsiveness in both off- and on-conditions were also performed. There were no familial PD cases. Ten age- and sex-matched controls (mean age: 57 ± 7 years) were also recruited. Both patients and controls were examined by the same physician (L.P.). An inclusion criteria for all participants was the Mini Mental State Examination (MMSE) > 24 . Patients were subdivided into mild PD (PD1) ($n = 11$, mean age: 60 ± 5.3 years) and advanced PD (PD2) ($n = 11$, mean age: 63 ± 6.1 years) (Table 1). Patients were on therapy with L-dopa plus benserazide or carbidopa, dopaminoagonists or neuroprotectors. Therapy was not modified before submitting patients to MRI. Both patients and controls gave their written informed consent to the study. The study was approved by 'Progetto Stategico', The Italian Health Ministry, in 2007.

Image acquisition

Magnetic resonance imaging protocol was carried out on a 1.5 T system (Signa EchoSpeed; GEMS, Milwaukee, WI, USA) using a standard head coil. To exclude other CNS diseases, sagittal T1-weighted fast spin echo (TR/TE/excitations 540/18 ms/2) images, echo train length 2, acquisition matrix 256×256 , field of view (FOV) 24×24 cm and axial-oblique, parallel to the intercommissural plane, proton-density and

T2-weighted spin echo (SE) (TR/TE/excitations 2500/30/100 ms/1) images were acquired with 5-mm slice thickness, no gap, acquisition matrix 512×256 , FOV 24×18 cm. Two additional unenhanced axial-oblique T1-weighted SE (TR/TE/excitations 800/20/1) sequences were obtained, with and without a saturation pulse (flip angle 950° , 1.2 kHz below water frequency), acquisition matrix 512×256 , FOV 24×18 cm, using a graphic prescription identical to the long TR images. The percentage of MT was quantified by calculating the MTR on the basis of the following formula: $MTR = [M_o - M_s]/M_o \times 100$, where M_s and M_o indicate the mean signal intensity of a given area with and without a saturation pulse, respectively. A higher value is related to a higher MT. The MRI protocol was carried out by one operator (P.F.); the regions of interest (ROI) were independently localized by two operators (N.T., Pi.C.), both blinded to the clinical presentation. Our investigation was aimed to explore the widest brain areas in both white and grey matter with the smallest number of ROIs. ROIs were also chosen on the basis of our and others previous work [11–13,15]. Thus, we selected a total of 38 ROIs, 19 ROIs in each hemisphere, that were measured on an independent console. The sampled regions are listed in Table 2.

To avoid the partial volume effect, ROI boundaries were traced, as far as possible, from the grey-white matter junction and not very close to the edge of the selected targets. Particular attention was paid to avoid lesions in both white and grey matter. To correctly locate samples in brainstem and basal ganglia, we utilized the coordinates of the ROIs placed on the T2-weighted sequence, which were set in the same position and with identical graphic prescription of the T1-weighted images.

Statistical analysis

Data were analysed to test for significant differences in MTR values amongst patients with PD1, PD2 and controls (Software R version 2.1, R Development Core Team, Vienna, Austria). We performed intraclass correlation coefficient (ICC) as a measure of inter- and intra-observer reliability. For each ROI, left- and right-hand MTR values were collected. ANCOVA test was performed to evaluate MTR values adjusted for age differences. The controls are matched for age to PD1 and PD2. We separately tested differences in age between controls and PD1 ($P = 0.411$), controls and PD2 ($P = 0.070$) and PD1 and PD2 ($P = 0.342$). Subsequently, once we found no significant differences with age and MTR values, we applied two-way ANOVA for testing differences in MTR amongst patients with PD; one factor was given by the left- and right-hand side of

Table 1 Demographic and clinical details of patients with Parkinson's disease (PD) and controls

	Controls	PD1	PD2
Number	10	11	11
Mean age ($\pm$ SD) (years)	57 ± 7	60 ± 5.3	63 ± 6.1
Sex (m)	4	5	4
Mean duration of disease (months)		14 ± 2.5	81 ± 16.3
MMSE	28.5	27.9	26.6
Mean H&Y		1.25	3.125
Stage 1		11	
Stage 2			5
Stage 3			5
Stage 4			1
Mean UPDRS		19.125	63.142

MMSE, Mini Mental State Examination; H&Y, Hoehn and Yahr; UPDRS, Unified Parkinson's Disease Rating Scale.

