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Deep brain stimulation does not enhance neuroinflammation in multiple system atrophy

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ABSTRACT

Slowly progressive, levodopa-responsive multiple system atrophy (MSA) may be misdiagnosed as Parkinson's disease (PD). Deep brain stimulation (DBS) is mostly ineffective in these patients and may even worsen the clinical course. Here we assessed whether neuropathological differences between patients with MSA who were treated with DBS of the subthalamic nucleus because of a misleading clinical presentation and typical disease cases may explain the more benign disease course of the former, and also the rapid clinical decline after surgery. The post-mortem assessment included the subthalamic nucleus, the globus pallidus, the thalamus and the putamen in five patients with MSA who received DBS and nine typical disease cases. There was no evidence for distinct neuroinflammatory profiles between both groups that could be related to the surgical procedure or that could explain the rapid clinical progression during DBS. Patients who received deep brain stimulation displayed a higher proportion of α-synuclein bearing neuronal cytoplasmic inclusions in the putamen compared with typical cases, while the number of surviving neurons was not different between groups. Our findings suggest that DBS does not induce neuroinflammatory changes in patients with MSA, at least several years after the surgery. We further hypothesize that the peculiar pattern of α-synuclein pathology may contribute to differences in the clinical phenotype, with a greater proportion of neuronal inclusions in the putamen being associated to a milder, "PD-like" phenotype with sustained levodopa response and slower disease progression.

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1. Introduction

Multiple system atrophy (MSA) is a fatal orphan neurodegenerative disorder that manifests in a variable combination with autonomic, parkinsonian, cerebellar, and pyramidal features (Fanciulli and Wenning, 2015; Gilman et al., 2008). The pathological hallmark is the accumulation of misfolded alpha-synuclein (α-syn) in oligodendrocytes, forming glial cytoplasmic inclusions (GCI), and to a lesser extent in neurons, typically as neuronal cytoplasmic inclusions (NCI) (Inoue et al., 1997; Kato and Nakamura, 1990; Lin et al., 2004; Nishie et al., 2004; Papp et al., 1989; Papp and Lantos, 1992). The median disease duration is 6–10 years (Gilman et al., 2008).

Current consensus diagnostic criteria include two classical phenotypes, MSA with predominant parkinsonism (MSA-P) and MSA with predominant cerebellar ataxia (MSA-C). Clinical heterogeneity has been repeatedly described in the literature, ranging from an asymmetrical, slowly progressive levodopa-responsive phenotype that may last up to two decades (Jellinger, 2012; Masui et al., 2012; Petrovic et al., 2012), to the very aggressive so-called 'minimal change' variant, which may lead to death within 5 years after having reached most of the clinical milestones by year 3 (Ling et al., 2015). Factors that have been associated with poor survival in MSA are older age at onset, female gender, MSA-P subtype, shorter period from onset to first clinical milestone, stridor within the first three years after symptom onset and early autonomic failure (Giannini et al., 2016; Low et al., 2015; O'Sullivan et al., 2008; Wenning et al., 2013).

In MSA, the neurodegenerative process is most pronounced in the substantia nigra (SN), putamen, globus pallidus (GP, particularly in MSA-P), vermis, cerebellar hemispheres and inferior olivary nucleus (particularly in MSA-C) (Fearnley and Lees, 1990; Wenning et al., 1997), while significant involvement of the thalamus, subthalamic nucleus (STN), cerebellar dentate nucleus and anterior horn cells is rare (Ozawa et al., 2004).

The relevance of recognizing the more benign slowly progressive, levodopa-responsive variant, lies in the fact that these patients can be misdiagnosed as having Parkinson's disease (PD) with significant levodopa-related motor complications and undergo deep brain stimulation (DBS) surgery. In this regard, we recently reported the poor clinical outcome of STN-DBS in a series of five post-mortem confirmed MSA patients. These patients were initially considered as having PD with motor fluctuations and underwent DBS surgery, followed soon after the operation by a rapid deterioration and the appearance of clinical features suggestive of MSA (Meissner et al., 2016).

The present study investigated whether neuropathological differences between these MSA-DBS cases and a cohort of typical MSA-P patients might account for the more benign disease course of the MSA-DBS cases, and also for their rapid clinical decline after DBS.

