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Neurol Sci (2003) 24:149–150
DOI 10.1007/s10072-003-0103-5

^{123}I-Ioflupane/SPECT binding to striatal dopamine transporter (DAT) uptake in patients with Parkinson's disease, multiple system atrophy, and progressive supranuclear palsy

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Abstract We used SPECT and the tracer ^{123}I-Ioflupane to measure dopamine transporter (DAT) binding in the caudate nucleus and the putamen of 70 patients with Parkinson's disease (PD), 10 with multiple system atrophy (MSA-P type), and 10 with progressive supranuclear palsy (PSP). Data were compared with 12 age-matched control subjects. We found significant reductions in mean striatal values in all three forms of parkinsonism. However, decrements were significantly greater in PSP (0.51±0.39, $p<0.01$) compared with MSA-P (0.70±0.33) and PD (0.95±0.38). No differences were found between MSA and PD. Putamen/caudate ratios were greater in PSP (0.83±0.12, $p<0.01$) than in PD (0.51±0.11), suggesting a more-uniform involvement of dopamine nerve terminals in both caudate nucleus and putamen. Our results confirm that DAT binding can provide an accurate and highly sensitive measure of dopamine degeneration. PSP patients may show a different pattern of neuronal loss compared with MSA and PD.

Degenerative parkinsonian syndromes are characterized by progressive loss of dopaminergic neurons in the substantia nigra. Neuroimaging studies allow in vivo assessment of the nigro-striatal dopaminergic system and the extent of neuronal loss in these disorders. Tracers that bind selectively to dopamine cells have been applied together with positron emission tomography (PET), and more recently single-photon emission computed tomography (SPECT), for clinical and research purposes. Historically, ^{18}F-fluorodopa/PET was considered the gold standard for assessment of dopaminergic denervation [1, 2]. There are, however, technical limitations to the use of this tracer. Firstly, PET is available at few centers. Secondly, it is likely that dopa-decarboxylase activity is upregulated early in the disease process, resulting in an underestimation of dopaminergic neuronal loss. Therefore tracers that utilize the SPECT technique and bind selectively to the dopamine transporter (DAT) may be better suited and provide more-accurate estimation of degeneration. Several tracers that bind to DAT and utilize SPECT are available; they are all cocaine derivatives. The most widely used are [^{123}I]β-CIT and ^{123}I-Ioflupane ([^{123}I]FP-CIT) [3, 4]. The main advantage of ^{123}I-Ioflupane is that a steady state allowing SPECT imaging is reached 3 h after a single bolus injection of the radioligand, compared with the 18–24 h required for [^{123}I]β-CIT. Therefore DAT imaging with ^{123}I-Ioflupane can be completed the same day.

Several studies have demonstrated the usefulness of ^{123}I-Ioflupane SPECT imaging in the diagnosis of parkinsonism. A sensitivity of 97% has been reported for the identification of nigrostriatal degeneration in Parkinson's disease (PD) [5]. Limited data are available on DAT binding in other degenerative parkinsonisms, such as progressive supranuclear palsy (PSP) or multiple system atrophy (MSA) [6].

Patients and methods

We studied DAT binding with ^{123}I-Ioflupane SPECT in 70 PD patients (age 62±13 years, disease duration 5±4 years), 10 MSA-P patients (age 60±8 years, disease duration 4±2 years), and 10 PSP patients (age 64±8 years, disease duration 4±3 years). UPDRS scores were obtained in all patients in "off" condition.

Brain SPECT was performed with a dedicated triple detector gamma camera (Prism 3000, Philips) equipped with ultra-high-resolution fan beam collimators. ^{123}I-Iofluopane (DATScan) (110–140 MBq) was administered intravenously 30–40 min after thyroid blockade. SPECT studies were acquired 3–4 h later and reconstruction was performed by applying an iterative algorithm, followed by tridimensional filtering. Transaxial sections were attenuation corrected and reoriented with respect to the canthomeatal plane. Four adjacent sections, including striatum and occipital cortex, were summed in a single 2.56-cm thick slice for quantitative analysis. Irregular regions of interest (ROIs) were drawn on both striata, putamina, heads of caudate nuclei, and mesial occipital cortices. Iofluopane specific binding ratio was expressed as (striatal ROI counts – occipital ROI counts)/(occipital ROI counts) and calculated for the whole striatum, putamen, and caudate head of each hemisphere. We also calculated putamen/caudate ratios for each subject.

Results

We found significant decrements in DAT binding in the striatum of PD, MSA-P, and PSP patients compared with healthy controls. Mean striatal values were 0.95±0.38 in PD, 0.70±0.33 in MSA, and 0.51±0.39 in PSP. Healthy controls have striatal uptake values >1.7. An example of ^{123}I-Ioflupane SPECT in a control subject, a PD patient, and a PSP patient is

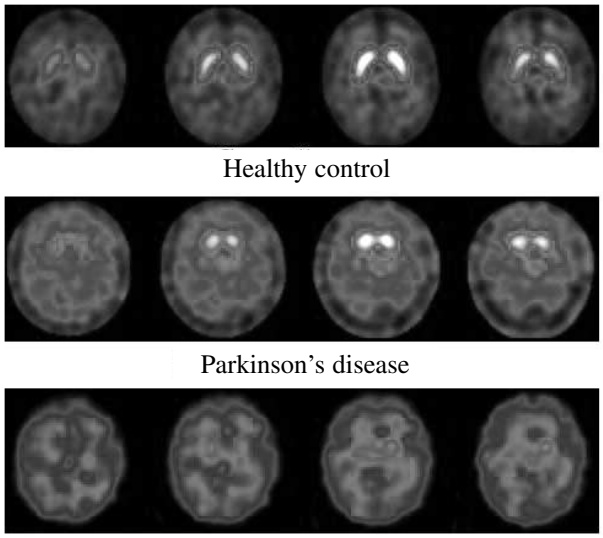

Fig. 1 ^{123}I-Ioflupane SPECT in a control subject, a PD patient, and a PSP patient

shown in Fig. 1. We found that striatal values were significantly lower in PSP than PD ($p<0.01$). No differences were found between MSA-P and PD. Putamen/caudate ratios were greater in PSP (0.83±0.12, $p<0.01$) than in PD (0.51±0.11) and MSA-P (0.60±0.12). In PD patients we found a significant correlation with UPDRS motor scores ($p<0.001$). No correlation was found in the MSA and PSP cohorts.

Discussion

We found significant decrements of striatal DAT in PD, MSA-P, and PSP patients. We can confirm that ^{123}I-Ioflupane SPECT binding is a sensitive measurement of the dopaminergic system in degenerative parkinsonsim. Moreover, we found a different pattern of degeneration between PD and PSP. A similar finding has been previously reported with the tracer ^{18}F-fluorodopa in PSP patients [2]. The greater decrements in the putamen than in the caudate nucleus in PD patients is consistent with neuropathological data showing that the bulk of nigral loss affects the dopaminergic neurons projecting to the putamen. PSP is characterized by a more-profound and diffuse dopaminergic loss.

We were able to show a significant correlation between striatal uptake and UPDRS motor symptoms in PD, suggesting that ^{123}I-Ioflupane SPECT is also a specific marker of disease progression. The absence of a significant correlation in other forms of parkinsonism was probably related to the fact that the majority of our MSA and PSP patients had already marked disease at the time of SPECT scanning.

The differential diagnosis of different forms of parkinsonism is not easy, particularly at an early stage. Although a recent report suggests that in a specialized tertiary center diagnostic accuracy can be as high as 90%, clinical pathological studies in the 1990s found the accuracy of clinical diagnosis to be only 76% [7], and the rate of misdiagnosis at early stages of the disease may exceed this figure. In a recent community based study conducted in a general practice, the diagnosis of clinically probable PD could only be confirmed in 53% of patients taking antiparkinsonian drugs [8]. Such misdiagnosis can lead to inappropriate management strategies that may include further unnecessary investigations.

In conclusion, studies of the pre-synaptic dopaminergic system may prove clinically useful in assessing the presence and extent of nigro-striatal loss in different forms of degenerative parkinsonism. Additional neuroimaging investigations, such as ^{123}I-IBZM for striatal dopamine receptors or 1,ST magnetic resonance imaging, may help to characterize these patients. The finding of a uniform and profound DAT loss in the whole striatum seems to be specific to PSP.

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Neurol Sci (2003) 24:151–152
DOI 10.1007/s10072-003-0104-4

Brain flow changes before and after deep brain stimulation of the subthalamic nucleus in Parkinson's disease

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Abstract Deep brain stimulation (DBS) of the subthalamic nucleus (STN) markedly improves motor symptoms and reduces medication needs in Parkinson's disease (PD) patients. However, its effect on brain function has remained unclear. We used SPECT and the tracer ECD to measure regional cerebral blood flow before and 6 months after DBS of the STN in 22 PD patients and 13 healthy controls. PD patients were divided into good and poor responders to DBS, if changes in "off" UPDRS motor scores after surgery were >60% or <40%, respectively. Statistical analysis was performed using the SPM99 software. At baseline, all PD patients showed significant perfusion reductions in cortical areas (premotor frontal, parietal, and occipital). After DBS, changes were normalized only in the good responders, while cortical defects in the poor responders were unchanged. No flow decrements were detected in basal ganglia and thalamus in both groups, suggesting that DBS does not have a "lesion-like" effect. We conclude that good surgery outcome is associated with normalization of cortical flow abnormalities in PD.

Deep brain stimulation (DBS) has gained increasing importance in the treatment of patients with advanced Parkinson's disease (PD) in the last few years. Targets considered for implantation have been the nucleus ventrointermedius of the thalamus (Vim), the internal globus pallidus (Gpi), and the subthalamic nucleus (STN) [1–4]. Despite some controversy, the STN target has become first choice in the majority of centers worldwide. However, the exact mechanism of action of this surgical technique on "in vivo" neuronal activity needs to be further elucidated.

Imaging studies can be used to support clinical examination in the screening of possible candidates [5] and in follow-up after surgery. We currently use perfusion SPECT together with magnetic resonance imaging (MRI), clinical and neuropsychological evaluation in the selection of PD patients for STN-DBS.

Patients and methods

We studied 22 PD patients before and 6 months following DBS of the STN and 13 age-matched controls. All PD patients presented with marked fluctuations and dyskinesia at baseline. They all had MRI after surgery that confirmed the electrode placement in the STN bilaterally.

Brain SPET studies were performed 30–60 min after intravenous injection of 740 MBq Tc-99m ECD, by means of a dedicated triple detector gamma camera (Prism 3000, Philips), equipped with ultra-high-resolution fan beam collimators. Reconstruction was performed by applying an iterative algorhythm on projection data, followed by tridimensional filtering. Transaxial sections were attenuation corrected and exported to a dedicated workstation for Statistical Parametric Mapping (SPM99) analysis implemented in MATLAB 5.3 environment.

After realignment, the sections were spatially normalized and smoothed with a 10-mm FWHM Gaussian filter. SPM analysis considered a threshold of $P<0.001$ for significance and considered clusters of at least 50 voxels. Significant voxels within a cluster were converted to Talairach and their location was investigated.

PD patients were divided into two groups based on changes in "off" UPDRS motor scores following DBS. Good responders had UPDRS improvements >60% (age 61±7 years, disease duration 14±6 years, "off" UPDRS-III 44±8), while poor responders had changes <40% (age 62±8 years, disease duration 13±3 years, "off" UPDRS-III 43±9).

Results

Clinical characteristics of PD patients after surgery were as follows: good responders (n=13) off UPDRS-III 13±6, % post/pre 72±9% (range 60%–86%); poor responders (n=9) off UPDRS-III 32±10, % post/pre 28±18% (range 8%–40%).

SPM imaging analysis of baseline cerebral blood flow data revealed mild and symmetric reductions of ECD uptake in occipital, posterior temporal, and parietal cortex in PD patients versus controls (Brodmann areas 18, 39, 7, 40, and 21). Before surgery, the same pattern of perfusion decrements was observed in good and poor responders to DBS versus controls.

After surgery, PD patients with good response showed a normalization of the previously observed pattern of cortical hypoperfusion (no significant perfusion differences versus controls during DBS). PD patients with poor motor improvement showed a persistent and significant perfusion impair-

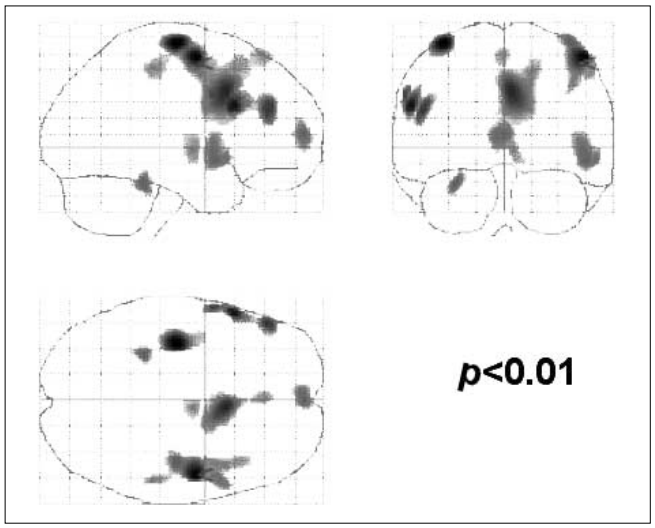

Fig. 1 Perfusion impairment during DBS in PD patients with poor motor improvement

ment during DBS in posterior cortex of both hemispheres, similar to that observed on pre-surgery SPET studies (Broadmann areas 39, 40, 7, and 8) (Fig. 1). No changes were observed in both groups during DBS-STN in the basal ganglia and the thalamus.

Conclusions

DBS produces remarkable clinical benefit to PD patients, however the effects of this high-frequency stimulation on brain function have remained unclear. Three different possible explanations have been proposed. It was first postulated that DBS acted by blocking local neuronal activity, because DBS produced similar clinical effects to destructive lesioning. A second proposed mechanism was preferential activation of inhibitory axon terminals that synapse on and inhibit projection neurons. A third alternative is that stimulation could directly excite projection neurons, increasing, rather than decreasing, their firing rate. This proposal suggests that DBS may be driving body and axons at regular frequencies that override rhythmic firing patterns associated with tremor or irregular PD firing patterns. Several imaging studies have addressed this issue and results are conflicting. Authors have used regional blood flow or glucose metabolism, assuming that their changes provide an index of neuronal activity. Therefore, a SPECT-measured flow or metabolic response could indicate a change of input to that region or alterations in local interneuronal activity. A recent study in patients undergoing DBS of the Vim thalamus showed increased blood flow at terminal fields of thalamocortical projections, suggesting that DBS stimulates and does not inactivate projection neurons in Vim thalamus [6].

In our study, given the absence of flow reductions in the basal ganglia and the thalamus, we conclude that DBS-STN does not produce a "lesion-like" effect. We believe that our findings support the view that DBS stimulates and does not inactivate projection neurons in the STN. Indeed, in the good responders DBS was associated with a "normalization" of functional defects in cortical areas. We speculate that this results from high frequency stimulation overriding the abnormal and irregular pattern of firing of STN neurons. Patients showing the least response to treatment (poor responders) did not show this characteristic pattern change.

Evidence is available suggesting that electrical stimulation leads to activation in the stimulated site and in turn to increased output. Microdialysis in Gpi found increased levels of glutamate during STN, suggesting activation of glutamatergic output from STN to Gpi [7].

In conclusion, the question of excitation versus inhibition may not have a simple answer and it is possible that high-frequency stimulation inhibits neuronal activity only near the stimulation site, while it activates axonal elements leaving the target structure [8]. Indeed, parkinsonian symptoms are likely to be the consequence of abnormalities in the pattern of neuronal firing and DBS probably works through a modification of this activity.

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Neurol Sci (2003) 24:153–154
DOI 10.1007/s10072-003-0105-3

Cognitve estimation: comparison of two tests in nondemented parkinsonian patients

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Abstract The Time and Weight Estimation test (STEP) and the Cognitive Estimation Task (CET) are two recently devised tests for the assessment of cognitive estimation. In the present study, we compared their performance in 30 nondemented idiopathic parkinsonian (PD) patients, also evaluated with the Frontal Assessment Battery (FAB) as an index of executive impairment, with the aim of verifying the putative frontal circuitry of cognitive estimation processes. Six patients (20%) showed a pathological performance on either or both tests. After division of the PD sample into tertiles based on the FAB score, no significant difference was detected by either estimation test. Furthermore, the two questionnaires were unrelated to each other. Thus, deficits of cognitive estimation ability appear to be mild in PD without dementia and do not correlate with executive impairment. Unexpectedly, the CET and the STEP seem to have no unique underlying construct.

Cognitive estimation tasks can only be answered via a multistep reasoning process and not by relying directly on previously acquired knowledge. Due to their relevant planning and reasoning components, cognitive estimation problems are thought to be mainly under the control of the prefrontal cortex. However, availability and accessibility of semantic memory traces, working memory, calculation, visual imagery, and visuospatial manipulation of mental images are necessary for both the elaboration and checking of quantitative estimates. Contradictory results have been obtained as to the specificity of cognitive estimation tasks for frontal dysfunction in both focal lesion and dementia patients [1–3]. Such tests have also often been shown to be unrelated to other frontal tasks in different clinical samples [1, 4].

The Time and Weight Estimation Test (STEP) [5] and the Cognitive Estimation Task (CET) [6] are two tests of cognitive estimation ability recently devised in Italy and independently tested in frontal lobe lesion and Alzheimer disease patients. They show sensitivity to both types of brain dysfunction compared with normal subjects [6, 7]. However, these types of patients are not ideal for the investigation of cognitive and neural correlates of estimation ability, which might be better explored in patients with a more-selective and extensive dysfunction of the frontal lobes. A complete and pure frontal syndrome is a frequent neuropsychological complication of idiopathic Parkinson's disease (PD) without dementia [8].

The aim of the present study was to assess and compare the performance of nondemented PD patients on both the STEP and the CET, in order to verify their putative frontal locus. We also aimed to provide further insight into the neuropsychological profile of PD through the evaluation of this hitherto neglected subtle cognitive aspect.

Study subjects were patients with idiopathic PD without dementia, which was defined by a Mini Mental State Examination (MMSE) adjusted score <24. Other exclusion criteria were past or current major neuropsychiatric disorders, including severe depression, as defined by a score ≥20 on the Geriatric Depression Scale (GDS), and a history of alcohol abuse. Neurological impairment was evaluated with the Unified Parkinson's Disease Rating Scale (UPDRS)-part III. Assessment of memory, visuospatial and executive functions was performed using a Story Recall (SR) test, Benton's Judgment of Line Orientation (JOL)—short form, and the Frontal Assessment Battery (FAB). All patients were also administered the two cognitive estimation questionnaires. The STEP is composed of two distinct ten-item sections focusing on time (e.g., How long does it take to have a shower?) and weight (e.g., How heavy is a pair of jeans?) estimations, respectively. Scores from 0 (bizarre estimation) to 3 (best estimation) are given for each answer, for a total maximum score of 60. Normative data are available [5]: the cut-off value for normality is ≥40 (the higher the score, the better the performance); no adjustment for gender, age, or education is needed. The CET includes 20 questions addressing the estimation of various quantitative parameters (e.g., What is the height of a traffic light? How many camels are there in Italy?). Each item scores from 0 (best estimation) to 2 (bizarre estimation), for a total maximum score of 40. Unpublished normative data are available: the cut-off value for normality is <19 (the higher the score, the worse the performance); raw scores must be adjusted for gender.

Thirty nondemented PD patients, 19 men and 11 women, were included in the study. They had a mean age of 66.1±7.3 years and a mean education of 6.8±4.0 years. Mean disease duration was 6.6±4.6 years and mean UPDRS-III score was 27.1±9.8. Their neuropsychological mean scores were as follows: MMSE=28.9±1.7, FAB=13.3±2.6, SR=10.0±2.4, JOL=11.5±1.3, and GDS=9.0±5.7. Mean adjusted CET and STEP scores were 14.2±3.6 and 42.8±5.3, respectively. Two patients (6%) showed a pathological performance on the CET only, and 3 (10%) on the STEP only; while 1 (3%) had an impaired performance on both. Correlation analysis was statistically significant between the CET and the UPDRS-III score (r=0.40), the MMSE (r=-0.50), the SR (r=-0.52), and the JOL (r=-0.47). The STEP slightly correlated only with age (r=-0.37) and with the MMSE (r=0.39). No significant correlation was found between the two cognitive estimation

tests (r=-0.29) and between either test and the FAB (r=-0.31 for the CET, 0.21 for the STEP). The study sample was also divided into tertiles on the basis of the FAB scores. Patients in the first tertile, i.e., those with the most-prominent executive impairment (n=8, mean FAB score=9.5±2.0), were significantly older and had slightly lower scores on the MMSE and the visuospatial task than both the second (n=13, FAB=12.8±0.8) and the third (n=9, FAB=16.4±0.9) tertiles. Neurological and memory features were similar for the three subgroups. No statistically significant difference was found for the three tertiles on the STEP. CET raw scores appeared significantly worse for the most-frontal patients than for the other two tertiles, but the difference disappeared after covarying for the MMSE score.