Table 2 Magnetization transfer ratio (MTR) values in different brain areas of patients with mild (PD1), advanced Parkinson's disease (PD2) and controls

	PD1	PD2	Controls		
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	<i>F</i> statistic	<i>P</i> -value
Pons	38.26 $\pm$ 0.96	37.71 $\pm$ 1.75	39.18 $\pm$ 1.01	5.17	0.0096
Midbrain					
Pars compacta	33.29 $\pm$ 1.62	32.01 $\pm$ 1.51	35.42 $\pm$ 2.04	12.75	<0.0001
Pars reticulata	34.19 $\pm$ 2.26	34.23 $\pm$ 1.46	35.92 $\pm$ 1.52	4.85	0.0125
Red nucleus	35.78 $\pm$ 0.90	35.49 $\pm$ 1.48	36.64 $\pm$ 1.69	2.88	0.0668
Basal ganglia					
Putamen	27.75 $\pm$ 1.50	27.99 $\pm$ 0.90	29.18 $\pm$ 1.28	5.87	0.0055
Caudate	27.60 $\pm$ 1.13	27.34 $\pm$ 1.30	28.54 $\pm$ 1.43	3.81	0.0299
Pallidum	32.47 $\pm$ 1.97	32.81 $\pm$ 1.58	33.83 $\pm$ 1.38	2.91	0.0649
Thalamus					
Total	33.14 $\pm$ 1.39	32.24 $\pm$ 1.38	33.14 $\pm$ 1.39	2.24	0.1203
Medial	29.86 $\pm$ 1.47	31.40 $\pm$ 1.56	31.54 $\pm$ 2.20	0.03	0.9736
Lateral	35.25 $\pm$ 1.34	33.93 $\pm$ 1.57	35.27 $\pm$ 1.36	4.55	0.0160
Anterior	34.54 $\pm$ 1.54	33.41 $\pm$ 2.66	34.54 $\pm$ 1.54	1.74	0.1874
Posterior	31.89 $\pm$ 1.71	31.69 $\pm$ 1.04	31.89 $\pm$ 1.70	0.09	0.9175
Supratentorial white matter					
Paraventricular	37.64 $\pm$ 1.00	36.98 $\pm$ 1.66	39.05 $\pm$ 1.44	9.06	0.0005
Centrum semiovalis	37.71 $\pm$ 1.03	37.92 $\pm$ 1.55	39.85 $\pm$ 1.27	4.59	0.0154
Frontal	38.07 $\pm$ 1.23	37.31 $\pm$ 3.12	39.21 $\pm$ 1.48	3.26	0.0477
Occipital	36.38 $\pm$ 1.66	36.14 $\pm$ 2.53	36.61 $\pm$ 1.67	0.21	0.8076
Parietal	37.77 $\pm$ 1.03	37.91 $\pm$ 1.55	39.05 $\pm$ 1.27	7.51	0.0016
Corpus callosum					
Genu	40.01 $\pm$ 0.84	39.09 $\pm$ 1.63	40.33 $\pm$ 1.73	2.00	0.1481
Splenium	39.90 $\pm$ 1.01	39.55 $\pm$ 2.54	40.26 $\pm$ 1.09	0.68	0.5135

P-values are referred to statistical significance of *F* in the ANOVA.

the ROI, the other factor was the group of patients with PD. Three tests were corrected for each ANOVA (controls versus PD1, controls versus PD2, PD1 versus PD2).

The mean and the standard deviation (SD) of the observed MTR values for each ROI, the *F* statistic and the corresponding *P*-value for PD1 and PD2 groups were estimated (Table 2). Bonferroni correction was used for all *post hoc* tests in each ANOVA and its value of $P < 0.05$ was considered significant (Fig. 1). A Pearson linear correlation coefficient and its statistical significance were computed between MTR measurements and UPDRS scale for all patients with PD.