2. Methods

2.1. Human brain samples

Formalin-fixed and paraffin-embedded material of 5 MSA-DBS and 9 typical MSA-P cases was assessed. Written informed consent was obtained prior to autopsy for the collection of the brain and the use of clinical and post-mortem data from all subjects or their legal representatives. Human brain samples were obtained from the brain banks in Marseille, Lyon, Strasbourg and Bordeaux (DC-2014-2164).

2.2. Immunohistochemistry

Brain regions included for comparative analysis were the putamen and GP (studied within sections demonstrating the lentiform nucleus), since these regions are severely affected in MSA; the thalamus, because it is classically spared by the degenerative process; and the STN, being the target of the DBS surgery. Dewaxed 4 μm thick coronal paraffin sections were either stained with hematoxylin eosin and Cresyl violet for the semiquantitative assessment of neuronal loss or processed for immunohistochemistry as follows.

Immunostaining was performed with antibodies against α-syn (mouse monoclonal antibody, clone LB509, 1:100; Invitrogen by life technologies), glial fibrillary acidic protein (GFAP, polyclonal rabbit, 1:4000; Dako), CD68 (monoclonal mouse anti-Human, clone PG-M1, ref. M 0876 DAKO) to label microglia and CD3 (polyclonal rabbit anti-Human, ref. A 0452 DAKO), a marker of mature T lymphocytes.

After antigen retrieval and blocking with 5% normal goat serum containing 0.05% Tween in phosphate-buffered saline (PBS) for 30 min at room temperature, sections were incubated overnight with the primary antibody. Immunoreactions were revealed with the appropriate secondary antibody, a ready-to-use goat anti-rabbit or goat anti-mouse EnVision-HRP enzyme conjugate (Dako) followed by the highly sensitive diaminobenzidine plus (Dako) as substrate chromogen for GFAP or a high sensitivity AEC (3-amino-9-ethylcarbazole) substrate-chromogen system (Dako) for α-synuclein, CD3 and CD68. Finally, sections were counterstained with Mayer's hemalum and mounted in a suitable mounting medium.

The number of immunopositive cells was obtained using a computerized image analysis system (Mercator V6.50, Explora Nova) linked to a Leica microscope type DM-6000B. Quantitative evaluation was carried out on one section per structure and boundaries on each structure were first delineated at low magnification (×2.5). Quantification was performed at ×20 magnification on the whole thalamus, STN and GP except for the putamen where, from a random start position, the computer-generated sampling grid placed the counting frames. Within each frame, cells were counted only if the totality of the cell was inside the frame taken into account. Results were expressed as an average of immunopositive cells per mm^2. To minimize the inherent variability in the immunohistochemical procedures, all sections from all patients were processed simultaneously for a given antibody and structure. Putaminal degeneration was assessed by counting the number of surviving neurons in the putamen.

2.3. Statistics

Comparisons between groups were done using a Student's *t*-test whenever the variable had a normal distribution, while a Mann-Whitney *U* test was used otherwise. The proportion of the total α-syn inclusion load represented by NCI was compared between groups by using a Fisher exact test (cut-off value of 10% of the total burden). For the comparison between brain structures (NCI, GCI, total α-syn inclusion load, CD3, CD68 and GFAP positive cells), a one-way ANOVA or, if appropriate, a one-way ANOVA on ranks was performed including all 14 subjects. Post-hoc Tukey or Dunn tests were performed whenever appropriate. A p-value < 0.05 was considered significant. If not indicated otherwise, results are presented as mean ± standard deviation. Statistical analyses were performed with GraphPad Prism Software, version 6.

3. Results

3.1. Patient characteristics

A detailed clinical description of the MSA-DBS cases is reported elsewhere (Meissner et al., 2016). Mean age at disease onset was lower in the MSA-DBS group (50.4 ± 6.3 years) compared to the MSA-P control group (56.9 ± 9.5 years, $p < 0.05$), while mean age at death was similar between groups with 61.6 ± 7.1 and 62.3 ± 8.5 years respectively. Accordingly, mean disease duration was higher in the MSA-DBS group (11.2 ± 3.0 years) compared to the MSA-P control group (5.4 ± 2.5 years, $p < 0.005$, Table 1).

The response during the pre-surgical levodopa challenge was excellent in all five MSA-DBS patients, ranging from 49 to 88%