Overall, our sample of nondemented PD patients did not show a significant deficit of cognitive estimation ability. While deliberately excluding PD patients with dementia (which usually appears in the advanced stages of the disease), our final study sample showed relatively mild disease duration and severity, and thus mild cognitive (i.e., frontal) deficits. The limited number of abnormal performances observed does not allow clear-cut assumptions about the relative specificity of the two tests for "anterior" dysfunction. However, both comparison and correlation analysis seems to point to a substantial lack of correlation with frontal functions as assessed with the FAB, in agreement with previous data arguing against a purely or prominent dysexecutive nature of cognitive estimation deficits [2, 3]. According to our findings, performance on the CET instead reflects a global factor of cognitive functioning, while the STEP seems to rely on an intellectual ability independent of those considered in the present study. More in-depth investigations of the multiple neuropsychological functions contributing to estimation processes are needed. Further studies correlating performance on the two questionnaires with a more-extensive assessment of executive functions are also recommended, as no element of the FAB deals specifically with reasoning capacity.

Unexpectedly, we found a low degree of congruency between the two cognitive estimation tests, suggesting that they do not have a unique underlying construct. We found no reciprocal correlations and dissociated performances were observed in 20% of our patients. Other systematic comparisons of the STEP and the CET should be performed in other clinical samples,to clarify their specificities and define their most-appropriate use in psychometric practice. Finally, the prevalence of cognitive estimation deficits should be assessed in PD patients in more-advanced stages of the disease, with and without dementia, in order to establish their clinical relevance.

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Neurol Sci (2003) 24:155–156
DOI 10.1007/s10072-003-0106-2

Proteome analysis of mesencephalic tissues: evidence for Parkinson's disease

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Abstract Proteome analysis is a powerful methodology to investigate protein expression in tissues involved in diseases not linked to particular genetic defects. To date, this technique has a limited number of applications in the field of neurodegenerative disorders. We decided therefore to investigate by this approach autoptic mesencephalic tissues of patients with idiopathic Parkinson's disease as well as control specimens from healthy subjects.

The tight association of proteins to form insoluble aggregates is considered the basis of a number of neurological diseases, both familial and sporadic. Self-aggregation of α-synuclein, wrong folding of the prion protein, defective cleavage of the amyloid β-peptide, and hyperphosphorylation of the tau protein all lead to changes in the dynamics of cytosolic proteins and eventually to the formation of macroscopic aggregates [1]. An analysis of water dynamics in ex vivo specimens of Parkinson's disease (PD) midbrains has shown a differential deposition of cytosolic proteins compared with age–matched controls [2]. In order to investigate the role of protein expression in the etiopathogenesis of PD we used proteome technology, which combines two-dimensional electrophoretic protein separation with protein identification by immunochemistry or mass spectrometry (MS). In two-dimensional electrophoresis, proteins are first separated on a pH gradient on the basis of their isoelectric point, and then a standard SDS-PAGE separation discriminates on the basis of the molecular weight. Two-dimensional gels may be blotted onto PVDF (hydrophobic polyvinylidene difluoride) membrane and incubated with antibodies against proteins of particular interest in PD (in this study α-synuclein, ubiquitin, and parkin). Alternatively, the identification of single spots on the two-dimensional gel may be achieved by endoproteinase digestion of excised spots and characterization of the peptide mixture by MS. Furthermore, two-dimensional maps are able to underline post-translational modifications or different levels of protein expression between parkinsonian and control tissues eventually linked to different molecular pathways.

Material and methods

Specimens of human substantia nigra from four patients with a known history of PD and from four control subjects without neurological diseases were provided by the Netherland's Brain Bank.

Tissues were homogenized in a lysis solution (urea/thiourea/CHAPS and protease inhibitor mix) and centrifuged to remove insoluble fractions. The protein content was estimated according to Bradford [3].

Two-dimensional electrophoresis was performed according to Jacobs et al. [4]. First-dimension isoelectric focusing required 13-cm IPG strips (non-linear pH gradient 3–10) and was performed on an IPGphor (Amersham Biosciences) at 16°C. The second dimension, SDS-PAGE, was performed on 12.5% separating polyacrylamide gels (45 mA/gel, 16°C). Gels were stained with Coomassie brilliant blue R250 (Amersham Biosciences) or electroblotted onto a PVDF membrane (1 h at 1.5 mA/cm^2) with a semidry transfer cell (Amersham Biosciences). Primary antibodies tested (monoclonal anti-α-synuclein and anti-ubiquitin) were purchased from Zymed Laboratories and Research Diagnostic (anti-parkin) and were diluted 1:500. The antigen-antibody interaction was carried out for 1 h at room temperature. Immunodetection was achieved by a peroxidase-linked secondary antibody (diluted 1:500) and chemiluminescence.

Coomassie-stained spots were manually excised and subjected to trypsin digestion in order to perform an MS analysis. Each spot was destained, desiccated, reswollen, and digested with modified porcine trypsin overnight at 37°C. Peptides were extracted by sonication and directly analyzed by MS. Database search with the peptide masses was performed against the SWISS-PROT database using the PeptIdent search algorithm (http://www.expasy.ch/tools/peptident.html).

Results and discussion

Recent studies [5] on familial PD report the crucial role of some proteins such as α-synuclein, ubiquitin, parkin, and UCH-L1. In particular, α-synuclein and ubiquitin are detected in Lewy bodies (LB), the pathological hallmark for idiopathic PD [7].

By the use of two-dimensional electrophoresis followed by western blotting we assessed the immunoreactivity of PD and control mesencephalic specimens with respect to α-synuclein, ubiquitin, and parkin. A particularly interesting observation was the presence in PD samples of a set of spots (pI=6–8, molecular weight=29 kilodaltons) that are cross-reactive for anti-α-synuclein and anti-ubiquitin antibodies. This component in control brains appears to be immunoreactive only for α-synuclein, there is no ubiquitin reactivity; its occurrence is associated with LB and it has been recently assigned to diubiquitinylated, phosphorylated α-synuclein

[6]. The final result of this molecular pathway may be the massive formation of ubiquitinylated α-synuclein, the core of LB. It is noteworthy that α-synuclein has also been recently detected only in neuromelanin granules of PD post-mortem midbrain specimens and not in control specimens [8]. There were no appreciable differences between pathological and healthy samples with respect to parkin immunoreactivity.

In order to build a map of protein extracted from human midbrain specimens, we tried to identify by peptide mass fingerprinting proteins that were expressed differently in PD and control maps. Although there are differences in the PD and control gels, there were 12 proteins whose expression level does not appear to be statistically different. However, Mn-SOD and dihydropteridine reductase were increased twofold in PD with respect to controls.

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Neurol Sci (2003) 24:157–158
DOI 10.1007/s10072-003-0107-1

Effects of dopaminergic stimulation on peripheral markers of apoptosis: relevance to Parkinson's disease

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Abstract We investigated the effects of dopaminergic stimulation on anti-apoptotic protein Bcl-2, pro-apoptotic enzyme caspase-3, and anti-oxidant/anti-apoptotic enzyme Cu/Zn superoxide dismutase (SOD) in human lymphocytes exposed to dopamine (DA). The same determinations were also carried out in parkinsonian patients treated with L-dopa. Caspase-3 activity and Cu/Zn SOD levels tended to increase when lymphocytes were exposed to low or intermediate doses of DA, while a decrease was observed, particularly in caspase-3 activity, with the higher DA dose. Bcl-2 levels were unaffected. In patients, we observed a negative correlation between Cu/Zn SOD levels and daily intake of L-dopa, which also tended to be negatively correlated with caspase-3 activity, but not with Bcl-2. Our results show that dopaminergic stimulation is associated with complex changes in regulatory proteins of apoptosis.

Introduction

Defective regulation of apoptosis (*programmed cell death*) may play a primary role in the pathogenic mechanisms of Parkinson's disease (PD). In contrast to necrosis – a passive mechanism of cell death – apoptosis is an active process triggered by the activation of specific cellular programs, which gradually evolves through a series of defined steps [1]. Apoptosis can be initiated by a variety of conditions, some of which are certainly implicated in the pathogenesis of PD, such as impaired activity of mitochondrial enzyme complex I [2]. Signs of apoptotic cell death and increased levels of pro-apoptotic molecules have been found in the substantia nigra of PD patients [3]. In addition, toxins used to replicate PD in animals induce apoptosis [4]. Another important issue is the relationship between dopaminergic stimulation and apoptosis. L-Dopa, still the most-used drug for PD therapy, and/or dopamine (DA) may promote oxidative stress and trigger apoptosis under experimental conditions [5, 6], while DA agonists exert the opposite effect [7]. Due to the difficulty of studying the pathogenic mechanisms of PD in the central nervous system of patients, peripheral blood cells – particularly lymphocytes – have been used as a source of information on bio-molecular aspects relevant to the pathogenesis of PD [8]. The aim of our study was to investigate the effects of dopaminergic stimulation on intracellular proteins involved in the regulation of apoptosis, by measuring anti-apoptotic protein Bcl-2, anti-oxidant/anti-apoptotic enzyme Cu/Zn superoxide dismutase (SOD), and pro-apoptotic enzyme caspase-3 in isolated, human lymphocytes exposed to increasing concentrations of DA. We also measured Bcl-2, Cu/Zn SOD, and caspase-3 in lymphocytes of PD patients treated with different doses of L-dopa.

Materials and methods

The *in vitro* study was conducted on lymphocytes isolated by density gradient centrifugation from 30-ml venous blood samples of 8 healthy volunteers – 4 females and 4 males – ranging in age between 26 and 33 years (mean±SD: 32±4.5 years). After isolation, lymphocytes were counted on a cell counter (Beckman Coulter, USA), split into aliquots, and re-suspended in RPMI-1640 medium. Increasing amounts of DA – 0.1, 1, or 500 μM – were added to the aliquots, which were then incubated for 24 h at 37°C in the dark. After incubation, cells were pelleted with a final centrifugation and, after discarding the supernatant, frozen at –80°C, until assay.

For the *in vivo* study, 20 PD patients (10 males and 10 females; mean age: 65.9±8 years; UPDRS score-subscale III: 23.0±9.7) were enrolled. Patients were receiving L-dopa, as monotherapy, with a daily intake ranging between 200 and 1.350 mg/day (mean±SD: 545±260 mg). After signing an informed consent, all patients gave a 20-ml blood sample. Lymphocytes were isolated, counted, pelleted, and stored at –80°C, prior to the biochemical assays.

Lymphocyte pellets, from both *in vitro* and *in vivo* studies, were re-suspended in ice-cold phosphate-buffered saline (PBS) and homogenized by ultrasound. Homogenates were ultracentrifuged and resulting supernatants were used for the assays. Cu/Zn SOD and Bcl-2 concentrations were assayed using enzyme-linked immunosorbent assay (ELISA) colorimetric kits (Bender MedSystems, Austria), while a fluorometric assay was used for the determination of caspase-3 protease activity (Molecular Probes, USA).

Results

In vitro study

As shown in Fig. 1, incubation of lymphocytes with DA induced a slight, non-significant increase in caspase-3 activity when the intermediate, 1 μM, dose was used; conversely, a massive decrease was associated with the highest dose of DA (500 μM). Cu/Zn SOD levels showed a slight increase with the lowest dose of DA (0.1 μM), followed by a significant reduction when the 1 or 500 μM doses were tested. No changes were found in Bcl-2 levels.

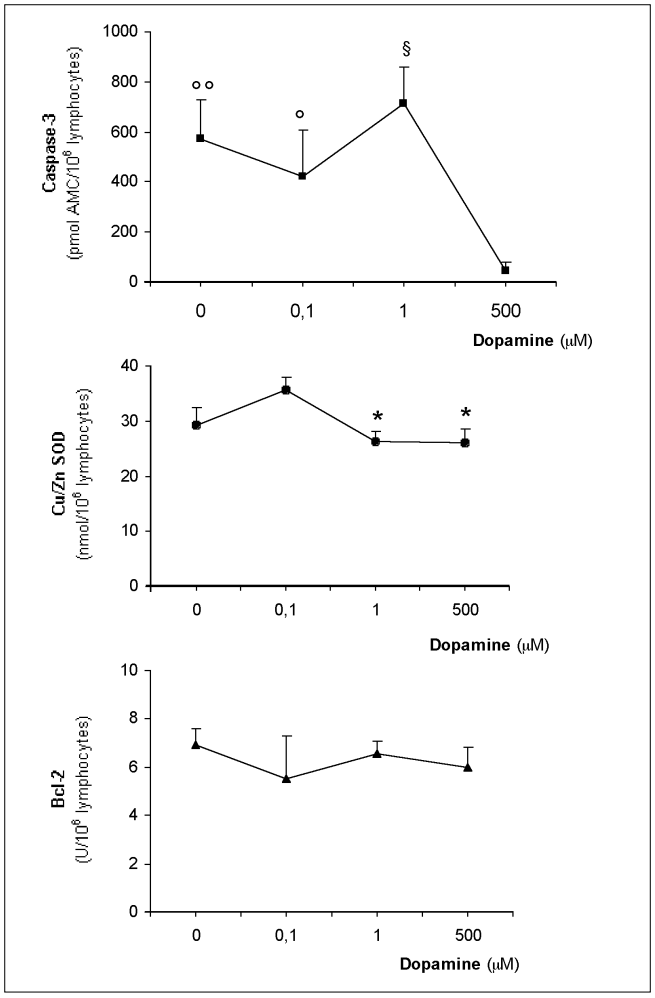

Fig. 1 Caspase-3 activity, Cu/Zn superoxide dismutase (*SOD*) and Bcl-2 concentrations in isolated, human lymphocytes incubated for 24 h with increasing amounts of dopamine. *AMC*, 7-amino-4-methyl-coumarin; °p<0.05, °°p<0.01, §p<0.001 vs. 500 μM; *p<0.005 vs. 0.1 μM (Student's t-test)

In vivo study

We found a significant, negative correlation between the daily intake of L-dopa and the lymphocyte levels of Cu/Zn SOD (y=140.2–0.1x, r=0.50, p<0.03). The drug dosage also tended to be negatively correlated with caspase-3 activity, although full statistical significance was not reached (r=0.41, p=0.066). No correlation was observed with Bcl-2 levels.

Discussion

The results of our study show that dopaminergic stimulation affects the intracellular levels of proteins involved – with different roles – in the regulation of the apoptotic cascade. Both in vitro and in vivo results suggest that exposure of lymphocytes to increasing concentrations of DA or L-dopa decreases the intracellular levels of Cu/Zn SOD, an anti-oxidant enzyme that counteracts the pro-apoptotic effect of the superoxide anion. Similar changes seem to affect the activity of caspase-3, one of the final effectors of the apoptotic process. In this case, however, a bi-phasic response was observed in the in vitro study, with the intermediate dose of DA inducing a slight increase in the enzyme activity, followed by a dramatic decrease associated with the maximal dopaminergic stimulation. The exact meaning of this finding is unclear and will require further investigation. An increase in caspase-3 activity is usually, although not necessarily, associated with apoptotic cell death, while the functional meaning of a decrease in caspase activity is unclear. In our experimental setting, it may be hypothesized that the exposure to exceedingly high concentrations of DA would impair the physiological function of the intracellular enzymatic machinery, including proteases, such as caspase-3. Neither the *in vitro* nor the *in vivo* study showed any modification in the level of Bcl-2, a major anti-apoptotic protein that acts at the mitochondrial level, suggesting that this mechanism of defense is not affected by dopaminergic stimulation.

In conclusion, our results show that dopaminergic stimulation is associated with complex changes in the intracellular levels of apoptosis regulatory proteins, both *in vitro* and *in vivo*. This may play a role in the therapeutic response to dopaminergic agents, as well as in the progression of the disease.

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Neurol Sci (2003) 24:159–160
DOI 10.1007/s10072-003-0108-0

DJ-1 (*PARK7*), a novel gene for autosomal recessive, early onset parkinsonism

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Abstract Four chromosomal loci (*PARK2, PARK6, PARK7,* and *PARK9*) associated with autosomal recessive, early onset parkinsonism are known. We mapped the *PARK7* locus to chromosome 1p36 in a large family from a genetically isolated population in the Netherlands, and confirmed this linkage in an Italian family. By positional cloning within the refined *PARK7* critical region we recently identified mutations in the *DJ-1* gene in the two PARK7-linked families. The function of DJ-1 remains largely unknown, but evidence from genetic studies on the yeast *DJ-1* homologue, and biochemical studies in murine and human cell lines, suggests a role for DJ-1 as an antioxidant and/or a molecular chaperone. Elucidating the role of DJ-1 will lead to a better understanding of the pathogenesis of DJ-1-related and common forms of Parkinson's disease.

By genome-wide scan and homozygosity mapping, we identified the *PARK7* locus in a large consanguineous family with four affected individuals, belonging to a genetically isolated population in the south west of the Netherlands [1]. Linkage to the same region was later confirmed in a consanguineous pedigree from central Italy with three affected members [2]. The phenotype in both families is characterized by parkinsonism of early onset (ranging from age 27 to 40 years), good levodopa response, and slow progression. Behavioral and psychic disturbances and dystonic features (including blepharospasm) are also present, and a positron emission tomography study in the Dutch family showed dopaminergic presynaptic dysfunctions [1–3].

Fine mapping studies and a positional cloning strategy led us to the identification of homozygous mutations in the *DJ-1* gene showing complete cosegregation with the disease haplotype and absence from large numbers of control chromosomes: a ~14-kb deletion removing a large part of the *DJ-1* coding region in the Dutch family and a missense mutation (Leucine166Proline, L166P) in the Italian family (Fig. 1) [4].

The expression of the *DJ-1* gene is abolished by the homozygous deletion in the patients of the Dutch family, indicating that the loss of DJ-1 function is pathogenic. The L166P mutation is also likely to severely affect the function of DJ-1 because: (1) it replaces a highly conserved residue in the DJ-1 protein, (2) it destabilizes the carboxy-terminal a-helix of the DJ-1 protein as predicted by structural models, and (3) it dramatically changes the subcellular localization of the DJ-1 protein in transfection experiments [4].

The human *DJ-1* is organized in eight exons distributed over 24 kb. The first two exons are non-coding and alternatively spliced in the mRNA. *DJ-1* is ubiquitously and highly expressed in the brain areas and extra-cerebral tissues. The human DJ-1 protein has 189 amino acids and belongs to the ThiJ/PfpI family. The exact function of the DJ-1 protein is unknown, but previous reports suggested an involvement in multiple cellular processes, including oncogenesis, regulation of RNA-binding protein complexes, sperm maturation and fertilization in rodents, and regulation of androgen receptor-mediated transcriptional activity [4]. Interestingly, studies in murine and human cell lines showed that the DJ-1 protein is converted into a more-acidic variant in response to oxidative stress, suggesting a role as an antioxidant [5]. Moreover, the yeast *DJ-1* homologue is transcribed during the oxidative stress response and during the response to protein misfolding (which in turn is associated with oxidative stress), raising the question of whether DJ-1 also plays a role as a molecular chaperone [6, 7]. DJ-1 might therefore be involved in the cellular response to stress at multiple levels. It might directly react to stress signals by chemical shifts and/or change in multimerization state; it might also modulate the gene expression of the stress response at transcriptional and/or post-transcriptional levels [4]. Although an involvement of human DJ-1 in the oxidative stress response, or in the response to protein misfolding, remains to be

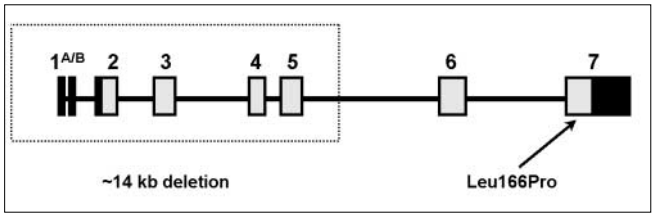

Fig. 1 Genomic structure of the *DJ-1* gene showing the two mutations identified [4]. *Black* and *grey boxes* indicate non-coding and coding exonic sequences, respectively

proven, the proposed model is intriguing in the light of the oxidative stress and protein misfolding documented in the brains of patients with Parkinson's disease (PD).

Mutational analyses in large series of patients with early onset PD are currently in progress to evaluate the frequency of *DJ-1* mutations, pinpoint functionally important domains in the DJ-1 protein, and allow accurate genotype-phenotype correlation studies. Although brain material from patients with *DJ-1*-related forms is not currently available, the presence of the DJ-1 protein is being investigated in brains from patients with Lewy body disease, as well as other neurodegenerative diseases. These studies might provide clues on the involvement of DJ-1 in common forms of neurodegeneration. Lastly, functional studies are in progress to elucidate further the role of DJ-1, identify its interacting partners, and explore possible relationships with the proteins encoded by the other genes firmly implicated in PD: *α-synuclein* and *parkin* [8]. Understanding the role of DJ-1 in the brain and the *DJ-1*-related disease might shed light on the mechanisms of brain maintenance and the pathogenesis of classical PD.