Results

The mean disease duration was 14 ± 2.5 months for the PD1 group and 81 ± 16.3 months for the PD2 group. No statistical difference was found with regard to gender (ANCOVA $F = 1.6140$, $P = 0.2149$) and age ($\chi^2 = 0.0776$, $P = 0.7806$) amongst groups. ICC ranged from 0.68 to 0.86 for inter- and intra-observer reliability ($P < 0.05$). MTR values in normal subjects were lowest in the striatum (caudate: mean = 28.54; putamen: mean = 29.18) and highest in the corpus callosum (genu: mean = 40.33; splenium: mean =

40.26) (Table 2). As determined by the Bonferroni test, in the PD1 group significant reductions in MTR values in SNc ($P < 0.01$), SNr ($P < 0.05$), putamen ($P < 0.01$), PWM ($P < 0.05$) and parietal WM ($P < 0.01$) were found (Fig. 1). Patients with PD2 showed significant reductions in MTR values in the same areas already seen in PD1: SNc ($P < 0.01$), SNr ($P < 0.05$), putamen ($P < 0.05$), PWM ($P < 0.01$) and PaWM ($P < 0.01$) (Fig. 1). Moreover, in the PD2 group also other areas showed significant differences in MTR compared to controls regarding caudate ($P < 0.05$), frontal white matter (FWM) ($P < 0.05$) and pons ($P < 0.01$). Whilst MTR differences were revealed when comparing PD2 group with both the controls and the PD1 group for LTh ($P < 0.05$) (Fig. 1). The Pearson linear correlation coefficient did not show any correlation between MTR values for each brain region and age or UPDRS scores in both PD1 and PD2 groups.

Discussion

The present study, which is the first-ever to examine multiple areas from mild to advanced PD, showed that (i) in patients with PD, MTR values were significantly reduced compared to controls both in grey and white

Figure 1 Box plots of the magnetization transfer ratio (MTR) values in brain areas involved in both mild Parkinson's disease (PD1) and patients with advanced Parkinson's disease (PD2) compared to controls (CTR). Statistical significances of the differences in the means amongst groups for each brain location are reported in the plots (*post hoc* analysis: Bonferroni test). The first line shows all areas in which both PD groups have a statistical difference from controls. The second line shows all areas in which only PD2 has a statistical difference from controls. Note that in LTh PD2 has different value from both PD1 and controls. SNr, pars reticulata; SNc, pars compacta; PWM, periventricular white matter; PaWM, parietal white matter; FWM, frontal white matter; LTh, lateral thalamus).

matter and (ii) in advanced PD, MTR value reductions showed a wider distribution than in mild PD. MTR analysis has been proved to reflect histological differences, allowing to detect subtle brain tissue abnormalities undetectable with conventional MR imaging techniques [8]. Low MTR, in particular, indicates a reduced capacity of the macromolecules in brain tissue to exchange magnetization with the surrounding water molecules, which seems to be strongly associated with

the degree of tissue damage in several disorders of the CNS, including PD [16]. In patients with advanced PD, a more diffused occurrence of MTR abnormalities was observed compared to early PD. Abnormalities in advanced PD involved brainstem (SNc, SNr and pons), basal ganglia (putamen and caudate), diencephalon (lateral thalamus) and supratentorial WM (frontal, parietal and periventricular WM). These findings support the hypothesis that a progressive spread of neu-

rodegeneration from the brainstem to supratentorial structures in PD advance in a predictable manner [17]. Our finding of a widespread diffusion of MTR abnormalities in advanced PD is also in line with the observation reported in literature of a significant increase in the *in vivo* levels of ^{11}C (R)-PK11195 in the brain of patients with PD. This ligand is a PET marker of peripheral benzodiazepine sites, which are selectively expressed by activated microglia. This binding has been found to be altered both in subcortical and cortical areas, suggesting the widespread microglial activation associated with the pathological process in PD [18].

In 1980s, SNc changes were initially reported as the sole lesion in studies utilizing cMRI. In the present study, we found alterations in same region [19]. The evidence of the involvement of SNc has been subsequently confirmed by MTR studies [13]. Our finding confirms that SNc is involved in the early phase of disease. Moreover, we found that in PD1 and PD2, SNr also presents MTR abnormalities compared to control subjects, whilst in PD2 alone pons revealed MTR alterations. Degeneration of pigmented nuclei and brainstem structures observed by pathological studies would likely corroborate our data.

A growing attention has been devoted over the last years to the *in vivo* study of white matter in PD. Periventricular hyperintensities have been reported by cMRI in patients with PD. These lesions have been indicated as a putative marker for a particular clinical subtype of PD characterized by a worse outcome [20] and reduced responsiveness to levodopa [21]. Several factors have been suggested to contribute to these WM hyperintensities in PD, including structural changes affecting the small intraparenchymal cerebral arteries and arterioles, altered cerebral blood flow autoregulation, amyloid angiopathy and changes in haemodynamic status, which are not directly related with cerebrovascular risks [22]. In line with these findings, a previous study of our group on patients with PD without dementia, found periventricular WM alterations by using MT analysis [11]. Conversely, lower MTR values in the WM have been found only in PD with dementia using a single ROI to investigate WM [15]. In our present study, a more extensive analysis of WM was carried out.