Acknowledgements We thank the families studied for their cooperation and understanding, and the members of the "Italian Parkinson Genetics Network" for their contributions. The work described in this paper was supported in part by the Parkinson Disease Foundation/National Parkinson Foundation (USA), the Princes Beatrix Foundation (NL), and the "Ministero dell'Istruzione, Universita' e Ricerca" (MIUR, IT).

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Neurol Sci (2003) 24:161
DOI 10.1007/s10072-003-0109-z

The diagnostic importance of the isolated supranuclear downward gaze ophthalmoplegia in progressive supranuclear palsy

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The early clinical diagnosis of progressive supranuclear palsy (PSP) is very difficult [1, 2, 3]. Great importance has been attached to the presence of downward supranuclear gaze palsy that, unlike the upward gaze palsy, is very specific. Its appearance is however, as a rule, less early [4].

A survey of the literature did not confirm the importance attributed to this clinical symptom [6, 7, 8]. Therefore it seemed useful to examine further cases.

We examined 35 randomized patients with a clear-cut diagnosis of PSP. Only 4 had downward supranuclear gaze paralysis present at disease onset.The symptom does not appear to be very specific and early and its clinical diagnostic importance is controversial.

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Neurol Sci (2003) 24:162–163
DOI 10.1007/s10072-003-0110-6

Dopaminergic degeneration and perfusional impairment in Lewy body dementia and Alzheimer's disease

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Abstract The clinical differentiation of Lewy body dementia (LBD) from Alzheimer's disease (AD) may be difficult. The aim of the present study was to assess the dopamine transporter function and the perfusional pattern in LBD and AD in vivo. Twenty patients with probable LBD and 24 with probable AD underwent on 2 separate days a brain perfusional SPECT with 99mTc-ECD and a SPECT with ^{123}I-FP-CIT, a ligand of dopamine transporter. In LBD a significantly ($p<0.0005$) lower ratio of specific (bilateral caudate nucleus, putamen) to non-specific (occipital cortex) ^{123}I-FP-CIT binding than in AD was reported. Perfusional data (SPM analysis) showed a significant ($p<0.001$) decrease of temporo-parietal blood flow in AD versus LBD, whereas in LBD a significant ($p<0.001$) occipital hypoperfusion with respect to AD was reported. Our findings confirm that dopaminergic nigrostriatal function is impaired in LBD. The selective occipital hypoperfusion in LBD needs to be further investigated.

Lewy body dementia (LBD) is widely recognized as the second most-common form of degenerative dementia after Alzheimer's disease (AD). The main clinical features include parkinsonism, persistent visual hallucinations, and fluctuating cognitive impairment. Clinical distinction from AD may be difficult because of a wide overlapping of the symptom profiles. It should also be mentioned that the occurrence of parkinsonism in AD is not uncommon; the timing of appearance of parkinsonism and dementia, as well as the neuropsychological testing, do not allow with enough sensitivity the distinction between AD and LBD. Accurate clinical diagnosis of LBD is important because such patients respond with very severe adverse effects to neuroleptics, they may be particularly responsive to cholinesterase inhibitors, and their parkinsonism can be successfully treated with levodopa in most cases. Finally LBD may have a different rate of disease progression from AD. The major neurochemical difference between AD and LBD is in the dopaminergic metabolism. A post-mortem study showed in LBD a reduction in dopamine concentration in the putamen as well as a reduced binding to the dopamine uptake sites in comparison with no change in AD [1]. In this study the nigrostriatal function was investigated by means of dopamine transporter (DAT) imaging (^{123}I-FP-CIT SPECT) in LBD and AD to assess the potential usefulness of such an approach for in vivo distinction of the two most-common forms of degenerative dementia. In the same study populations, regional cerebral blood flow (rCBF) measurements, as evaluated by 99mTc-ECD SPECT, were compared in order to investigate the perfusional pattern in LBD and AD.

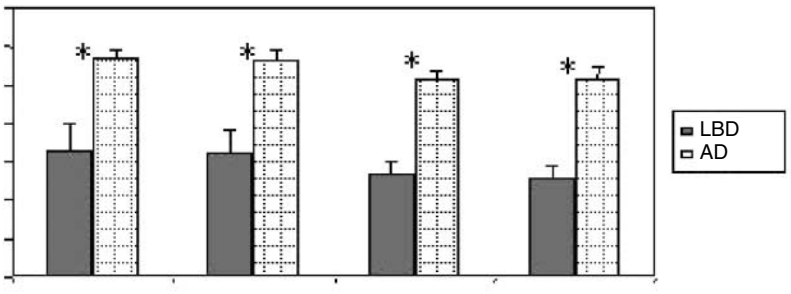
Materials and methods

Twenty patients who fulfilled the consensus criteria for probable LBD (14 female, 6 male, mean age 70.5±5.3 years) [2] and 24 with probable AD (NINCDS-ADRDA criteria) (14 female, 10 male, mean age 67.4±5.9 years) [3] were included in the study. For each patient a number of tests was performed: the Mini Mental State Examination (MMSE), the Cambridge Cognitive Function Examination (CAMCOG), the Neuropsychiatry Inventory, and the Unified Parkinson's Disease Rating Scales, motor part (UPDRS). All patients underwent on 2 separate days (maximum interval 1 week) a brain SPECT with 99mTc-ECD and a brain SPECT with ^{123}I-FP-CIT. Scanning took place between 3 and 4 h after injection of ^{123}I-FP-CIT (185 MBq) and 1 h after injection of 99mTc-ECD (900 MBq). For ^{123}I-FP-CIT binding values, regular circular regions of interest were used to calculate the average striatal (caudate nucleus, putamen) to non-specific (occipital lobes) radioactivity ratios, for both hemispheres. Perfusional data were analyzed by Statistical Parametric Mapping (SPM99).

Results

There were no significant differences between the two groups with respect to family history of PD or AD, past history of psychiatric disorders, history of smoking and alcohol consumption, and years of education. There were no significant differences between patients with LBD and those with AD in the results of neuropsychological investigations (Mini Mental State Examination, mean value±SD LBD 21.0±1.8, AD 20.6±2.3). In LBD a significantly ($p<0.0005$) lower ratio of specific to non-specific ^{123}I-FP-CIT binding was found than in the AD group (Fig. 1).

SPM analysis showed in AD a significant decrease of rCBF in the temporo-parietal cortex compared with LBD ($p<0.001$). When LBD patients were compared with AD, a significant decrease of rCBF was observed in the occipital areas ($p<0.001$).



Right	C/O	1.65±0.37	2.85±0.12	*p<0.0005
Left	C/O	1.61±0.31	2.84±0.12	*p<0.0005
Right	P/O	1.33±0.18	2.57±0.12	*p<0.0005
Left	P/O	1.29±0.15	2.59±0.14	*p<0.0005

Fig. 1 ^{123}I-FP-CIT SPECT results in Lewy body dementia (*LBD*) and Alzheimer's disease (*AD*) patients

Discussion

This study confirms that LBD and AD could be distinguished in vivo by DAT imaging. This observation is in agreement with pathological findings of a consistent loss of substantia nigra neurons and depletion of striatal dopamine content in LBD [1]. In accordance with previous in vivo investigations [4, 5], our findings indicate that dopaminergic nigrostriatal function is impaired in LBD. The report of occipital hypoperfusion in LBD could be linked with one of the main features of the disorder, that of visual hallucinations. However, according to a previous report [6], LBD patients without hallucinations, although few in number, had in the present study a similar pattern of occipital hypoperfusion to those with hallucinations. Also no correlation was reported between occipital hypoperfusion and visuo-spatial abnormalities. It is possible that the occipital changes reported in LBD might be linked to dopaminergic abnormalities within some components of the visual pathway [7]. Alternatively occipital hypoperfusion might reflect impairment of saccadic eye movement [8], or the presence of autonomic failure [9]. Further studies including parkinsonian patients without dementia will further define the functional nature of occipital changes in LBD.

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Neurol Sci (2003) 24:164–165
DOI 10.1007/s10072-003-0111-5

Presence of β-arrestin in cellular inclusions in metamphetamine-treated PC12 cells

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Abstract Cellular inclusions containing ubiquitin and α-synuclein were observed in PC12 cells treated with metamphetamine (MA). To study the possible involvement of β-arrestin in inclusion formation, we treated PC12 cells with MA for different times and analyzed the ubiquitin proteosome pathway (UPP). We found that β-arrestin is ubiquitinated in the MA-treated PC12 cell line. The involvement of β-arrestin in UPP was further supported by electron microscopy and by confocal microscopy, which documented the presence of β-arrestin in these Lewy body-like inclusions. Our experiments reveal an interesting and previously unappreciated connection between β-arrestin and ubiquitination and suggest that β-arrestin could be involved in the development of the inclusion bodies.

The neurodegenerative diseases are marked by the presence of an abnormal accumulation of mutant or damaged proteins that leads to selective neuronal dysfunction. Parkinson's disease (PD) is a prototypical neurodegenerative disease characterized by resting tremor, rigidity, hypokinesia, and postural instability. Pathological examination shows loss of dopamine-producing neurons in the substantia nigra pars compacta, which results in a drastic depletion of dopamine in the striatum [1]. The main pathological hallmark of this disorder is a pronounced accumulation of intracytoplasmic protein aggregates, called Lewy bodies, which contain α-synuclein and specific components of the ubiquitin-proteasome pathway. (UPP) [2]. Recent studies clearly indicate that the UPP plays a crucial role in the pathogenesis of neurodegenerative diseases because the UPP is involved in cellular quality control by degrading misfolded or damaged proteins that could form potentially toxic aggregates. Efficient dopamine sequestration is probably the main mechanism by which nigral cells protect themselves from the deleterious effect of dopamine oxidation. In familial forms of PD, a loss of the normal function of the UPP promotes the accumulation of dopamine in the cytoplasm [3]. This leads to oxidative stress at nigrostriatal terminals, where most neurotransmitters are synthesized and stored. This could be the final deleterious event that triggers the death of nigral dopaminergic neurons in PD. Further evidence that oxidative stress participates in the loss of nigral neurons in PD comes from studies using the parkinsonism-inducing toxin (MPTP) and the psychostimulant metamphetamine (MA), which induce nigro-striatal damage in experimental animals. The active metabolite of MPTP, MPP^+, and MA are taken up into dopaminerigic terminals by the plasma membrane dopamine transporter (DAT), leading to the redistribution of dopamine to the cytoplasm, promoting nigral cell loss and clinical symptoms of PD. Like MPP^+, administration of MA induces cytoplasmic and intranuclear inclusion bodies, both in nigral and striatal neurons [1]. This effect can be reproduced in vitro in undifferentiated PC12 cells. Both in striatal neurons and in PC12 cells the inclusions appear as membranous whorls containing ubiquitin immunoreactivity. Confocal microscopy analysis of PC12 cells treated with MA for 24 h shows that α-synuclein co-localizes with ubiquitin within the whorls [4]. Based on these observations it was suggested that PC12 cells might represent a useful in vitro model system to investigate the molecular mechanisms that underlie the loss of dopaminergic neurons in PD.

Using this model our investigation aimed to identify the possible role of G protein-coupled receptor (GPCR) signaling in the modulation of inclusions formation. This working hypothesis is based on the recent data showing that UPP can be modulated, at least in part, by GPCR signals [5]. Using the β_2-adrenergic receptor (β_2-AR) as a model, it was documented that upon agonist stimulation the receptor is ubiquitinated and that this process is mediated by binding to the regulatory protein β-arrestin. A rapid and transient ubiquitination of β-arrestin was also demonstrated [5, 6]. Here we hypothesize that following agonist stimulation the DA receptor (which is a GPCR) binds to β-arrestin and that β-arrestin binding can be a prerequisite for receptor ubiquitination.

Arrestin is a regulatory protein involved in GPCR regulation and signaling, acting at different steps of signal propagation. This protein was first identified as a regulatory protein important for the homologous desensitization and internalization of the majority of GPCRs. Agonist stimulation also leads to rapid receptor phosphorylation by specific receptor kinases (G protein-coupled receptor kinases, GRKs) and this phosphorylation increase the affinity for β-arrestin binding, which functionally uncouples the receptor from G protein activation (homologous desensitization). β-Arrestin binding also promotes internalization of numerous GPCRs. Internalized receptors are either recycled back to the surface after dephosphorylation or are degraded [7]. In some cases, β-arrestin works as a signaling protein for the GPCR-stimulated intracellular response. For example, our laboratory documented that agonist stimulation of the mGlu1 receptor induces the redistribution of β-arrestin to intracellular vesicles that are distinct from those where the receptor is internalized. This indicates that β-arrestin is not involved in receptor internalization. In this case β-arrestin is rather important for the

mGlu1 receptor-stimulated MAPK activation. The expression of β-arrestin dominant negative mutant inhibits ERK1/2 phosphorylation, suggesting that endogenous β-arrestin mediates the activation of MAPK [8]. More recently the involvement of β-arrestin in the ubiquitination and degradation of the β_2-AR has been shown. Receptor ubiquitination is not crucial for its internalization, but is essential for proper trafficking to lysosomes for degradation, whereas the ubiquitination of β-arrestin is essential for rapid receptor internalization [5].

To study the possible involvement of β-arrestin in inclusion formation, we treated PC12 cells with MA for different times and analyzed different proteins involved in signaling and UPP by immunological techniques. Initial experiments indicated that β-arrestin is ubiquitinated in the MA-treated PC12 cell line. We observed that exposure to the PC12 cell line MA for 5 min induced the ubiquitination of β-arrestin, which appeared as a band of higher molecular size after immunoprecipitation and immunoblot. Ubiquitination of β-arrestin was rapid and transient. In this case β-arrestin can act as an adapter protein to bring an E3 ligase to the activated receptor (presumably the DA receptor).

The involvement of β-arrestin in UPP was further supported by our experiments with light transmission electron microscopy and confocal microscopy. The analysis of PC12 cells after MA treatment and of striatal neurons from MA-treated mice by transmission electron microscopy revealed the presence of β-arrestin in these Lewy body-like inclusions. Confocal microscopy confirmed the formation of inclusions positively stained for β-arrestin in MA-treated PC12 cells. In untreated cells, the distribution of β-arrestin is diffuse in the cytosol, while after MA treatment we observed the redistribution of β-arrestin in membranous whorls.

Conclusions

Our experiments reveal an interesting and previously unappreciated connection between β-arrestin and ubiquitination. They document that β-arrestin is localized in inclusion bodies associated with neurodegenerative diseases and could likely be involved in the development of these pathological correlates. Further studies are needed to clarify the mechanisms that drive the localization of β-arrestin in these inclusions and whether the DA receptors play a role in controling these processes. β-Arrestin could represent a potential molecular target for controling the formation of inclusion bodies in experimental models of PD.

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Neurol Sci (2003) 24:166–167
DOI 10.1007/s10072-003-0112-4

Dementia, ataxia, extrapyramidal features, and epilepsy: phenotype spectrum in two Italian families with spinocerebellar ataxia type 17

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Abstract We observed two families with a dominantly inherited complex neurological syndrome with onset in adulthood. Family F included 9 affected in four generations. One patient showed prominent anticipation of onset age. Onset was with cerebellar signs followed by dementia, psychiatric symptoms, seizures, and extrapyramidal features. Family M included 14 affected individuals in five generations. Presenting symptoms were either psychiatric and cognitive impairment or a cerebellar syndrome. Extrapyramidal features, dysphagia, incontinence, seizures, and myoclonus may occur. In both families magnetic resonance imaging showed marked atrophy of the brain and cerebellum. Molecular analyses demonstrated an expanded CAG/CAA repeat in the in the TATA box-binding protein (TBP) gene (SCA17).

There is a wide genetic heterogeneity within autosomal dominant spinocerebellar ataxias (SCAs), and 19 different loci have been identified. An expanded trinucleotide cytosine-adenine-guanine (CAG) repeat has been shown in most of the cloned genes [1]. An expanded CAG repeat has been described in the TATA box-binding protein (TBP) gene [2], a general transcription initiation factor, in four Japanese pedigrees (*SCA17*). We describe the phenotype of two Italian families with this mutation.

The first kindred (Family F) originated from Campania and included 9 patients in four generations. The clinical features of the four personally examined patients are shown in Table 1. The patients in III generations had disease onset in the 3rd to 4th decade, whereas in the only patient in the IV generation the disease began at 3 years. The mean age at death was 54 years in 7 deceased patients. Cerebellar signs, such as gait ataxia, dysarthria, and dysmetria, were the first symptoms in all patients. Dementia was early and severe; psychiatric symptoms, such as mood depression, insomnia, and delusions, were frequent. Increased tendon reflexes, abnormal involuntary movements, seizures, and hypoacusia may occur. Incontinence and dysphagia were late features. Magnetic resonance imaging (MRI) showed atrophy of the cerebrum and cerebellum in all patients. Peripheral nerve conduction studies demonstrated a mild sensorimotor axonal neuropathy.

The clinical picture of patient 4 was peculiar, with ataxia and dysarthria presenting at the age of 3 years, fast progression with loss of independent gait and sphincter control, dysphagia, spasticity, growth delay, grand mal seizures, and death at the age of 15 years.

The second pedigree (Family M) originated from Calabria and included 14 patients in five generations. The clinical features of the 7 personally examined patients are shown in Table 1. The age at onset was usually in the 3rd-4th decade. No differences in age at onset were found between generations. The mean age at death was 56 years in 7 deceased patients. Psychiatric features, such as depression, personality changes, aggressiveness, negligence of personal hygiene, delusional thoughts, hallucinations, and alcoholism, were present in the majority of patients at onset. Cognitive impairment was an early feature. Ataxia, dysarthria, rigidity, and dystonia developed successively. Perioral dyskinesia, brisk tendon reflexes, and seizures may occur and late stages were characterized by anarthria, dysphagia, and incontinence. In some cases the disease began with a cerebellar syndrome followed by dementia and other neurological features. MRI showed marked atrophy of the brain and cerebellum.

Genomic DNA was prepared from peripheral blood according to standard procedures after informed consent. The presence of a CAG repeat in *HD*, *SCA1*, *SCA2*, *SCA3*, *SCA6*, *SCA7*, *SCA12*, and *DRPLA* genes was excluded by polymerase chain reaction (PCR), as previously described [3]. In addition, we excluded linkage to other autosomal dominant spinocerebellar ataxias (SCA4, SCA5, SCA6, SCA11, SCA13, and SCA14 loci), familial Alzheimer disease (APP, PS-1, PS-2, FTDP-17, BRI, PI12, FND loci), and parkinsonism (PARK1, PARK2, PARK3 loci).

Western blot analyses, with either anti-polyglutamine 1C2 and 1F8 monoclonal antibodies (mAbs), showed in all the analysed patients from both families, beside a normal band corresponding to wild type TBP, a band corresponding to the expanded protein. These findings were confirmed using anti-TBP mAb 3G3.

The fragment of the *TBP* gene containing the CAG/CAA repeat was amplified by PCR and separated by electrophoresis on a 3% agarose gel, as previously described [2]. PCR analysis of the CAG repeat region within the *TBP* gene showed an expanded band in all patients.

Neuropathological examination of 1 patient from the family M showed cortical, subcortical, and cerebellar atrophy, Purkinje cell loss and gliosis, degeneration of the inferior olive, marked neuronal loss, and gliosis in the caudate nucleus and in medial thalamic nuclei. Neuronal intranuclear

Table 1 Clinical features of the patients

Family	F				M						
Patient	3	2	1	4	5	30	22	18	17	7	10
Sex	F	M	M	F	M	F	M	F	F	F	F
Age at onset (years)	35	34	23	3	20	35	22	53	33	26	33
Age at examination (years)	44	44	32	14	60	44	51	63	54	40	42
Age at death (years)	–	44	–	15	62	–	52	–	–	–	49
First symptom	A	A	A	A	P	P	P	D	P	P	A
Ataxia	+	+	+	+	+	+	+	+	+	+	+
Dysarthria	+	+	+	+	+	+	+	+	+	+	+
Dementia/psychiatric symptoms	+	+	+	+	+	+	+	+	+	+	+
Dystonia/rigidity	+	+	+	+	+	+	+	+	+	+	+
Urinary incontinence	+	+	–	+	+	–	+	+	+	+	+
Epilepsy	+	–	+	+	+	+	–	+	+	+	+
Slowing on EEG	–	–	NP	+	+	–	–	+	NP	+	+
Atrophy on MRI	+	+	+	+	NP	+	+	NP	+	+	+

A, ataxia; *P*, psychiatric symptoms; *D*, dementia; *NP*, not performed; *MRI*, magnetic resonance imaging; *EEG*, electroencephalogram

inclusions staining with anti-TBP and anti-polyglutamine IC2 mAbs were found.

SCA17 appears to be a rare disease since, after the original description [2], two families have been found in a survey of 604 German cases of familial or sporadic ataxia [4], 1 among 162 European families with dominant ataxia [5], and 1 further patient among 202 Portuguese and Brazilian families with ataxia [6].

Both our families showed an expanded CAG repeat within the TBP gene (*SCA17*). Disease onset was in the 3rd to 4th decade in most cases, but in family F we found a case with a young-onset severe phenotype, with marked anticipation of onset age, not yet described in SCA17. The phenotype of family F is characterized by progressive cerebellar ataxia, dementia, psychiatric and extrapyramidal features, epilepsy, mild axonal neuropathy, and cerebral and cerebellar atrophy. This clinical picture is quite similar to that already described in the other reported families [2, 4, 5, 6]. In family M, behavioral symptoms and frontal impairment dominated the early stages of the disease, preceding ataxia, rigidity, and dystonic movements (Table 1). Since ataxia is not the presenting symptom in most patients, the diagnosis of SCA was difficult. The characteristics of this family broaden the clinical picture of SCA17.

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Neurol Sci (2003) 24:168–169
DOI 10.1007/s10072-003-0113-3

Frequency of familial aggregation in primary adult-onset cranial cervical dystonia

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Abstract Family history of definite/probable dystonia was studied in 36 probands with primary adult-onset cranial/cervical dystonia. Of the 157 relatives who were examined, 8 from 7 families and 11 from 10 families were diagnosed as having definite or probable dystonia, respectively. The frequency of familial occurrence of definite dystonia was 19.4%, 33% when considering both definite and probable dystonia. There was a tendency for relatives affected by either definite or probable dystonia to have the same type of dystonia as the index patient. Similar segregation ratios were found for parents, siblings, and children with either definite or probable dystonia. These observations raise the possibility that probable dystonia represents formes frustes/mild phenotypes of dystonia rather than another movement disorder.

Primary adult-onset dystonia is a clinically heterogeneous movement disorder in which dystonia is the primary and sole abnormality directly attributable to the condition [1]. Although many cases are apparently sporadic, the condition is known to aggregate in families [2–4]. The proportion of patients with a positive family history varies in different studies from 9% to 27% [2–6], depending on whether familial occurrence was assessed by history or examination of at-risk relatives. Even the latter method, however, is open to bias, because diagnosis of dystonia is based solely on clinical grounds and may often be difficult [7]. It is unclear whether subtle motor findings, such as increased blinking, unusually tight hand gripping with writing, or other activities, and isolated focal postural/action tremor represent formes frustes/mild phenotypes of dystonia, or other movement disorders. To accommodate the level of diagnostic uncertainty, the categories of definite and probable dystonia have been introduced, but the latter category has never been validated. In this study we evaluated the frequency of family history of definite/probable dystonia in an Italian series of probands with primary adult-onset cranial/cervical dystonia.

The study was approved by the ethics committee. Inclusion criteria were a diagnosis of cranial, cervical, or limb dystonia (alone or in combination) according to published criteria [1]; age at dystonia onset (defined as time of first symptoms) >20 years; and duration of disease >1 year. Exclusion criteria were clinical and laboratory findings (including serum ceruloplasmin estimation and imaging studies) suggesting secondary causes of dystonia [1]. No patient was specifically referred for familial dystonia.

For the purposes of the family study, all available first-degree relatives giving informed consent were examined in their homes by a neurologist trained in movement disorders. Family members underwent complete neurological examination, including triggering maneuvers for dystonic movements or postures in asymptomatic subjects. In particular, subjects were asked to repeatedly open/close their eyes or their mouth, look upward/downward, speak, walk, read, and perform manual tasks, including spontaneous and dictated writing. In accordance with published criteria [1], definite dystonia was diagnosed when slow dystonic movements and definitely abnormal postures occurred at rest or were activated by specific tasks. Definite blepharospasm, (BSP) was defined as tonic or clonic episodes of involuntary eyelid closure, associated with signs of orbicularis oculi muscle contraction, such as lowering of the brows beneath the superior orbital margin (Charcot's sign); definite oromandibular dystonia (OMD) was diagnosed when spasms of the tongue, mandibular, and floor of the mouth muscles were associated with involuntary jaw deviation, closing or opening; definite cervical dystonia (CD) was diagnosed when slow dystonic movements or abnormal neck postures were associated with head jerks. A diagnosis of definite limb dystonia was established when slow dystonic movements and definitely abnormal postures occurred at rest or were activated by specific tasks. Increased blinking with no evidence of Charcot's sign, unusually tight hand gripping with writing, and isolated focal postural action tremor were not used as a diagnostic criterion because their diagnostic value remains unclear. These cases were assigned a diagnosis of probable dystonia. Coexistence of postural tremor and dystonia in the same body region did not exclude the diagnosis of definite dystonia [1]. Diagnoses made on site were confirmed through direct or videotape examination by a senior neurologist expert in dystonia, blinded to the previously assigned diagnoses. Segregation analysis was performed by Weinberg's proband method, assuming multiple incomplete ascertainment.

During a 6-month-period, 36 probands with primary adult-onset cranial-cervical dystonia (20 with focal BSP, 10 with focal CD, and 6 with segmental cranial/cervical dystonia) attended the movement disorders clinic of our department. There were 26 women and 10 men aged 57.2±17.4 years (mean±SD); their duration of dystonia (mean years±SD) and education level (mean years of schooling±SD) were 7.8±10.3 years and 5.8±3.8 years, respectively. The 36 probands produced a potential population of 263 first-degree relatives, of whom 196 were alive and 157 were examined. In particular, 18 of 72 parents, 82 of 123 siblings, and 57 of 68 children were examined. Eight relatives from 7 families and 11 from 10 families were diagnosed as having definite or probable dystonia, respectively. The frequency of familial occurrence of definite dystonia was 19.4% (20% in the focal BSP group, 17% in the segmental group, and 20% in the focal CD group). Considering both definite and probable

Table 1 Demographic and clinical features of index patients and affected relatives

Index patients			Affected relatives (relation, age, type of dystonia)	
Patient no.	Sex, age	Type of dystonia	Definite dystonia	Probable dystonia
1	F, 41	CD	Mother, 73, CD	
2	F, 63	BSP	Daughter, 42, BSP	
			Daughter, 38, BSP	
3	M, 69	BSP+CD	Son, 40, BSP	Brother, 77, head tremor
4	M, 75	BSP	Brother, 86, BSP	Daughter, 39, increased blinking
5	F, 50	CD	Daughter, 22, CD	Mother, 78, head tremor
				Brother, 58, abnormal upper limb posture (not fixed)
6	F, 53	BSP	Brother, 49, ULD	Brother, 55, increased blinking
7	F, 75	BSP		Sister, 71, shoulder elevation
8	F, 81	CD		Sister, 88, head tremor
9	M, 67	BSP		Daughter, 37, increased blinking
10	M, 71	BSP	Daughter, 43, OMD+CD	Daughter, 40, increased blinkig
11	F, 51	CD		Brother, 71, increased blinking
12	F, 78	BSP		Brother, 61, abnormal neck posture (not fixed)

BSP, blepharospasm; *OMD*, oromandibular dystonia; *CD*, cervical dystonia; *ULD*, upper limb dystonia

dystonia, the frequency of familial occurrence of dystonia was 33% (19 affected relatives from 12 families).

Demographic and clinical features of index patients and affected relatives are shown in Table 1. Among affected relatives, 6 of 8 with definite dystonia (75%) and 7 of 11 with probable dystonia (63%) had the same type of dystonia as index patients ($p>0.05$). Segregation ratios (based on examined first-degree relatives) were 0.25 (parents), 0.10 (siblings), and 0.35 (children) for definite dystonia; 0.25 (parents), 0.23 (siblings), and 0.15 (children) for probable dystonia. There was no significant difference among the proportion of affected parents, siblings, and children, within either definite or probable dystonia. Likewise, no significant difference was found between definite and probable dystonia with regard to the proportion of affected parents ($p=0.4$), siblings ($p=0.4$), and children ($p=0.3$).

Discussion

Our sample of probands with primary adult-onset cranial/cervical dystonia had demographic and clinical features similar to those of previous cranial-cervical dystonia series [2–6]. The familial occurrence of definite dystonia in first-degree relatives was about 20%, lower than that reported in previous studies assessing familial occurrence of dystonia by clinical examination [2–4]. However, our estimate did not take into account deceased relatives (cranial dystonia tends to develop in elderly subjects, and most can be deceased), and was based only on the category of definite dystonia. Considering both definite and probable dystonia, the frequency of familial occurrence of dystonia in first-degree relatives was 33%, close to those previously reported [2–4]. Due to the lack of mapped gene(s) associated with late-onset dystonia, validation of the category of probable dystonia is not possible. However, we observed a tendency for relatives affected by probable dystonia to have the same type of dystonia as the index patients. In addition, the similar segregation ratios found here for parents, siblings, and children with probable dystonia are consistent with an autosomal dominant transmission and reduced penetrance or polygenic inheritance. The same observations were made for definite dystonia [2–4]. This raises the possibility that probable dystonia represents formes frustes or mild phenotypes of dystonia rather than another movement disorder.

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Neurol Sci (2003) 24:170–171
DOI 10.1007/s10072-003-0114-2

Bedtime cabergoline in Parkinson's disease patients with excessive daytime sleepiness induced by dopamine agonists

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Abstract Excessive daytime somnolence is a common adverse effect of dopamine-agonist treatment of Parkinson's disease (PD). Many factors, such as age and sleep disturbances, could be involved in the pathogenesis of this phenomenon. However, pharmacokinetic factors have never been considered. In this open, prospective, pilot study, nine consecutive non-demented PD patients in early disease stages on monotherapy treatment with dopamine agonists and with no significant sleep problems, were enrolled. They were selected based on the presence of excessive daytime sleepiness induced by the dopaminergic treatment. A fast switch-over from the dopamine agonist currently used to a single equivalent dose of cabergoline, a long-acting dopamine agonist, administered at bedtime was performed. All patients were evaluated by means of UPDRS and Epworth Sleepiness Scale (ESS). A significant 70% reduction of daytime sleepiness was observed during the 3-month study compared with baseline. Data from this study suggest that both pharmacodynamic and pharmacokinetic mechanisms are involved in the pathophysiology of dopamine agonist-induced sleepiness.

Introduction

Sleep disturbances, such as insomnia, parasomnias, and excessive daytime sleepiness, with or without sudden irresistible sleep attacks, are common in Parkinson's disease (PD), occurring in up to 98% of all parkinsonian patients and reducing the quality of life. [1] In a recent prospective survey of 683 consecutive PD patients without dementia conducted in Canada, excessive daytime sleepiness, an inappropriate and undesirable sleepiness during waking hours, was present in 51% of patients [2]. Most of them were under treatment with L-dopa, dopamine agonist monotherapy, or both. Excessive daytime sleepiness may be evident in PD prior to medication; however, it increases with treatment duration [3]. Sleep fragmentation and difficulty in maintaining sleep are the major risk factors for the appearance of diurnal somnolence in parkinsonian patients. However, daytime somnolence is also observed in PD patients with a normal night-time sleep pattern, suggesting that an impaired arousal system might be the cause of this phenomenon [4].

Excessive daytime sleepiness may occur at any stage of PD, but it gradually worsens as disease progresses, suggesting that impaired alertness may be directly related to the duration or severity of the disease. Moreover, increased daytime somnolence has long been recognized as a side effect of dopaminergic drugs, particularly dopamine agonists and levodopa. It can appear during dopaminergic treatment in patients without prior sleep disorders and/or excessive daytime sleepiness. All dopamine agonists can induce this adverse effect, suggesting that it might be pharmacodynamic class-effect. The involvement of pharmacokinetic factors has never been considered. It is a common clinical observation that many parkinsonian patients, treated with dopamine agonists, complain of episodes of excessive daytime sleepiness soon after taking a single dose, suggesting a probable pharmacokinetic factor related to the peak plasma levels of the dopamine agonist.

The aim of this study was to verify whether the substitution of short half-life dopamine agonists, such as pramipexole, ropinirole, and pergolide, with cabergoline, a dopamine agonist with a very long plasma half-life, given at bedtime, could improve the excessive daytime sleepiness induced by these drugs.

Patients and methods

Nine consecutive PD patients (6 males and 3 females) in the early stages of the disease were enrolled in this open-label, pilot study. The clinical characteristics of the study population were: age (mean±SD) 62.5±5.4 years, Hoehn-Yahr stage 1.8±0.5, and disease duration 2.2±1.3 years. Patients were selected based on the following inclusion criteria: (1) early disease stage (Hoehn-Yahr ≤3), (2) treatment with dopamine agonist monotherapy, (3) daytime sleepiness as evidenced by the Epworth Sleepiness Scale (ESS) ≥10. Exclusion criteria were: (1) presence of daytime sleepiness prior to dopamine agonist treatment, (2) insomnia or other sleep disturbances at baseline, (3) cognitive impairment (Mini Mental State Examination <24), (4) depression, (5) concomitant diseases associated with sleep disturbances and/or daytime sleepiness, (6) intake of drugs able to interfere with sleep-wake cycle. Four patients were taking ropinirole, three patients were on pramipexole, and the remaining two were taking pergolide.

Patients were evaluated at baseline by means of the ESS and the Unified Parkinson's Disease Rating Scale (UPDRS-motor examination), and then they were switched to an equivalent dose of cabergoline given once a day at bedtime. The cabergoline dose equivalent was as follows: 1 mg equivalent to 0.7 mg of pergolide, 4 mg of ropinirole, and 1 mg of pramipexole [5]. Subsequently, patients were evaluated monthly for 3 months, using the ESS and UPDRS-motor score. If necessary, the daily dose of cabergoline was adjusted by the end of the 2nd month of treatment, and maintained unchanged until the end of the study.

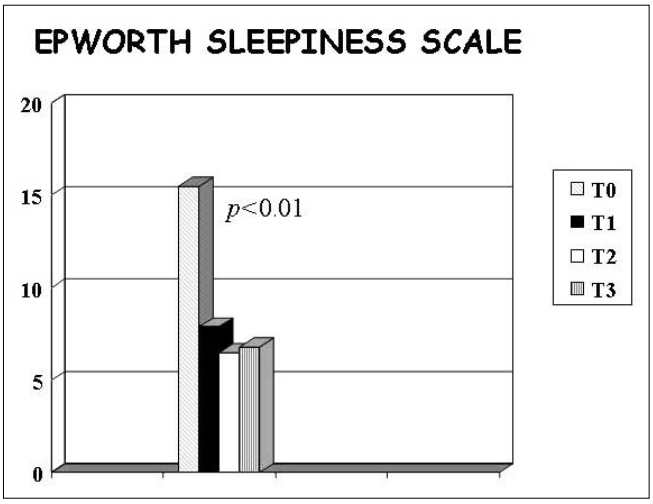

Fig. 1 Epworth Sleepiness Scale scores at baseline (T0) and at 1 month (T1), 2 months (T2), and 3 months (T3) after cabergoline substitution in nine parkinsonian patients

Results

All patients but one reported a subjective improvement of diurnal somnolence. At the end of the study the ESS scores were 70% lower (15.5±4.7 at baseline vs. 6.8±2.1 at final visit, $p<0.01$). This significant improvement was already evident after the 1st month of cabergoline treatment and was maintained almost unchanged throughout the 3-month study. The motor performance, evaluated via the UPDRS-motor score, did not change during the study. In four patients the initial cabergoline dose was slightly increased during the 1st month of the study to achieve the best control of motor symptoms. The final cabergoline dose (mean±SD) was 4.7±1.8 mg/day. No significant side effects were observed changing from the previous dopamine agonist to cabergoline.

Discussion

Data from this study suggest that daytime sleepiness induced by treatment with dopamine agonists with a short half-life can be partly reversed by substitution with cabergoline given at bedtime. It is well known that disease-related factors, such as duration and severity of PD, advanced age, cognitive impairment, depression, and duration of levodopa therapy [6], together with the presence of nocturnal sleep disturbances, are relevant to the appearance of diurnal hypersomnolence in parkinsonian patients. None of these factors was present in our patients.

Pharmacodynamic factors associated with the dopaminergic treatment are also crucial for inducing excessive daytime sleepiness. In controlled clinical trials with ropinirole, pramipexole, pergolide, and cabergoline, somnolence occurred as an adverse event in a higher proportion of PD patients compared with placebo and levodopa [6]. This observation favors the view that daytime sleepiness occurring during dopamine agonist therapy is a pharmacodynamic class effect. However, the improvement of diurnal hypersomnolence seen in our study when ropinirole, pramipexole, and pergolide were substituted with cabergoline at bedtime can not be explained by a pharmacodynamic mechanism.

The main difference among these drugs lies on their pharmacokinetic features. Cabergoline has a very long half-life (65 h), while ropinirole, pramipexole, and pergolide have shorter half-lives [7]. We hypothesize that the risk for sleepiness is higher when the peak plasma levels of these drugs are reached, a problem that can be easily avoided by using an agonist with a long half-life, like cabergoline, that can be given once a day in the evening. Pharmacokinetic factors can also play a role in the occurrence of sleep attacks induced by dopamine agonists, a phenomenon sometimes associated with excessive daytime sleepiness. It has been reported that some PD patients were aware of a sudden onset of sleep at the time they took their medication [2]. Further studies with larger numbers of patients are necessary to better understand the role of pharmacokinetic factors in the pathophysiology of dopamine agonist-induced daytime sleepiness.

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Neurol Sci (2003) 24:172–173
DOI 10.1007/s10072-003-0115-1

Decreased plasma and cerebrospinal fluid content of neuroactive steroids in Parkinson's disease

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Abstract The levels of the neuroactive steroids allopregnanolone (THP) and 5α-dihydroprogesterone (DHP) were quantified in the plasma of 11 (group 1) and in the liquor of 12 (group 2) Parkinson's disease (PD) patients using a gas-chromatographic/mass-spectrometric method. When compared with controls, both groups showed a significant decrease in DHP and THP concentrations. These decreases could be a useful marker of PD. Moreover, in view of the importance of GABA-ergic transmission to substantia nigra (SN) neurons and GABA-ergic modulation exerted by the two neuroactive steroids, our data indicate a global dysregulation of the SN GABA-ergic system in PD patients. Moreover, a lack of neuroprotective factors (i.e., GDNF, BDNF), promoted by DHP, may contribute to dopaminergic cell death.

This is a pilot study aimed at exploring possible neuroactive steroid alterations in Parkinson's disease (PD). Neuroactive steroids are a subclass of steroids that can be synthesized in the central nervous system independent of peripheral sources [1]. The neurosteroid allopregnanolone (THP) and 5a-dihydroprogesterone (DHP) potently modulate neuronal activity by allosterically regulating GABA action at $GABA_A$ receptors or by changing specific $GABA_A$ receptor subunit gene expression, respectively [2]. Moreover, DHP promote gene expression of neurotrophic factors (i.e., BDNF and GDNF) [3].

The substantia nigra (SN) shows the highest levels of THP and its precursor DHP in human postmortem brain [4], suggesting a fundamental role for neurosteroids in this brain area. Moreover, a few groups have reported some effects of steroids on the dopaminergic system [5]. These observations prompted us to consider an involvement of neuroactive steroids in the pathophysiology of PD. The aim of this study was to measure in plasma and cerebrospinal fluid (CSF) of PD patients the levels of THP, DHP, and their precursor progesterone.

With this purpose, we collected plasma from 11 (group 1) and CSF from 12 (group 2) male PD patients consecutively referred to the Movement Disorder Unit of the Neurology Department of Tor Vergata University. They were considered PD patients according to the Brain Bank criteria [6]. Their age, disease duration, and clinical score according to the Unified Parkinson's Disease Rating Scale (UPDRS) are reported in Table 1. Group 1 was under dopaminergic treatment, whereas Group 2 underwent a 2-week drug withdrawal. Twelve age-matched healthy subjects were controls for group 1, while CSF was collected from 13 age-matched healthy subjects as controls for group 2.