Our findings suggest that MTR, which is more sensible to the WM damage compared to cMRI [23], can disclose brain damage unveiling WM changes in PD. On this basis, we hypothesize that these WM changes, detected by MTR, may be correlated with cognitive impairment which in turn becomes clinically relevant later, at least in a subgroup of patients. In fact, WMH in the deep white matter may contribute to dementia in PD [15] and initial cognitive impairment, as observed

in Mild Cognitive Impairment [24]. These MTR determined alterations of WM, which cannot be evaluated in a cross-sectional study, need to be verified by a longitudinal assessment of the same PD cohort.

We found a differential involvement of basal ganglia nuclei in mild versus advanced PD. It has been recently reported that an asymmetrical lateral ventricular enlargement in PD, compared to controls, is associated with disease progression and could depend on changes in the surrounding structures such as the hippocampus, caudate, putamen, thalamus and cortex [25]. In fact, in our study, we observed a MTR reduction of putamen which had already been evident in mild PD, whilst abnormalities of caudate and lateral thalamus were only observed in advanced PD. These results are in agreement with previous studies reporting volume reduction of putamen in both advanced PD and early PD [26] and of caudate [27] utilizing MRI-volumetric analysis. It is stimulating that, differently from other regions, the thalamus exhibits a significant difference between the PD1 and the PD2 group. According to Braak *et al.*, [28] thalamus is a target of the extra-nigral PD-related inclusion Lewy body pathology. The fact that Lewy bodies are lacking in the thalamus of healthy controls as well as in elderly indicates that Lewy bodies and Lewy neurites are not associated with normal ageing. Interestingly, Lewy bodies are present only in specific thalamic nuclei [29]. In the Braak's classification, the thalamic involvement has been found only in the late stages. Thus, in our study, it is conceivable that PD1 (mild) does not reach a level of thalamus involvement, differently from PD2 group (advanced).

Moreover, the presence of a widely distributed structural damage detected by MTR has been already demonstrated in the basal ganglia in both PD and Parkinsonisms [12]. Thus, it can be hypothesized that MT changes in the striatum, lateral thalamus and frontal WM involve the classical neuronal circuit (basal ganglia/thalamus/cortex) controlling motor activity in both physiological and pathological conditions [30,31].

We observed that regional MTR values did not correlate with UPDRS scores in both PD groups. We think that different explanations concur to determine this topic. First, the UPDRS was developed as a core assessment tool for impairment and disability in PD [32], but insufficient information is available on the ability of the UPDRS to discriminate between disease categories of clinical pertinence. To date, operative definitions of 'minimal', 'mild', 'moderate' and 'severe' stages of PD have not been explicitly defined [33]. Secondly, because of 'floor effect', the UPDRS's utility is potentially limited mainly in the early phase, and

therefore this scale did not reveal correlations in mild PD. Thirdly, the low number of patients included in this study could not highlight correlations between regional MTR values and the wide ranges of UPDRS score of both PD groups.

We are aware of the potential weaknesses of this study. First, given that manual transfer was used for ROIs, it is possible that the accuracy is not the absolute best. Secondly, the effort to explore WM in PD through ROI placement might not have been enough, and a whole brain MTR evaluation should be included in further studies. Finally, in patients with PD performing MRI exam is usually difficult. For that reason, the series, with and without MT saturation, have been made consecutively, without an interval period, to minimize head motions. Moreover, to perform the basic artefacts that could impair the evaluation of ROI's ratios, subtraction sets between slices with and without MT saturation have been executed to look for rim artefacts.

Furthermore, our study has shown significant differences amongst controls and group of patients. However, these results should be cautiously taken, because our analysis does not provide a tool to predict in a single patient the morphological correlate of the pathology. Further study, using a larger population, will be probably helpful to address this ambitious goal.

Regardless of these limitations, this study, which is the first-ever to examine multiple areas in the mild to advanced stages, has shown widespread MTR value changes over the mild to advanced stages. Therefore, the present findings should be further investigated.

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