We quantified the levels of progesterone, DHP, and THP in both groups using a gas-chromatographic/mass-spectro-

Table 1 Neuroactive steroid levels (mean±SD) in plasma and cerebrospinal fluid (CSF) of Parkinson's disease (PD) patients and controls

	Plasma		CSF	
	Controls (n=12)	PD (n=11)	Controls (n=13)	PD (n=12)
Progesterone	4.30±0.95	3.62±0.58 (NS)	3.22±1.12	3.09±0.66 (NS)
THP	2.63±1.19	0.85±0.81*	2.67±1.14	0.96±0.39**
DHP	3.90±1.62	1.69±0.92*	3.05±1.05	0.95±0.77**
	Main factor group: *df* 1/21; F=30.89; p<0.001		Main factor group: *df* 1/23; F=23.16; p<0.001	
	Main factor steroids: *df* 2/42 (ε=0.99); F=26.28; p<0.001		Main factor steroids: *df* 2/46 (ε=0.88); F=23.73; p<0.001	
	Interaction *df* 2/42 (ε=0.99); F=3.33; p<0.05 *p<0.05; **p<0.01 post hoc Tukey test		Interaction *df* 2/46 (ε=0.88); F=12.32; p<0.001	
Age (years)	62.35±5.67	61.43±6.32	63.22±6.02	65.00±5.25
Disease duration (years)	–	5.35±3.45	–	5.54±4.21
UPDRS score	–	38.12±19.87	–	39.01±20.11
L-Dopa equivalent	–	535±352	–	Wash-out

THP, allopregnanolone; *DHP*, dihydroprogesterone

metric (GC-MS) method, which identifies and quantifies with accuracy very small amounts (fmol) of neurosteroids [7]. Briefly, approximately 5,000 dpm of [^{3}H]-progesterone was added to the plasma and liquor to monitor recovery. After extraction with 3 ml of ethyl acetate (performed twice), and separation by thin-layer chromatography [i.e., carbon tetrachloride/methanol {99:1, v/v), cyclohexane/ethyl acetate (3:2, v/v)], 7 pmol of progesterone was added as internal standard to the eluate containing DHP or THP, and 10 pmol of 3a, 5a-THP was added to the eluate containing progesterone as internal standard. These eluates containing THP and progesterone were lyophilized and derivatized with heptafluorobutyric acid anhydride (HFBAA). While the eluate containing DHP was lyophilized and derivatized with methoxyamine hydrochloride. Derivatized steroids were analyzed using a Finnigam Trace GC/MS equipped with capillary column (HP-35MS, length 30 m, internal diameter 0.25 mm, film thickness 0.25 mm), in the positive ion chemical ionization mode and ions at 496 and 510 m/z were selectively monitored. The recovery of tritiated steroids ranged from 80% to 90%. The detection limit was approximately 10 fmol.

Neurosteroid concentrations in the plasma and CSF of the two groups were separately analyzed with a two-way ANOVA utilizing factor "between" (PD group versus control group) and factor within: "steroids levels" (progesterone, DHP, and THP levels). Greenhouse-Geisser correction was used when more than two levels were present. Post hoc Tukey test was used to assess single differences.

A significant decrease of DHP and THP levels of the PD patients compared with controls was found, as shown in Table 1. In contrast, no significant changes of progesterone were found.

Discussion

While the role of neuroactive steroids, mainly DHP and THP, has been extensively studied in neuropsychiatric disorders (i.e., depression, stress anxiety) [2], their role in brain degeneration is still unexplored. This is the first report giving evidence for a potential role of neuroactive steroids in the pathophysiology of a degenerative disease such as PD. We found a remarkable decrease in DHP and THP, but not in their precursor progesterone, in the periphery (group 1) and more importantly in the central nervous system (group 2). The more-cautious explanation of these findings could relate the decrease of THP and DHP in the CSF to the degenerative process involving the nigral area. Thus the THP and DHP decrease may represent a biochemical marker of dopaminergic cell loss. However, it is relevant from the clinical point of view that the same decrease may be observed in the plasma. The lack of a concomitant decrease of the precursor progesterone suggests a reduced activity of the converting enzyme system both in the periphery and in the central nervous system, as observed in genetic dysregulation of enzyme systems.

A tentative explanation, relating neurosteroid decrease and dopamine loss with a causal link, should take into account neurosteroid actions in the central nervous system. THP is the most-potent positive modulator at the $GABA_A$ receptors, acting in a benzodiazepine-like manner; moreover it regulates the gene expression of $GABA_A$ receptors subunit through its immediate precursor DHP, which also promotes gene expression of neurotrophic factors. Therefore, the described decrease of these neuroactive steroids may produce dysregulation of the GABA-ergic transmission involved in the striato-nigral feedback loop, regulating dopamine release [5, 6]. A similar dysregulation might in turn produce an overactivity induced oxidative stress of dopaminergic elements. In addition, a reduction of neurotrophic factors, like GDNF or BDNF, may potentiate oxidative stress damage.

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Neurol Sci (2003) 24:174–175
DOI 10.1007/s10072-003-0116-0

Continuous apomorphine infusion and neuropsychiatric disorders: a controlled study in patients with advanced Parkinson's disease

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Abstract The aim of this study was to asses whether patients with Parkinson's disease (PD) develop cognitive and psychiatric complications more frequently during prolonged therapy with continuous apomorphine infusion compared with standard oral treatment. Thirty consecutive PD patients with severe motor fluctuations were included in the study. Twelve patients accepted the treatment with subcutaneous continuous apomorphine infusion, while the remaining 18 preferred to continue with oral dopaminergic therapy. The two groups were evaluated with neuropsychological, psychiatric, and motor tests at baseline and after 1 year. The off daily duration and the levodopa dosage were significantly reduced in infused patients. The neuropsychiatric assessment did not change in both groups compared with baseline, except for a significant improvement of mood in the apomorphine group.

Introduction

Although L-dopa remains the gold standard for treatment of Parkinson's disease (PD), the development of severe long-term motor complications represents one of the most-challenging problems facing the practicing neurologist [1]. Continuous subcutaneous apomorphine infusion can significantly improve motor fluctuations in patients with advanced PD [2, 3]. However, few studies have investigated the occurrence of cognitive and psychiatric disorders in parkinsonian patients undergoing continuous apomorphine infusion. An uncontrolled study reported the development of cognitive and psychotic complications during follow-up [4]. The objective of this study was to assess whether patients with PD develop cognitive and psychiatric disorders during continuous apomorphine infusion more frequently than patients on standard oral treatment.

Materials and methods

Consecutive patients attending the Parkinson Unit at the Department of Neuroscience of University of Messina, with PD defined by Gelb and Gillmann criteria [5] and a history of severe motor complications, were eligible for the study. Criteria for exclusion were a history of allergy to morphine and its derivatives, the presence of dopaminergic psychosis, the presence of orthostatic hypotension, the presence of pulmonary, liver, and cardiovascular diseases, a score lower than 24 on the Mini Mental State Examination (MMSE) and an age greater than 65 years. Thirty patients were included in the study, which was an open-label, blinded-rater, parallel-group trial. Twelve patients previously treated with an oral L-dopa preparation and other antiparkinsonian drugs accepted the subcutaneous continuous apomorphine infusion, while the remaining 18 preferred to continue standard oral dopaminergic therapy. Both groups were similar for demographic and clinical features (Table 1) and were evaluated at baseline and after 12 months using AIMS and daily on-off diaries during waking for at least 1 week before starting treatment and at the last control. At the same time neuropsychiatric evaluation was performed using MMSE, Rey-copy, Corsi, BPRS, and Beck Depression Inventory (BDI). Patients were weaned off dopaminergic treatment the evening before admission for initiation of apomorphine. They were pre-

Table 1 Demographic, clinical features, Brief Psychiatric Rating Scale (BPRS), Beck Depression Inventory (BDI), Abnormal Involuntary Movement Scale (AIMS), and off awake daily duration scores of the two groups. Values are means (SD)

	Apomorphine (n=12)	L-Dopa (n=18)
Age (years)	55 (9)	56 (8)
Illness duration (months)	122 (37)	111 (30)
Hohen and Yahr stage	3.8 (0.5)	3.7 (0.6)
L-Dopa dosage (mg/day)		
Baseline	775 (248, 64)	750 (239, 46)
Endpoint	350 (125, 83)**	850 (283, 25)**
Apomorphine dosage (mg/day)	100	
BPRS total		
Baseline	27 (7.6)	26 (7.4)
Endpoint	25 (7.8)	25 (7.4)
BDI		
Baseline	21 (6.2)	19 (2.8)
Endpoint	10 (2.6)*	20 (2.8)
AIMS		
Baseline	7.7 (1.2)	7.9 (1.3)
Endpoint	4 (0.6)**	8 (1.3)
Off awake daily duration (h)		
Baseline	5 (1.527)	6 (1.70)
Endpoint	2 (0.4)**	6.5 (1.51)

Statistically significant differences between end point and baseline
*p<0.001, **p<0.01

treated with the anti-emetic domperidone (60 mg daily) for 3 days before to perform an acute test with an apomorphine bolus. Blood pressure and electrocardiography were monitored in the first 10 min following the first subcutaneous bolus injection (2 mg). The infusion was started with a dose of 2 mg/h. A further increase of apomorphine dose and reintroduction of a lower dose of L-dopa was required in the next few days to obtain optimum benefit. All patients were finally treated with 100 mg apomorphine infusion daily, with a variable dosage of 6–8 mg/h. Patients who refused the infusion maintained their previous treatment with oral dopaminergic drugs and the dosage was changed in relation to the clinical condition, if necessary. The within and between-group differences were analyzed using an analysis of covariance (ANCOVA).

Results

Results are summarized in Table 1. Compared with pre-infusion L-dopa intake, L-dopa dosage was significantly reduced (by 55%) ($p<0.001$) in infused patients and unchanged in the control group. The off-awake duration was significantly reduced by 40% ($p<0.001$) and the AIMS improved significantly by 37% ($p<0.001$) compared with baseline in the infused group, but did not change in the control group. The neuropsychiatric assessment did not change significantly in both groups in comparison with baseline, except for a significant improvement ($p<0.01$) of BDI in the apomorphine group. Only 1 patient experienced nausea in the first 5 days of treatment in the apomorphine group. This adverse effect disappeared by increasing the dose of domperidone. Five infused patients developed small, itchy nodules at the injection sites. Dilution of apomorphine to 5 mg/ml and application of steroid ointment minimized this complication.

Conclusions

The development of severe incapacitating motor fluctuations and dyskinesias as a result of chronic L-dopa treatment represents one of the most-challenging problems in the treatment of advanced PD. Over the last few years several new pharmacological strategies, including high doses of novel dopamine agonists as well as COMT inhibitors, have been proposed to treat these complications, with poor efficacy.

Continuous subcutaneous apomorphine infusion is a highly effective treatment and allows a marked reduction of levodopa dosage, daily off time, and dyskinesias in patients with advanced PD. These findings are similar to those reported for deep brain stimulation (DBS) [6]. Our results show that in the short time of 1 year this treatment does not modify cognitive status significantly. Previous reports indicated that apomorphine treatment may induce a hypomanic euphoric state [7]. In our study mood was significantly improved, whereas it is known that DBS induces depression [8]. These results confirm that apomorphine is very efficacious in the management of motor complications in PD and does not induce cognitive impairment. Our results are only preliminary and a longer follow-up is necessary to confirm these findings.

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Neurol Sci (2003) 24:176–177
DOI 10.1007/s10072-003-0117-z

Natural history and clinical features of progressive supranuclear palsy: a clinical study

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Abstract Progressive supranuclear palsy (PSP or Steele-Richardson-Olszewski syndrome) is one of the most-common types of atypical parkinsonism. To characterize the natural history and the clinical features of PSP, we reviewed the records of 25 patients followed in our clinic since 1991, with a clinical diagnosis of PSP according to NINDS and Golbe criteria. Progressive onset of early bilateral bradykinesia and postural instability with falls during the 5th decade strongly support the diagnosis of PSP in our patients. Pseudobulbar symptoms are very common at onset and during the course of the illness.

Progressive supranuclear palsy (PSP or Steele-Richardson-Olszewski syndrome) is one of the most-common types of atypical parkinsonism. PSP is characterized by clinical heterogeneity, therefore this syndrome can be difficult to diagnose. The estimated prevalence is 1.3–4.9 per 100 000 inhabitants; the incidence is 0.3–1.1 per 100 000 inhabitants/year.

To characterize the natural history and clinical features of PSP, we reviewed the records of 25 patients followed in our clinic since 1991, with a clinical diagnosis of PSP according to NINDS and Golbe criteria [1]. These patients represent 4.1% of all patients followed in our Movement Disorders Outpatient Department. The male to female ratio was 1.5. We analysed symptoms at onset, gait disturbance, postural instability, falls, falls during the 1st year of the illness, bilateral bradykinesia, axial rigidity, supranuclear palsy, tremor, rigidity and/or bradykinesia, neck dystonia, unilateral dystonia, blepharospasm/eyelid opening apraxia, dysarthria, dysphagia, response to levodopa treatment, cognitive impairment, dementia, disease duration, age, and cause of death. The mean age at onset was 62±6.2 years. The deceased patients were 6, with a disease duration of 7.2±2.1 years. The disease duration in surviving patients was 7.28±3.3 years [2].

The most-common onset symptoms were bilateral bradykinesia (15/23 patients) and postural instability with falls (12/23 patients). Nine patients presented with dysphagia and dysarthria, 3 with tremor, 2 with cognitive impairment, 2 with blepharospasm/eyelid opening apraxia, and 1 with neck dystonia (Table 1).

Table 1 Symptoms at onset in 25 cases of progressive supranuclear palsy

Symptoms at onset	No. of cases
Bilateral bradykinesia	15
Gait disturbance, postural instability, falls	12
Dysphagia/dysarthria/hypophonia	9
Tremor	3
Blepharospasm/eyelid opening apraxia	2
Cognitive impairment	2
Neck dystonia	1

Table 2 Symptoms during the follow-up

Symptoms	No. of cases
Gait disturbance	25
Postural instability	25
Falls in the 1st year	10
Falls	25
Bilateral bradykinesia	25
Axial rigidity	19
Supranuclear palsy	25
Tremor, rigidity, or bradykinesia (at least two)	23
Tremor, rigidity, and bradykinesia	4
Neck dystonia	8
Unilateral dystonia	3
Blepharospasm/eyelid opening apraxia	7
Dysphagia	18
Dysarthria	22
L-Dopa treatment response	
Absent	16
Initial	2
Initial but poor	7
Cognitive impairment	9

Ten patients presented with falls in the 1st year of the disease. During the follow-up period (range 2–10 years) dysarthria and dysphagia were present, respectively, in 91% and 74% of patients and axial rigidity in 74%; 35% of the patients developed neck dystonia, 30% blepharospasm and eyelid opening apraxia; cognitive impairment was present in 30% of patients.

During the follow-up period all patients presented gait disturbance, postural instability with falls, bilateral bradykinesia, and supranuclear palsy. In addition, L-dopa treatment had low efficacy in all patients (Table 2).

Search for Tau protein mutations was performed in 8 patients. All presented the genotype A0\A0 [3, 4].

Data obtained in this study agree with Golbe and NINDS diagnostic criteria and with previous studies reported in the literature. In our cohort of patients, pseudobulbar features and speech and swallowing problems were commonly encountered. Our study also emphasizes the high frequency of dystonic features, such as neck dystonia, blepharospasm, and eyelid opening apraxia, and the importance of these potentially treatable problems in PSP. Finally, we observed that in several patients differential diagnosis from other types of parkinsonism was possible only after 1 year of the disease.

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Neurol Sci (2003) 24:178–179
DOI 10.1007/s10072-003-0118-y

Excessive daytime somnolence in Parkinson's disease. Follow-up after 1 year of treatment

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Abstract Excessive daytime somnolence (EDS) and quality of sleep were studied in 25 parkinsonian patients at baseline, when they had not yet received any antiparkinsonian medication, and after 1 year of treatment with dopaminergic drugs. EDS was measured by the Epworth Sleepiness Scale (ESS) and sleep quality by the Pittsburgh Sleep Quality Index (PSQI). At baseline, the ESS score was not different from that of age-matched healthy controls. The mean ESS score increased significantly after 1 year of follow-up, being more than 10 in 12 patients. The mean PSQI also increased significantly after 1 year of treatment, but there were no differences in the number of "bad sleepers" at baseline and at follow-up. In conclusion, EDS seems to emerge during the course of the illness, at least in a proportion of PD patients, and could represent another clinical correlate of the interaction between the ongoing neurodegenerative process and the side effects of drugs.

Insomnias (disorders of initiating or maintaining sleep), parasomnias (vivid dreams, nightmares, nocturnal vocalizations, sleeptalking, nocturnal hallucinosis), and excessive daytime sleepiness (EDS), defined as an inappropriate and undesirable sleepiness during waking hours, are common finding in Parkinson's disease (PD) [1]. EDS has received renewed interest after recent reports suggested that parkinsonian patients taking dopaminergic drugs may have "sleep attacks" while driving [2]. Current evidence suggests that individuals with PD may fall asleep because they experience EDS and find themselves in situations in which resistance to sleep is decreased [3].

Whether these disorders, particularly EDS, are related to the disease process itself or to complications of dopaminergic therapy is unclear. We previously demonstrated that de novo PD patients are no different from sex- and age-matched healthy controls in clinical measures of EDS [4]. To better understand the role played by treatment or disease-related factors in the pathogenesis of EDS, we restudied these PD patients after 1 year of dopaminergic treatment.

Patients and methods

We studied 25 PD patients (15 men, 10 women; aged 64±8.1 years; symptoms duration 2.6±2.2 years; H and Y stage: 13 patients stage I, 10 patients stage II, 2 patients stage III) at baseline, when they had not yet received any antiparkinsonian medication, and after 1 year of treatment with dopaminergic drugs. Motor disability was rated by means of subset III of the UPDRS, which was assessed in the morning, before any medication was taken. Mini Mental State Examination (MMSE) was used as a screening procedure for dementia, and Beck Depression Inventory (BDI) was used for evaluation of depression. EDS was measured by the Epworth Sleepiness Scale (ESS) [5], a measure of general level of daytime sleepiness. A score more than 10 is considered abnormal, and a score more than 16 is indicative of severe EDS. The quality of sleep was measured by the Pittsburgh Sleep Quality Index (PSQI) [6], a self-rated questionnaire expressing a global score that represents the sum of seven component scores, each of which addresses a specific aspect of subjective sleep quality. An overall score >5 can distinguish poor (PSQI >5) from good (PSQI <5) sleepers. Comparisons between means were performed with Student's *t*-test.

Results

At baseline, the ESS score was not different from that of healthy controls [1, 4]. Twelve patients were treated with L-dopa alone, 3 patients with L-dopa plus pramipexole, and 10 with dopamine agonist monotherapy (pramipexole n=4, ropinirole n=3, pergolide n=3). The ESS score increased significantly ($p<0.01$) after 1 year of follow-up (Table 1). Furthermore, 12 patients at the follow-up visit had an abnormal ESS score (>10) compared with only 3 patients at baseline. However, no patient had a score more than 16 either at baseline or at follow-up.

Although mean PSQI score also increased significantly after 1 year of treatment, the number of "bad sleepers", defined as those patients showing a total score more than 5, was the same at baseline and at follow-up (Table 1). There was no significant difference in mean UPDRS score after 1 year of dopaminergic treatment with respect to baseline, although in some patients there was a clear improvement in motor disability. There were also no differences in ESS,

Table 1 Motor disability, excessive daytime somnolence (EDS), and sleep quality at baseline and at follow-up

	Baseline	Follow-up
UPDRS III	19.2±8.4	20.8±7.12
Beck Depression Inventory	9±5.9	9.3±5.7
MMSE	26.8±5.9	26.3±3.3
ESS	6±2.9	9±3.2*
No. of patients with a score >10	3	12
PSQI	5±2.7	6.1±3.9**
No. of patients with a score >5	15	15

ESS, Epworth Sleepiness Scale; *PSQI*, Pittsburgh Sleep Quality Index; *MMSE*, Mini Mental State Examination)
*$p<0.01$ versus baseline; **$p<0.05$ versus baseline

PSQI, or UPDRS scores when the patients were examined according to the different treatment strategies (L-dopa versus dopamine agonists).

Discussion

An inappropriate and undesirable sleepiness (EDS) during waking hours is a frequent finding in PD patients. EDS has recently been associated with "sleep attacks" while driving in PD patients [2], and dopaminergic drugs have been implicated as a possible causative mechanism. Recent trials on dopamine agonists have indeed reported somnolence as a significant adverse event of treatment, probably as a class-related phenomenon [7]. In agreement with these observations, we have shown in a previous report that de novo PD patients have ESS scores similar to those of sex- and age-matched healthy control subjects [4]. Hence, EDS does not seem to be a trait of untreated PD. However, in the present study we observed a significant increase in ESS score after 1 year of follow-up. This effect was particularly evident in 12 of the patients whose ESS scores reached values above 10, whereas the score remained stable and within normal limits in the remaining 13 patients. These results should be interpreted cautiously and it must be emphasized that polysomnography is the best method for the objective study of sleep-wake disturbances. However, ESS has been shown to correlate with MSLT and nightime polysomnography, and may be considered as an objective, easily administered measure of EDS [5].

EDS seems to emerge during the course of the illness, at least in a proportion of PD patients. Onset of EDS might simply reflect a worsening of nocturnal sleep quality in PD patients due to increasing severity of illness. However, although mean PSQI score increased significantly at follow-up, there was no increase in the number of "bad sleepers" (PSQI >5) between baseline and follow-up measurements. In addition, as all patients were receiving dopaminergic treatment, motor disability did not deteriorate but actually improved in some patients, so that this explanation seems unlikely. However, since the increase in the ESS score was observed only in a proportion of PD patients, EDS could represent another clinical correlate of the interaction between the ongoing neurodegenerative process and the side effects of drugs, similar to other phenomenon, such as dyskinesias and hallucinations. Further studies are needed to better characterize those patients who may be more prone to the development of this disabling symptom.

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Neurol Sci (2003) 24:180–1181
DOI 10.1007/s10072-003-0119-x

Neuroprotective effects mediated by dopamine receptor agonists against malonate-induced lesion in the rat striatum

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Abstract In rats, intrastriatal injection of malonate, a reversible inhibitor of the mitochondrial enzyme succinate dehydrogenase, induces a lesion similar to that observed following focal ischemia or in Huntington's disease. In this study we used the malonate model to explore the neuroprotective potential of dopamine agonists. Rats were injected intraperitoneally with increasing concentrations of D_1, D_2, or mixed D_1/D_2 dopamine agonists prior to intrastriatal injection of malonate. Administration of increasing doses of the D_2-specific agonist quinpirole resulted in increased protection against malonate toxicity. Conversely, the D_1-specific agonist SKF-38393, as well as the mixed D_1/D_2 agonist apomorphine, conferred higher neuroprotection at lower than at higher drug concentrations. Our data suggest that malonate-induced striatal toxicity can be attenuated by systemic administration of dopamine agonists, with D_1 and D_2 agonists showing different profiles of efficacy.

Metabolic abnormalities associated with selective neuronal damage have been implicated in a number of neurological disorders, including Parkinson's disease, Huntington's disease, and cerebral ischemia. However, the precise mechanisms underlying the neuronal damage are still poorly understood. Direct injection of malonate, a reversible inhibitor of the mitochondrial enzyme succinate dehydrogenase (SDH), into the corpus striatum of rats induces neuropathological changes very similar to those observed in Huntington's disease or following focal ischemia [1]. Therefore, this procedure has been used to investigate the pathogenetic mechanisms of these disorders, which are characterized by selective striatal vulnerability.

Inhibition of SDH by malonate induces an energy crisis in the neuron that enhances the susceptibility to other potentially toxic agents such as dopamine. The striatum is richly innervated by dopamine afferents and exposure to elevated levels of dopamine can cause neuronal death, presumably as a result of the inherent instability of the catechol moiety that can form reactive oxygen species (ROS) [2]. Accordingly, intrastriatal injection of malonate has been associated with local, massive release of dopamine and ROS formation [3].

Dopamine release and uptake can be modulated by occupation of dopamine receptors with specific agonists [4]. Accumulating evidence has suggested that dopamine agonists might have neuroprotective properties against neurodegenerative diseases, through the activation of dopamine receptors and/or mechanisms independent of receptor stimulation [5].

The aim of this study was to examine the potential neuroprotective potential of selective and non-selective dopamine agonists against malonate-induced lesions in rats. In particular, we studied the effect of systemic pretreatment with the D1 agonist SKF-38393, the D2 agonist quinpirole, and the mixed D1/D2 agonist apomorphine.

Male Sprague-Dawley rats, weighing 250–300 g, were anesthetized with 50 mg/kg sodium thiopental followed by an intraperitoneal injection of R-(+)-SKF-38393 (2 or 10 mg/kg), (-)-quinpirole hydrochloride (1 or 5 mg/kg), R-(-)-apomorphine (0.5 or 2.5 mg/kg), or saline, and were placed in a stereotaxic frame. Thirty minutes later, 2 µl of 0.75 M malonate in 0.1 M phosphate-buffered saline (pH 7.4) was injected at 0.5 µl/min into the right striatum (0.7 mm anterior, 2.8 mm lateral, and 5.0 mm ventral with respect to the bregma and dura). Three days after surgery, rats were killed by decapitation; brains were immediately removed, frozen on dry ice, and stored at -80°C. Serial, coronal sections (25 µm) were cut throughout the entire striatum using a cryostat and mounted on polylysine-coated slides.

The anatomical and functional extent of the striatal lesion was evaluated using a metal-enhanced histochemical staining method for cytochrome oxidase activity. The area of lesion on each slide was quantified using a video-based analysis system (SCION Image 1.62, Scion Corporation, Frederick, Mass., USA). To determine the lesion volume (mm^3), area measurements were summed and multiplied by the intersectional distance (100 µm). Nissl staining of every fourth section was also carried out, to ascertain the correct positioning of the intrastriatal injection. Statistical analysis was carried out using analysis of variance (ANOVA) coupled with the Fisher's post-hoc test. The minimum level of statistical significance was set at $p<0.05$.

Intrastriatal injection of 1.5 µmol of malonate into the corpus striatum produced a large lesion that encompassed almost the entire striatum (mean±SD: 50.6±5.7 mm^3), as measured by the absence of cytochrome oxidase staining. Pretreatment of the animals with the D2 dopamine receptor agonist quinpirole induced a dose-dependent decrease in the lesion volume; 24% (38.5±4.5 mm^3) and 36% (32.5±2.2 mm^3) reduction was observed following injection of 1 and 5 mg/kg of the agonist, respectively (Fig. 1). Conversely, protection against malonate-induced striatal lesion was more

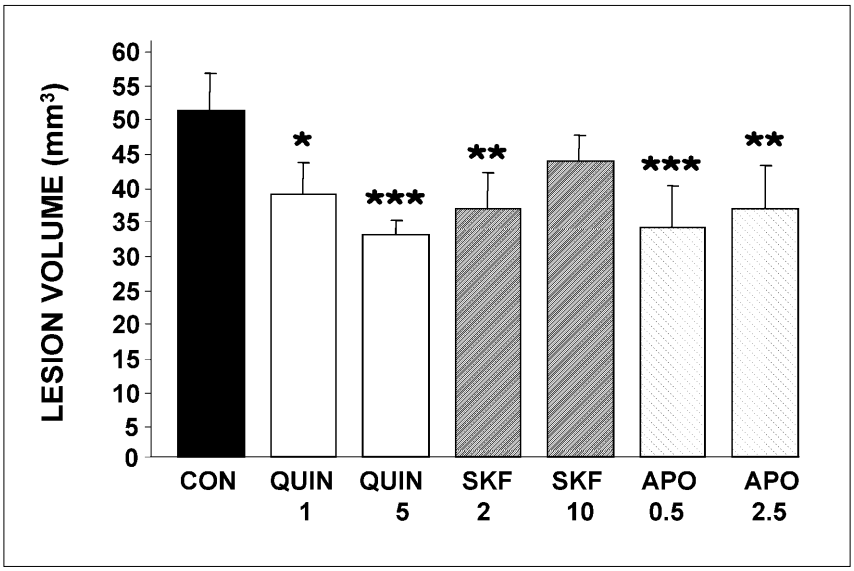

Fig. 1 Volume of malonate-induced striatal lesion after treatment with dopamine receptor agonists. *Bars* represent the means±SEM. **p*<0.005, ***p*<0.001, ****p*<0.0001 versus control (Fisher's post hoc test; ANOVA F=5.5, p<0.0004)

pronounced at lower than at higher drug concentrations when animals were pretreated with the D_1 receptor agonist SKF-38393 (2 mg/kg: 36.3±5.3 mm^3, -28%; 10 mg/kg: 43±3.7 mm^3, -14%), and the mixed D_1/D_2 dopamine receptor agonist apomorphine (0.5 mg/kg: 33.6±6.1 mm^3, -34%; 2.5 mg/kg: 36.3±6.4 mm^3, -28%).

Discussion

Pre-treatment of the animals with selective or non-selective D1/D2 dopamine agonists reduced the lesion volume significantly, except when the higher dose of SKF-38393 was used.

The direct stimulation of D2 presynaptic autoreceptors by quinpirole and apomorphine, the latter being more active on the D2 receptor, presumably induces a decline of dopamine concentration at the striatal level [6], and thus may limit the contribution of the neurotransmitters to the malonate lesion. In addition, apomorphine may act as a radical scavenger, but also as a reducing agent, at higher drug concentrations [7]. Therefore, the neuroprotective profile of the drug likely reflects the balance between its D2 agonist, radical scavenging, and its radical-activating properties. Similarly, the inverse relationship between the neuroprotective effect and the dose of SKF-38393 observed in our study may be explained by the antioxidant or pro-dopaminergic/oxidant activities of the drug, depending on its concentration [8].

The particular spectrum of DA receptor agonist activity of each drug used in this study, as well as their individual antioxidant properties, most likely contributes to their specific dose-dependent neuroprotective profile. The present study provides evidence that dopamine agonists can exert neuroprotective effects in vivo against malonate toxicity in the rat striatum. Since energy impairment has been directly implicated in neurological disorders, such as Parkinson's disease, Huntington's disease, or cerebral ischemia, modulation of the dopaminergic system and further understanding of the precise mechanisms of action of dopamine receptor agonists may be promising areas of study for neuroprotective therapy.

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Neurol Sci (2003) 24:182–183
DOI 10.1007/s10072-003-0120-4

Amphetamines induce ubiquitin-positive inclusions within striatal cells

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Abstract The present study explores whether effects induced by amphetamine derivatives on striatal GABA cells might be connected with effects on dopamine (DA) metabolism. Methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") were administered to C57Black mice following a dosage regimen in which various doses of both drugs were injected i.p. at 2-h intervals. Neuronal inclusions produced under these experimental conditions were examined under electron microscopy. Drugs reducing DA availability prevented inclusion formation; conversely we observed that increasing DA synthesis or impairing physiological DA degradation enhanced the number of inclusions. The present study indicates that the presence of extracellular striatal DA is essential for the production of subcellular alterations induced by amphetamine derivatives. This is in line with a recent hypothesis connecting striatal DA release with degeneration of striatal GABA neurons.

Methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") produce nigrostriatal damage, which is likely to be triggered by the massive increase of free cytoplasmic dopamine (DA) promoted by both drugs [1]. In turn, this determines enhanced DA metabolism, leading to formation of highly reactive metabolites, such as DA-quinones. These by-products, apart from destroying DA axons, extend to the surrounding serotonin (5HT) terminals, which are also considerably damaged. Although there has been extensive study of amphetamine-induced toxicity to monoamine nerve terminals, no previous report specifically investigated whether these effects might extend to striatal GABA cell bodies. Thus, the present study explores a recent hypothesis suggesting the potential deleterious influence of increased striatal DA levels on striatal GABA neurons. This derives from original data obtained a decade ago by Filloux and Townsend [2], who evaluated the morphological effects produced by intra-striatal injections of exogenous DA on GABA cells. In keeping with this, Jakel and Maragos [3] in a recent review hypothesized that even endogenous DA triggers degeneration of striatal medium-sized neurons when these cells are partially impaired. Thus, in Huntington's disease, when the biochemistry of striatal cells is altered, endogenous DA might induce neuronal cell death. This would explain why post-natally derived GABA cell cultures expressing mutant huntingtin do not degenerate unless they are exposed to extracellular DA [4]. Recently, we described striatal inclusions occurring after multiple METH or MDMA administration [1]. Therefore, in the present study we evaluated the role of endogenous DA in triggering these phenomena. Both neurotoxins were administered to male C57 black mice (C57BL/6J), 9–10 weeks old, in multiple steps (each consecutive dose was injected i.p. 2 h apart). Each treatment group was composed of 10 animals. All mice were sacrificed 7 days after drug administration.

Both treatments led to morphological changes within striatal GABAergic cells, consisting of neuronal inclusions shaped as whorls of concentric membranes, which stained for various components of the ubiquitin-proteasome (UP) pathway. We measured the effects of various pharmacological agents in modulating DA metabolism and the ultrastructure of striatal GABA neurons. In particular, we administered the blocker of tyrosine hydroxylase a-methyl-*p*-tyrosine (a-MpT 150 mg/kg), the DA releaser reserpine (5 mg/kg), the DA precursor L-dopa (100 mg/kg), and the non-specific monoamine oxidase (MAO) inhibitor pargyline (4 mg/kg). We compared the effects induced by these treatments with those obtained in saline-injected mice.

In particular, we measured the number of striatal neurons by assaying GAD activity and measuring GAD-67 immunostained neurons, as well as striatal histochemistry (hematoxylin and eosin staining). To explore subcellular changes we used transmission electron microscopy combined with immunocytochemistry. Briefly, for light microscopy striatal sections were incubated with primary antibodies (Sigma Chemical, St. Louis, Mo., U.S.A.), overnight at 4°C or 2 h at room temperature, followed by 1 h incubation with secondary antibody (diluted 1:50). Primary antibodies for GAD 67, ubiquitin, and heat shock protein (HSP70) were diluted 1:1000.

For electron microscopy the brains were maintained in situ immersed in fixative solution (2% paraformaldehyde/0.1% glutaraldehyde in 0.1 M phosphate-buffered saline, pH 7.4) overnight at 4°C, and then removed from the skull. Caudate-putamen was dissected, post fixed, and embedded in Epon/Araldite. For immunoelectron microscopy, 50-nm-thick sections were stained using immunogold.

Both amphetamine derivatives did not produce striatal cell death. However, these treatments led to formation of inclusions in striatal cells, which prevailed in the dorsal striatum and were more abundant in the nuclear compartment (Fig. 1). Pre-treatment with a-MpT or reserpine abolished

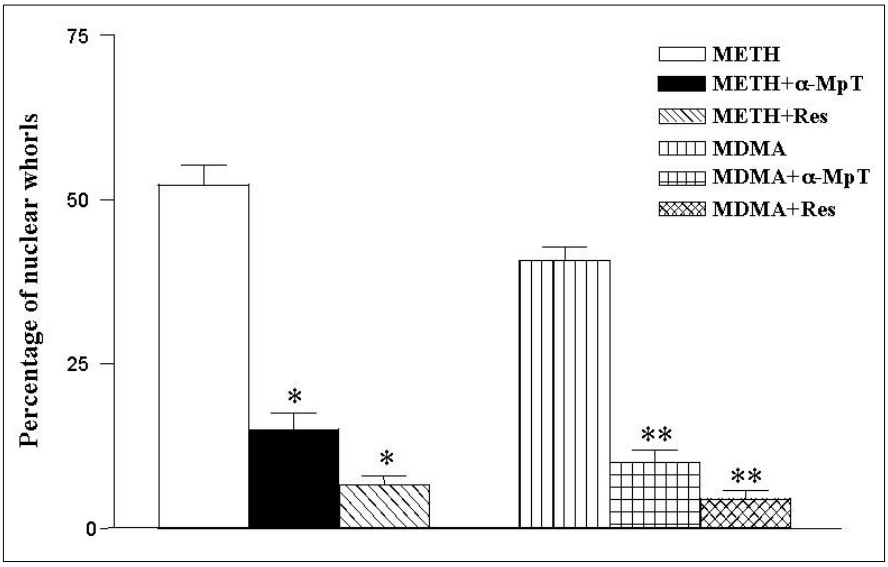

Fig. 1 Effects of various treatments on nuclear whorls in the dorsal striatum. Drugs reducing dopamine (DA) levels produce a protection against subcellular effects induced by methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA). This was achieved by either administering an inhibitor of tyrosine hydroxylase, α-methyl-*p*-tyrosine (a-MpT) or blocking vesicle uptake, reserpine (Res). *$p<0.05$ compared with METH, **$p<0.05$ compared with MDMA

these effects (Fig. 1), while the DA precursor L-dopa enhanced the number of inclusions, which were also increased by the blockade of physiological DA metabolism using the non-selective MAO inhibitor pargyline.

Discussion

Most neurological degenerative disorders are characterized by the presence of neuronal inclusions that contain various proteins belonging to the UP system. The UP system is also localized in the nucleus. Therefore, it is likely that the nuclear inclusions we found in GABA striatal cells might be due to the strong oxidative stress occurring in this cell compartment. It is known that DA itself produces an abnormal activity in the UP pathway, which works as an intracellular protein clearing system [5, 6]. Therefore, saturation of this multi-enzymatic system might be involved in the pathogenesis of neuronal nuclear inclusions. Interestingly, unlike Parkinson's disease (exclusive cytoplasmic inclusions), neuronal inclusions found in striatal cells show a preferential nuclear localization. This might be due to the specific composition of protein aggregates. In line with a recent report of Conway et al. [7], oxidation by DA derivatives of cytoplasmic a-synuclein protofibrils seems to be the final target to cluster cytoplasmic inclusions within DA neurons. Our hypothesis is that synuclein protofibrils do not migrate from the cytoplasm to the nucleus. In contrast, other oxidative products derived from DA seem to be present in striatal cells, allowing them to move from the cytoplasm to the nucleus or primarily clusters within the nucleus itself. Despite this different subcellular localization, both types of neuronal inclusions rely on the availability of DA as demonstrated by the present study.

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Neurol Sci (2003) 24:184–185
DOI 10.1007/s10072-003-0121-3

Deficit of short-term memory in newly diagnosed untreated parkinsonian patients: reversal after L-dopa therapy

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Abstract We assessed the effect of pathology and L-dopa therapy on attention, working memory, and executive functions, in newly diagnosed Parkinson's disease patients. Twenty-one consecutive outpatients who met the criteria for de novo Parkinson's disease, and were naive for L-dopa therapy, were observed for the first time. All patients underwent clinical and neuropsychological evaluations (cognitive decline, memory, executive control). Each patient was re-evaluated on standard L-dopa therapy. Serial Position Curves showed an increased primacy effect (5.18±2.07) and a decreased recency effect (13.35±5.51). These findings normalized after L-dopa therapy (3.50±1.72 and 16.20±3.09 respectively). The effect of L-dopa on working memory is discussed.

Introduction

There is disagreement in the literature about the role of L-dopa in modifying the cognitive profile in patients with Parkinson's disease (PD). The deficits observed in working memory and in executive functions are mostly due to deficits in striatum-frontalis projections [1]. A generally accepted interpretation of the specific deficit has not been reached. Memory impairment has been considered a consequence of a disturbance in recall strategies rather than a specific storage deficit [2]. The lack of firm evidence about the role of each neurotransmitter in determining such deficit has contributed to discussion on the unitary hypothesis versus multiple-system control mechanisms [3].

The aim of the study was to assess alterations of attention, working memory, and executive control abilities, in newly diagnosed patients with PD and to investigate the contribution of pathology and L-dopa therapy.

Subjects and methods

We studied the clinical and neuropsychological profile of 21 consecutive patients (8 female and 13 male, mean age 64.9 years range 43–83 years, mean education 7.4±4.34 years) observed for the first time in the outpatient service of the Neurological and Psychiatric Science Department, University of Florence. The enrolment period was November 1998 to March 2000. All patients met the criteria for de novo PD at the clinical examination and were naive for L-dopa therapy.

All patients have been clinically evaluated by UPDRS, Hoehn and Yahr, and Schwabb and England tests. They have been subjected to neuropsychological evaluation consisting of: (1) cognitive impairment evaluation: MMSE; Raven Progressive Matrices (1938), Phrasals Generation, Weigl's Sorting Test; (2) verbal and not-verbal short-term memory evaluation: Serial Position Curve, Two-syllabic Words Serial Repetition test, WMS, Block Tapping Test (Corsi), Rey Figure test copy and delayed recall; (3) executive and control functions evaluation: Wisconsin Card Sorting test modified by Nelson, Gibson Spiral Maze, Stroop colour-word test (Victoria version), Tower of London, Category Words Fluency test, Phonemic Words Fluency test, Fragmented Figures Assemblement.

Each patient was re-evaluated 1 year later, after standard L-dopa therapy. We again administrated those tests for which equivalent forms were available or those tests with minor "learning effect".

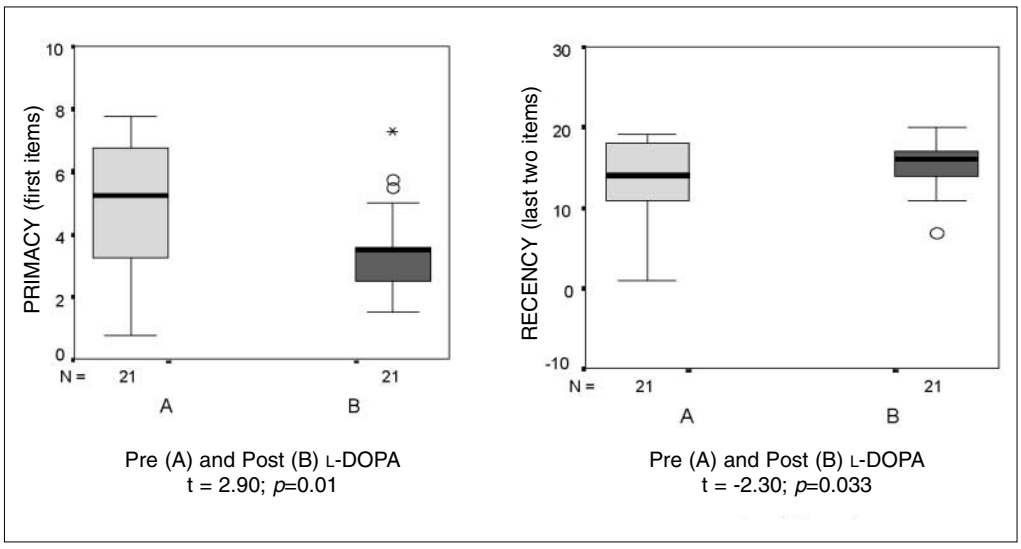

Fig. 1 Serial Position Curve

Results and discussion

Subjects investigated in this study were in the very early stages of the disease and minimal alterations of cognitive status were expected. Care was taken to exclude incidental dementia and none of the patients showed cognitive decay in the follow-up period. We selected subjects with PD who achieved a MMSE score of at least 24. Only 1 patient had a score of 22, but he was included because he had a score of 26 on the Raven Progressive Matrices 1938.

The cognitive profile of patients did not substantially change after starting L-dopa treatment. Sensitive tests of cognitive impairment showed a strong correlation between performances before and during L-dopa therapy.

There were no differences between Gibson spiral maze tests before and after L-dopa treatment, except for a trend in the number of mistakes (t=1.91, p=0.071). A statistically significant improvement in semantic verbal fluency after treatment (t=2.344, p=0.033) was observed; otherwise, reading fluency (Stroop test part A) was significantly reduced after L-dopa treatment.

On the serial positional learning curve we observed that patients, as a group, achieved a primacy score at Serial Position Curve beyond 75% of the normal distribution curve [4]; in contrast, they showed a reduction in recency effect, so that they were under 25% of the normal distribution. After 1 year of standard L-dopa treatment, this phenomenon reversed and the values of the curve overlapped again with those of the reference population. These data were statistically significant in the t-test for matched samples (t=2.901, p=0.01; t=2.308, p=0.033; primacy and recency, respectively).

The other tests, with equivalent forms administered before and during L-dopa treatment, were evaluated and no significant differences between the two performances were found.

Neuropsychological observations of parkinsonian patients have agreed on the consistency of control executive involvement [1]. This did not seem to be the case in our early stage patients. We found no alterations in tests quoted to evaluate both fronto-lateral and fronto-orbital structures, before and after L-dopa therapy.

Our data suggest a mild deficit of immediate memory and working memory, as observed by others [1, 5], ascribed to nigro-striatal afferent deficit [6]. Moreover, we found a dissociation of working memory deficit and strict control functions, which supports the hypothesis that the executive control system may not be a unitary process [3], but the synthesis of different sub-components, influenced by L-dopa in different ways. The presence of a working memory impairment linked to information processing load (greater deficit on visuospatial components) and the impairment of access to the semantic system (reduced fluency) may be due to an impaired setting of procedures [7]. Such deficit does not appear to be related either to go/no-go function (constancy of Stroop effect before and after L-dopa treatment) or to general planning functions (unchanging WCST). The presence of a double dissociation between primacy and recency on Serial Position Curve suggests a possible effect of L-dopa on both short- and long-term memory [8], probably linked to a balancing between retrieval ability and immediate memory buffers.

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Neurol Sci (2003) 24:186–187
DOI 10.1007/s10072-003-0122-2

Progressive supranuclear palsy: analysis of six cases

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Abstract We analyzed six cases of progressive supranuclear palsy (PSP) from 1 January 1998 to 31 June 2002. All patients underwent a complete range of laboratory tests, head computed tomographic (CT) scan or magnetic resonance imaging (MRI); three were evaluated by formal neuropsychological examination. All were taking L-dopa or dopamine agonists. At the onset of PSP the initial diagnosis was almost always Parkinson's disease. The head CT scan or MRI findings were non-specific, while neuropsychological testing disclosed cognitive impairment, with predominant subcortical-frontal involvement. We emphasize the still obvious current difficulty in diagnosing PSP at an early stage in clinical practice. Therefore it is essential to formulate better clinical diagnostic criteria, to permit correct management of the disease.

Introduction

Progressive supranuclear palsy (PSP), also called Steele Richardson Olszewski syndrome, was first described as a clinicopathological entity by these authors in 1964 [1], although a few single clinical or pathological reports were previously published. PSP is one of the most-common parkinsonian syndromes, with an estimated prevalence of 1.39 in 100 000 inhabitants [2]. However, this is probably a considerable underestimate, as diagnosed cases are often not recognized until the disease has run half its course and probably many more patients die without a diagnosis, or have a misdiagnosis, usually Parkinson disease (PD). Median survival time is 5.6 (range 2–16.6) years [3]. Currently, there is no biological marker for the diagnosis of PSP. Neuropathological examination remains the "gold standard" for its definitive diagnosis [4]. To improve the specificity and sensitivity of the clinical diagnosis of PSP accurate criteria have been published [5].

Materials and methods

We describe six patients (four men, two woman), with a mean age of 66 years (range 59–73 years), who fulfilled the NINDS-SPSP criteria for the diagnosis of PSP [5]. They were followed from 1 January 1998 until 31 June 2002. We performed a complete range of laboratory tests and serial orthostatic and clinostatic blood pressure measurements, to exclude other parkinsonian syndromes and extrapyramidal diseases. All patients underwent a head computed tomographic (CT) scan or magnetic resonance imaging (MRI). In three patients, with less deterioration of motor functions and expressive language, we were able to perform a formal neuropsychological examination with the Brief Mental Deterioration Battery (BBDM) [6] and other tests.

Results

The first clinical visit occurred at mean of 3.5 years (range 1–8 years) after the onset of the disease. During follow-up one patient died, after a disease duration of 7 years. Gait disorder, postural instability, and a history of falls were the most-common symptoms at onset of the disease; further initial complaints, mainly reported by relatives, were apathy, depression, memory deficits, and ideomotor slowing. Supranuclear vertical and/or horizontal palsy was the cardinal sign, with rigid akinetic parkinsonism and postural instability. Other signs were dysphagia, dysarthria, neck dystonia (predominant in extension), apraxia of lid opening, hyperreflexia, and Babinski's sign (Table 1).

All patients were taking L-dopa or dopamine agonists; five of these were originally diagnosed as having PD and one chronic vascular encephalopathy.

The clinical therapeutic response was altogether judged as poor. Head CT scan (2 pz) and MRI (4 pz) showed nonspecific signs, such as mild cerebral atrophy and ventricular enlargement. In the three patients who underwent formal neuropsychological examination, we noticed cognitive impairment, with predominant subcortical-frontal involvement (see patient 4 in Table 2). A 73-year-old woman, examined 12 years from the onset of PSP, had moderate dementia.

Table 1 Symptoms in our six patients with progressive supranuclear palsy (PSP)

Signs	Patients	*n* (%)
Gaze palsy	6	(100)
Bradykinesia	6	(100)
Axial rigidity	6	(100)
Limb rigidity	6	(100)
Postural instability and falls	6	(100)
Mild L-dopa response	3	(50)
Retrocollis	3	(50)
Anterocollis	1	(16)
Apraxia of lid opening	1	(16)
Hyperreflexia	4	(66)
Babinski's signs	1	(16)
Dysarthria	4	(66)
Dysphagia	3	(50)
Apathy	2	(33)
Depression	2	(33)
Dementia	1	(16)
Sphincter disturbances	3	(50)

Table 2 Brief Mental Deterioration Battery in patient 4 performed approximately 4 years from the supposed onset of PSP

Test	Raw score	Corrected score (for age and education)	Equivalent score	Cut-off
Rey's 15 words				
Immediate recall	26	32.1	1	
Delayed recall	6	7.8	2	
Immediate visual memory	9	10.7	0	
Multiple Features Target Cancellation Task				
Time execution	60 min			90
Correct identifications	10			12
"False alarms" errors	0			2
Simple analogies	5	7.5		15
Final result	-0.89			0
Supplementary tests				
Digit span				
Forward	4	4.5	2	
Backward	2			
Corsi span	3	3.25	0	
Verbal fluency (F.A.S.)	12	19.9	1	
Weigl's test	3	4.45	0	
Copying drawing	3	4.1	0	
Ideomotor apraxia (De Renzi test)	63			53

Discussion

The first interesting issue arising from our study is still an obvious difficulty in diagnosing PSP in the early stages in clinical practice. Five of our patients were initially diagnosed as having PD and one chronic vascular encephalopathy. The recent clinical PSP criteria of Litvan et al. [5] are the same as those of Golbe et al. [7], who in their clinicopathological study calculated at first visit a 49% sensitivity compared with a 97% specificity. Therefore it is essential to formulate better clinical diagnostic criteria, to permit the correct management of the disease.

However, postural instability with falls and supranuclear gaze deficits were the initial signs of PSP that were useful in the differential diagnosis with PD and other parkinsonian syndromes.

In four patients we noticed non-specific MRI findings, such as mild cortical atrophy and/or ventricular enlargement. We did not find more-specific PSP findings, especially mesencephalic atrophy and an increase in signal intensity in the periaqueductal region on T_2-weighted images [8]. In our patients the head CT scan and MRI examination by the neuroradiologist were to some extent invalid, because of the erroneous initial diagnosis or the early stages of illness in some patients. In those submitted to formal neuropsychological examination, we found cognitive impairment, with predominant subcortical-frontal involvement. The differential diagnosis was mainly with corticobasal degeneration, in which the praxic functions are in general more compromised.

Acknowledgement We thank Miss Federica Dell'Anna for preparing this manuscript.

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Neurol Sci (2003) 24:188–189
DOI 10.1007/s10072-003-0123-1

Neuroacanthocytosis: clinical, radiological, and neurophysiological findings in an Italian family

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Abstract We have studied three members of a family (mother and two siblings) where the mother and father were first cousins and who presented a history of progressive mental deterioration, hyperkinetic extrapyramidal disorders, and epileptic seizures. They underwent the following examinations: cupremia, cupruria, and level of ceruloplasmin, genetic analysis for SCA1, 2, 3, 6, dentato-rubric-pallido-luysian atrophy, and Huntington's disease, electromyography (EMG), electroencephalography (EEG), brain magnetic resonance imaging (MRI), and investigation of acanthocytes with scanning electron microscopy. Genetic analysis was negative in all patients and acanthocytes were positive. EMG showed an axonal neuropathy in one sibling, EEG showed epileptiform activity in the two siblings, and MRI showed cortical atrophy in all subjects. This family shows the great variability of neuroacanthocytosis and a dominant autosomal transmission, as described only once previously in the literature.

Neuroacanthocytosis has been described in the last few years as a familial chorea with slowly progressive dementia and hereditary abnormalities. It is characterized by: (1) onset in adolescence or early adulthood of involuntary generalized movements that generally start as oro-facial dyskinesia and spread to the wholebody; (2) mild mental deterioration in most but not all cases; (3) absent or attenuated tendon reflexes with signs of chronic axonal neuropathy and muscular atrophy due to denervation; (4) atrophy of the caudate nucleus and the putamen; (5) acanthocytosis. This last alteration seems to be due to an alterated composition of the fatty acids bound covalently to red blood cell membrane proteins. Hereditary transmission is autosomal recessive, except in one case described in New England. Mild acanthocytosis can not be detected without the use of a scanning electron microscope.

Materials and methods

We studied three members of a family, mother (65 years old) and two siblings (1 male 38 years old and 1 female 39 years old), where mother and father were first cousins and who presented a history of progressive mental deterioration, hyperkinetic extrapyramidal disorders, and epileptic seizures.

They underwent the following examinations: cupremia, cupruria, level of ceruloplasmin, genetic analysis for SCA 1, 2, 3, 6, dentato-rubric-pallido-luysian atrophy, and Huntington's disease, electromyography (EMG), electroencephalography (EEG), brain magnetic resonance imaging (MRI), and investigation of acanthocytes with scanning electron microscopy.

Results

Neurological examination showed tics, oro-facial dyskinesia, and dystonia of the limbs in all subjects; neuropsychological valutation showed a mild mental deterioration in all subjects. Cupremia, cupruria, level of ceruloplasmin, and all the genetic analyses were negative in all subjects.

EMG showed a motor-sensitive axonal neuropathy in the female sibling. EEG showed bitemporal epileptiform activity in the two siblings, but not in the mother. MRI scans showed a hypointensity of the white matter in the T_1-weighted images and a hyperintensity in the T_2-weighted images, without enhancement with gadolinium in the parietal lobe of the male sibling. Global cerebral atrophy was observed in all subjects. Scanning electron microscopy revealed acanthocytes in all subjects.

The siblings have been given antiepileptic therapy and neuroleptics, with a good control of epileptic seizures and sufficient control of involuntary movements at the beginning of treatment. However, there has been progression in the last year.

Conclusion

The family described here shows the large spectrum of clinical, neuroradiological, and neurophysiological abnormalities that can be associated with neuroacanthocytosis and the possibility, described only once previously in the literature, of an autosomal dominant heredity.

This variation in symptoms explains why neuroacanthocytosis is difficult to diagnose and, consequently, often remains underestimated.

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Neurol Sci (2003) 24:190–191
DOI 10.1007/s10072-003-0124-0

Psychometric properties of the Unified Parkinson's Disease Rating Scale and of the Short Parkinson's Evaluation Scale

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Abstract The internal consistency (Cronbach's alpha and item-total correlation) and construct validity (factor analysis, intercorrelations, and relationship with Hoehn and Yahr staging and Schwab and England's ADL scale) of the sections "motor examination" and "activities of daily living" of the Unified Parkinson's Disease Rating Scale (UPDRS) and of the Short Parkinson's Evaluation Scale (SPES) were analyzed in 59 subjects with Parkinson's disease (PD) with various degrees of disability. Our results indicate that the SPES is easier and quicker than UPDRS and that it maintains many psychometric properties similar to those of the UPDRS, but with the reduction of a number of items and ordinal levels of each item studied here (producing more homogenous sections than the original versions). The tremor items would be better represented as a separate section in both scales.

The Unified Parkinson's Disease Rating Scale (UPDRS) is one of the most widely used rating scales for assessing patients with Parkinson's disease (PD) [1]. It comprises six sections: (1) mentation, behaviour, and mood; (2) activities of daily living (ADL); (3) motor examination (ME); (4) complications of therapy; (5) modified Hoehn and Yahr staging (HY); and (6) Schwab and England's ADL scale (SE) [2]. Sections 2 and 3 have been most widely employed in clinical trials as outcome measure. Several studies have investigated the structure and metric properties of the UPDRS, showing that the scale meets the basic psychometric criteria for reliability and validity in PD [3–5], but highlighting some problems, particularly regarding the misplacement and the redundancy of some items in sections 2 and 3 [3, 4]. To address these drawbacks, a new short scale (the SPES) has recently been proposed, which shows a good inter-rater reliability and construct validity [6]. The SPES is a disease-specific scale, omitting the UPDRS items considered difficult to evaluate, redundant, or of minor clinical significance. It contains four sections: mental state (3 items), ADL (8 items), ME (8 items), and complications of therapy (5 items). Furthermore, the HY and a scoring of motor fluctuations are included. The SPES adopts a four-point ordinal scale for each item.

The aim of the present study was to thoroughly analyze the internal consistency and the construct validity of the sections ME and ADL of UPDRS, comparing them with the corresponding sections of the SPES, in a group of PD subjects with various degree of disability.

Materials and methods

Fifty-nine unselected patients (32 males and 27 females), with a mean age 68 years (SD 9) and duration of disease between 1 and 21 years (mean 7 years), were evaluated (in "on phase", if fluctuating) by neurologists familiar with the specific rating scales.

Only the sections UPDRS-ADL, UPDRS-ME, HY, and SE [2], SPES-ADL, and SPES-ME [6] were considered in the present study. The internal consistency of the UPDRS-ADL, UPDRS-ME, SPES-ADL, and SPES-ME was assessed by means of the Cronbach's alpha (a minimum of 0.90 is desirable for clinical application [7]) and the item-total correlation (ITC), which examines how well each item is correlated with the total score, omitting that item from the total (the Spearman coefficient r_s should be above 0.20 [8]). Construct validity of the same sections was analyzed through: (1) correlation of each section with scores of HY and SE, between UPDRS-ME and SPES-ME, and between UPDRS-ADL and SPES-ADL; and (2) factor analysis [principal component analysis and then orthogonal rotation of the matrix of factors (Varimax) to facilitate the interpretation of the principal factors extracted].

Results

The median (and interquartile range) of the scores was as follows: UPDRS-ADL 12 (9–16), UPDRS-ME 18 (14–24), SPES-ADL 7 (6–11), SPES-ME 8 (7–11), HY 2.5 (2–3), and SE 80 (70–90). The Cronbach's alpha scores were: UPDRS-ADL 0.87, UPDRS-ME 0.91, SPES-ADL 0.91, and SPES-ME 0.78. The ITCs were as follows: UPDRS-ADL r_s>0.60 for all items (except salivation and swallowing, r_s=0.38; and tremor and sensitive disturbances, r_s<0.20); UPDRS-ME r_s>0.65 for all items (except resting tremor and action tremor, r_s<0.20); SPES-ADL r_s>0.60 for all; SPES-ME r_s>0.57 for all items (except resting tremor and postural tremor, r_s<0.20). After the elimination of the items with very low homogeneity from the overall construct (r_s<0.20 in ITC), the Cronbach's alpha scores increased (UPDRS-ADL 0.91, UPDRS-ME 0.95, SPES-ME 0.90) and factor analysis gave the following results: (1) UPDRS-ADL (11 items): two

factors accounted for 67% of the variance, with the first explaining 56% and the other including salivation, falling, and freezing when walking (scarcely related to ADL); (2) UPDRS-ME (12 items): one factor accounted for 69% of the sample variance; (3) SPES-ADL (8 items): two factors accounted for 70% of the variance, with the first explaining 61%, and the other including eating and feeding; (4) SPES-ME (6 items): two factors accounted for 79% of the variance, with the first explaining 67%, and the other including speech and rigidity.

The HY correlated with each of the two sections of both instruments (UPDRS-ADL r_s=0.84, UPDRS-ME r_s=0.87, SPES-ADL r_s=0.83, SPES-ME r_s=0.82); the same applied for the SE (UPDRS-ADL r_s=-0.87, UPDRS-ME r_s=-0.87, SPES-ADL r_s=-0.81, SPES-ME r_s=-0.78). The correlations did not significantly change after the deletion of heterogeneous items in the two UPDRS sections, and slightly decreased for SPES-ME. The UPDRS-ADL highly correlated with SPES-ADL (r_s=0.90), as did the UPDRS-ME with SPES-ME (r_s=0.88).

Discussion

The UPDRS and SPES are multi-dimensional scales [1], as such they must be analyzed – from a psychometric point of view – section by section (each should cover one dimension only), and not as a single index. Thus, every section must separately demonstrate its reliability and validity. The internal consistency (a main aspect of the reliability) reflects the extent to which items measure various aspects of the same characteristics and nothing else. We calculated both the Cronbach's alpha and the ITC, because the first index is based not only on the magnitude of the correlations among the items, but also on the number of the items in the scale, whereas the latter gives information about the homogeneity of each item in relation to the characteristic in question. Only after deletion of the items related to tremor (and also to sensitive disturbances in UPDRS-ADL) did the Cronbach's alpha of all sections present a satisfactory internal consistency (≥0.90), in spite of the reduction of the number of items [7]. The low ITC of the deleted variables (<0.20) suggests that they analyze a different construct [8] and points to the advisability of their exclusion from the calculation of the total score of the corresponding sections.

As for construct validity, the high correlation among the sections ADL and ME of both UPDRS and SPES and with HY and SE strengthens the validity of both instruments (also in the reduced versions analyzed here) for measuring respectively the disability and the motor impairment of patients with PD [3–6]. This is the first study that independently verifies the psychometric properties of the SPES and that investigates its factor structure, analyzing separately ADL and ME sections. A previous paper [6] performed factor analysis on the two sections combined, but this procedure possibly represents a methodological limitation because it does not make sense to talk of homogeneity across different sub-scales composing a multidimensional instrument.

In conclusion, there is a need for a "simplified" PD scale and one with more-homogenous sections than UPDRS. The SPES is easier and quicker than UPDRS and maintains many psychometric properties similar to those of the UPDRS, notwithstanding the reduction of the number of items and ordinal levels of each item studied here. The tremor items would be better represented as a separate section in both scales.

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Neurol Sci (2003) 24:192–193
DOI 10.1007/s10072-003-0125-z

Levodopa pharmacokinetics and dyskinesias: are there sex-related differences?

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Abstract We examined the potential sex-related differences in levodopa pharmacokinetics and their relation with the presence of dyskinesias in a group of 115 patients (67 men, 49 women) with Parkinson's disease. The patients were given a standard oral dose of levodopa plus benserazide (100/25 mg). The area under the levodopa plasma concentration time curve, corrected for the levodopa test dose (in mg/kg body weight), (AUC_w) was significantly higher in women than in men, with a reduced oral clearance. No difference in the proportion of men and women experiencing dyskinesias was observed.

Introduction

Levodopa remains the most-effective treatment for Parkinson's disease (PD). However, as the disease advances, motor complications and dyskinesias develop, resulting from a complex interaction between the kinetic features of levodopa and the pathology of the disease itself [1]. Observational studies reported an increased incidence of dyskinesias among women [2]. Sex differences in levodopa pharmacokinetics and their possible relation to the development of dyskinesias have rarely been investigated [3]. The aim of the present study was to evaluate the possible effect of gender on the pharmacokinetic features of levodopa and their relation to the development of dyskinesias in a large population of patients on chronic levodopa therapy.

Subjects and methods

Patients attending the Laboratory of Neuropharmacology for levodopa therapeutic monitoring [4] from January 1998 to January 2002 were enrolled in the study. Inclusion criteria were diagnosis of PD according to the clinical criteria of the Parkinson's Disease Society Brain Bank [5], chronic (>3 months) levodopa plus carbidopa or benserazide therapy, and a positive response to an oral levodopa test [4].

On the morning of the study, the patients received an oral fasting dose (8 a.m.) of levodopa (100 mg) plus benserazide (25 mg) after a 12-h washout of levodopa and any concomitant antiparkinsonian drugs. A standard low-protein breakfast was allowed 90 min after dosing. Venous blood samples (2 ml) for measurement of plasma levodopa concentrations were drawn by an indwelling catheter at fixed times, and processed as reported previously [4]. Dyskinesias, when present, were rated on a scale from 0 (absent) to 4 (violent dyskinesias) [4].

The peak plasma levodopa concentration (Cmax) and the time to peak (*t*max) were the observed values. The area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal rule. Both Cmax and AUC were corrected for the levodopa test dose (in mg/kg body weight) ($Cmax_w$, AUC_w). The plasma elimination half-life (*t*1/2) was calculated by linear regression analysis of the terminal portion of the log-linear plasma concentration time curve. Clearance values (CL) were calculated as dosexf/AUC, assuming f=0.85 [6].

Statistical analysis was carried out by Student's *t*-test or the Mann-Whitney U-test, as appropriate. The chi-squared test was used to compare proportions of men and women experiencing levodopa-induced dyskinesias. Significance was set at $p<0.05$.

Results

One hundred and fifteen patients were enrolled in the study (67 men and 49 women) (Table 1). The two groups of patients were similar in age, body mass index, disease severity according to the Hoehn and Yahr scale (H and Y), disease

Table 1 Clinical characteristics and levodopa pharmacokinetic data in male and female patients

	Men (n=67)	Women (n=49)	*p* value
Age (years)	61±11	59±9	NS
Body weight (kg)	72±10	61±9	<0.001
Body mass index	24.5±2.4	23.8±3.7	NS
H and Y stage	2* (1.5–3)	2.5* (2–3)	NS
Disease duration (years)	6.2±4.9	6.4±4.0	NS
Levodopa therapy duration (years)	2.5* (0.5–5)	4.2* (0.8–7)	NS
Levodopa daily dose (mg/day)	300* (200–437)	300* (200–500)	NS
Number of patients with dyskinesia (%)	21 (31%)	17 (34%)	NS
$Cmax_w$ (μg/ml)	1.3±0.5	1.4±0.5	NS
*t*max (min)	40* (30–60)	30* (30–50)	NS
AUC_w (μg/ml min)	106±25	120±25	<0.003
*t*1/2 (min)	52* (46–63)	52*(47–61)	NS
CL (ml/min/kg)	8.1* (7.2–9.6)	7.3* (6.3–8.5)	<0.003

Values are expressed as mean±standard deviation or *median (25th–75th percentiles)

duration, duration of levodopa therapy, and daily dose, but were significantly different for body weight. Dopaminergic therapy was taken by 23 men and 9 women (NS).

Levodopa $Cmax_W$, *t*max, and *t*1/2 did not differ in the two groups (Table 1). Levodopa AUC_W was significantly higher in women than in men ($p<0.003$). CL was significantly reduced in women ($p<0.003$).

Dyskinesias were present in 38 of 115 patients (33%). No sex-related differences were observed (Table 1). From separate comparisons, patients with dyskinesias showed a longer disease duration than patients without dyskinesias (median 10 versus 3 years, $p<0.001$), more-severe PD symptoms (H and Y scale 3 versus 2, $p<0.001$), were receiving a larger daily levodopa dose (400 versus 200 mg, $p<0.001$), and were on drug treatment for a longer period (7.5 versus 1.5 years, $p<0.001$). No difference in levodopa pharmacokinetic parameters was observed between the two groups.

Discussion

The present data partly confirm and extend previous observations of a gender effect on oral levodopa disposition in PD patients. In line with recently reported findings [7], women showed a greater levodopa bioavailability than age-matched men, as evidenced by a higher AUC_w and a reduction in the apparent oral clearance of the drug. The sex-related difference in levodopa handling was independent of body weight and suggests that other factors should be examined [7]. In the presence of L-aromatic amino acid decarboxylase inhibitors, *o*-methylation of levodopa by catechol-*o*-metyltransferase enzyme (COMT) becomes a major elimination pathway. Sex-mediated differences in COMT activity [8] might partly account for the observed levodopa pharmacokinetic differences. In a prospective follow-up of the DATATOP cohort [2], women were found to be more prone to dyskinesias than men. However, the interpretation of this finding is complicated by the fact that women were initially treated with a higher dose (mg/kg) than men. More recently, Zappia et al. [3] found a greater percentage of dyskinesias in women than in men, with a greater levodopa AUC after a test dose. They pointed out a pivotal role of body weight in explaining sex-related differences in levodopa pharmacokinetics and suggested that women, being lighter than men, could be at higher risk for developing dyskinesias due to the higher chronic levodopa exposure. Contrary to these findings, the greater levodopa bioavailability in our female patients was not accounted for by differences in body weight and was not associated with a greater incidence of dyskinesias than in men. A longer duration of symptoms, more-severe clinical stage, a higher daily levodopa dose, and a longer duration of treatment were the main determinants of the presence of dyskinesias in our patients. However, no difference in levodopa pharmacokinetics, namely drug AUC_w and $Cmax_w$, was observed between the two subgroups of patients with or without dyskinesias.

Our results confirm in a large population of PD patients the finding of a greater levodopa bioavailability in women than in men. Sex-related differences in drug disposition, although statistically significant, were moderate and are likely to be of minor relevance in drug prescribing.

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Neurol Sci (2003) 24:194
DOI 10.1007/s10072-003-0126-y

Six years' experience in deep brain stimulation in Parkinson's disease: advantages and limitations of use of neurophysiological intraoperative microreading

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Introduction

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is increasingly used in the treatment of the cardinal symtoms of Parkinson's disease and to reduce the common complications of L-dopa therapy. There is some discussion about the technique to best localize the STN. Some neurosurgeons do not like neurophysiological monitoring because the microelectrode recording increases the risk of bleeding and prolongs the operation, while computed tomography/magnetic resonance imaging (CT/MRI) and macrostimulation allow adequate anatomical targeting.

We report the clinical results of a group of parkinsonian patients undergoing DBS without neurophysiological monitoring compared with a group with neurophysiological assessment.

Materials and methods

Twenty-two patients with Parkinson's disease (Hoehn-Yahr stage III) undergoing surgery in Vicenza between 1997 and 2002 were divided into two groups. Group A underwent surgery between 1997 and 2000 without neurophysiological monitoring, and included 10 males and 5 females, with a mean age of 63 years (range 53–74 years) and a mean duration of disease of 13 years (range 4–20 years). Group B underwent surgery between 2001 and 2002, with neurophysiological assessment, and included 4 males and 3 females, with a mean age of 55 years (range 38–67 years) and a mean duration of disease of 11.5 years (range 6–21 years).

In 2001 neurophysiological monitoring with Neurotrek was added to CT/MRI and ventriculography in our center to better localize the STN. This technique consists of a simultaneous recording from five high-impedance (>0.2 MW) microelectrodes. The central microelectrode is positioned in the theoretical target, two electrodes are positioned on the right side (one to the front and one to the back), and two electrodes are positioned on the left side (one to the front and one to the back), each 2 mm from the central electrode. Submillimetric changing of depth is praticable.

Electrophysiological results

The microelectrode recording gives a precise identification of the borders of the STN and determines its maximal length. Microrecording showed that 11 of 14 electrodes (78%) deviated from the theoretical target and that in 9 of 14 cases (64%) the deviation exceeded 1 mm. In 5 cases the macroelectrode was positioned to the lateral side, never to the medial side.

Clinical results

The 3-month clinical follow-up showed a significant reduction of UPDRS III score in the group with neurophysiological monitoring (-38%, $p<0.006$), while there was no significant difference in patients without intraoperative microrecording. As for dyskinesias and off-periods, a significant reduction of UPDRS IV score was observed in both groups, but was more pronounced in group B. The reduction of L-dopa therapy was significant only in group B.

Side effects

In group A there was worsening dysphonia (6 cases, 4 severe), local infection (3 cases), dystonia (1case), eyelid apraxia (2cases), left intracerebral hemorrhage (1 case), and suicide (1 case). In group B there was worsening dysphonia (2 cases, both severe), local infection (1 case), dysarthria (2 cases), eyelid apraxia (1 case), gait freezing (1 case), thrombophlebitis (1 case), and a transient confusional state (6 cases).

Conclusions

Our results demonstrate and confirm that microelectrode recording is a useful technique for anatomical targeting of the STN and consequently leads to better clinical results from DBS in Parkinson's disease. Since the operation is prolonged we recommend the correct selection of patients.

Neurol Sci (2003) 24:195–196
DOI 10.1007/s10072-003-0127-x

Sleep attacks in Parkinson's disease: a clinical and polysomnographic study

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Abstract The objective of the study was to evaluate daytime sleepiness, (the features of episodes of sudden sleep onset), i.e., so-called sleep attacks (SAs), in three male Parkinson's disease (PD) patients (mean age 66 years) on chronic therapy with ropirinole or pramipexole. A structured clinical interview, the Epworth Sleepiness Scale, and continuous 24-h ambulatory polysomnography were used to assess the features of SAs occurring in the patients in their normal home environments. The polysomnographic patterns characterizing SAs (sleep occurring against a background of wakefulness, and not preceded by a feeling of sleepiness or by other heralding symptoms) were analyzed. The results showed that SAs can be clearly documented through polysomnographic monitoring and really, but rarely, occur in PD. SAs seem to represent the extreme of the continuum of daytime sleepiness observed in PD patients.

Introduction

Daytime sleep attacks (SAs) have been reported in Parkinson's disease (PD) patients on dopamine agonist or L-dopa treatment [1]. However, the actual prevalence and ultimate significance of (SAs) in PD remain controversial. Some doubt has been expressed [2] over whether sleep really does occur suddenly and without heralding symptoms in these patients, and it has been hypothesized that SAs may, instead, be preceded by a sleepiness of which the patients are unaware.

Our knowledge of SAs in PD is based mainly on clinical findings, and polysomnographic (PSG) studies of this phenomenon are rare [3]. We report clinical and PSG findings in three PD patients with a clinical history of SAs, who underwent continuous 24-h ambulatory-PSG (A-PSG) monitoring.

Patients and methods

We investigated three male subjects aged 66, 60, and 72 years, all with idiopathic PD (mean illness duration 7.3±2.3 years) without dementia. They had mean Unified Parkinson's Disease Rating Scale (UPDRS) "on" and "off" scores of 23±9.6 and 40±12 respectively, and their median Hoehn and Yahr "on" and "off" stages were 2 and 2.5, respectively.

In addition to L-dopa 466±152 mg/day (range 300–600 mg/day), two of the patients were taking ropinirole (24 and 28 mg/day) and one pramipexole (2.1 mg/day). They had been on these therapeutic regimens for a mean duration of 3±1.7 years (range 2–5 years).

On enrolment in the study, all patients reported episodes of falling asleep during the day in the last 3 months. These episodes showed the features of so-called SAs, i.e, they occurred at inappropriate times, occurred suddenly against a clinical background of wakefulness, and they were not preceded by a feeling of sleepiness or by any other heralding symptom.

None of the patients reported any history of accidents occurring in association with their SAs. Furthermore, no episodes of sudden loss of muscle tone in relation to pleasant emotions were reported in any circumstances.

Each patient was assessed for daytime sleepiness through the administration of a structured interview and of the Italian version of the Epworth Sleepiness Scale (ESS) [4].Once the patients, and their relatives or carers, had been trained in keeping an accurate sleep log and in using an event marker to signal any subjective or objective symptom of sleepiness, intentional napping, or sudden involuntary falling asleep in daytime hours, the patients were prepared to undergo 24-h A-PSG monitoring. This began at 8 p.m. and took place at home.

Drug regimens and daily routines had remained stable for at least 2 weeks prior to the start of the study and they were maintained throughout the 24-h A-PSG monitoring session. The patients were also encouraged not to modify their usual daytime and nighttime activities and schedules during the A-PSG monitoring.

The recordings were made on flash card by means of an 11-channel ambulatory polysomnograph (Micromed MS 40). Electroencephalography was performed using a referential technique with nine electrodes (Fp2, Fp1, C4, C3 T4, T3, 01, 02) positioned in accordance with the International 10–20 System and referred to a common electrode placed in Fz. The display system provided a computerized recombination of the traces in referential or bipolar montages. The 10th and 11th channels were dedicated to an electrooculogram (bipolar montage with an electrode at right outer counter and the other at left outer counter 1 cm above and below the horizontal line, in order to allow the recording of both horizontal and vertical eye movements) and a submental electromyogram, respectively.

The PSG recordings were scored visually page by page from the screen in accordance with standard sleep scoring criteria [5]. Microsleep and naps were defined according to the International Classification of Sleep Disorders [6].

Results

The ESS score was within the normal range in one patient and severely abnormal in the other two. In all three patients, analysis of the 24-h A-PSG recording revealed two distinct patterns of falling asleep in daytime hours.

Pattern 1 involved gradual sleep onset corresponding to a

Table 1 Daytime sleepiness and nocturnal polysomnographic (PSG) findings

		1	2	3	Mean
ESS score		9	19	15	14.3±5
PSG sleep attacks		4	1	2	
PSG microsleeps		+	–	–	
PSG naps		3	5	6	
PSG naps (duration)	*Min*	60	158	60	20±16
Sleep latency	*Min*	28.5	17	1	15±13
TST	*Min*	299	185	247	243±57
TIB	*Min*	387	480	381	416±55
SE	*Min*	77	39	65	60±19
REM latency	*Min*	97.5	107	Na	68±59
WASO	*Min*	60.5	115	134	103±38

ESS, Epworth Sleepiness Scale

signaled voluntary nap, generally in the early afternoon, taken while the patients were sitting in a chair or lying down. The falling asleep pattern was one of alternating quiet wakefulness and drowsiness followed by the gradual occurrence of stage 2 NREM sleep, eventually followed by stage 3 NREM sleep.

Pattern 2 involved polygraphic patterns corresponding to the sudden occurrence of stage 2–3 NREM sleep recorded against background activity denoting wakefulness with eyes open.

These patterns occurred during the early afternoon in one patient and both in the early and in the late afternoon in the other two. In both these patients the sudden sleep onset occurred during leisure/relaxation time (while playing cards in one patient). In all cases, the episodes of suddenly falling asleep were witnessed by the caregiver.

Table 1 summarizes the ESS scores and 24-h A-PSG findings.

Discussion

Our data demonstrate that episodes of suddenly falling asleep against background activity denoting wakefulness did occur in all three PD patients investigated. These episodes are clearly distinguishable from the voluntary naps the patients took during A-PSG monitoring; the latter were characterized by a gradual sleep onset.

In line with the observations of Tracik and Ebersbach [3], the SAs we documented in our patients were not associated with cataleptic phenomena or characterized by sleep-onset REM periods (SOREMPs).

Polysomnographic documentation of SAs characterized by SOREMPs has been performed in another study [7], in one patient, and SOREMPs have been documented in sleepy PD patients during performance of the Multiple Sleep Latency Test [8].

The question of whether SAs and daytime sleepiness in PD patients are distinct phenomena, or manifestations of different stages in a single disease continuum, is still under debate, as is whether they constitute narcoleptic-like phenomena.

Our three PD patients all showed a high proportion of microsleeps and intentional naps on A-PSG recording. Two also had a high ESS score.

Taken together, these data indicate that SAs are an extreme manifestation of increased daytime sleepiness. Further studies are needed to establish whether the different SA patterns observed depend on factors such as disease severity, drug regimens, and genetic susceptibility.

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Neurol Sci (2003) 24:197–198
DOI 10.1007/s10072-003-0128-9

The use of entacapone in patients with advanced Parkinson's disease: 2 years' experience

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Abstract Since January 2000 we have administered entacapone (200 mg) to 75 patients with severe Parkinson's disease in combination with their routine levodopa dose. At baseline the mean UPDRS (item III) score was 38±6. After 3 months of entacapone therapy the patients presented a significant improvement of motor fluctuations; the mean UPDRS score (item III) was 20±4. This improvement was also statistically significant after 2 years of entacapone therapy.

Short Report

Parkinson's disease (PD) is a chronic neurodegenerative disease with the principal pathological abnormality of progressive loss of dopaminergic neurones projecting from the substantia nigra to the striatum [1]. During the first years of levodopa therapy, the patient experiences marked improvement in parkinsonian symptoms, functional capacity, and quality of life. However, after an initial period of good and continuous response to levodopa, levodopa dose-related motor complications, end-of-dose wearing-off, and dyskinesias emerge [2, 3].

To further smooth the response to levodopa and diminish the dose and frequency of administration of levodopa needed by PD patients, a second class of enzyme inhibitors has been used as an adjunct to levodopa therapy, the catechol-O-methytransferase (COMT) inhibitors [4]. Entacapone, used as an adjunct to each daily levodopa/DDC inhibitor dose, inhibits the formation of 3-O-methyldopa in the periphery, thereby increasing the bioavailibility and half-life of levodopa without affecting the peak plasma concentration (Cmax) or the time taken to reach Cmax (tmax) [5].

Since January 2000 we have administered entacapone to 75 patients with advanced Parkinson's disease in our neurological outpatient department. They presented marcate motor fluctuations characterized by sudden on and off states with frequency and media duration of 4±1 and 1±0.5 h daily, respectively. To confirm their symptoms, all patients were asked to fill in hourly diaries of their disturbances, reporting motor fluctuations, off and on periods with and without dyskinesias during the last week prior to administration of entacapone. The patients had been treated for 6±2 years with levodopa (800±100 mg daily) and dopaminoagonists (ropinirole 15±3 mg and pramipexole 2.1±0.18 mg). We utilized the UPDRS [6] to evaluate motor fluctuations. Statistical analyses were performed with Wilcoxon's signed rank test and Student's *t*-test.

At baseline the mean UPDRS (item III) score was 38±6. All patients started entacapone (200 mg) in combination with their routine levodopa dose. After 3 months they returned to our laboratory for follow-up. The patients showed a large significant reduction of motor fluctuations, the mean UPDRS (item III) score was 20±4. This value was statistically significant at 6, 12, 18, and 24 months after the introduction of entacapone therapy ($p<0.001$) (Table 1).

We also observed a mild increase of dyskinesias in 20 patients after 3 months of entacapone treatment. For this reason we decided to reduce levodopa (200±50 mg). Within 10 days of entacapone introduction, 3 patients experienced severe dyskinesias, so we reduced the levodopa dose (300±25 mg). However, patients continued to present marked dyskinesias and so after 1 month of entacapone treatment the drug was withdrawn. Furthermore, 20 patients presented with discoloration of the urine; 6 patients reported reversible and mild nausea, but they continued to take entacapone.

Our short report confirms the efficacy and safety of entacapone therapy in severe PD. Patients presented a significant reduction and severity of motor fluctuations, and this benefit was confirmed after 2 years of entacapone therapy.

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Table 1 Mean UPDRS (item III) score during entacapone therapy

Time (months)	0	3	6	12	18	24
Score mean value	38±6	20±4	19±2	21.5±3	20.7±3	21.3±5
p versus baseline		<0.001	<0.001	<0.001	<0.001	<0.001

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Neurol Sci (2003) 24:199–200
DOI 10.1007/s10072-003-0129-8

Cerebrospinal fluid biochemical markers in early detection and in differential diagnosis of dementia disorders in routine clinical practice

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Abstract Measurement of total tau and amyloid $\beta_{(1–42)}$ (Ab42) in cerebrospinal fluid (CSF) improves diagnostic accuracy of Alzheimer's disease (AD). We examined a consecutive patient sample referred to our center for diagnostic assessment of cognitive decline. CSF tau and Aβ42 were assayed each week as routine neurochemical analyses. There were 119 patients investigated. These included 61 with probable AD (35 mild AD, 26 severe AD), 24 with mild cognitive impairment (MCI), 14 with vascular dementia, 11 with Lewy body dementia, and 9 with fronto-temporal dementia. Mild AD showed significantly lower CSF Aβ42 levels and significantly higher CSF tau levels than the other diagnostic groups; 79% of MCI patients had pathological values for both biomarkers. We confirm that these biomarkers have a role in the clinical work-up of patients with cognitive deficits.

The clinical diagnosis of Alzheimer's disease (AD) substantially relies on the exclusion of other causes of dementia. There is a great need for biochemical diagnostic markers (biomarkers) that could aid in the diagnosis of AD early in the course of the disease. During the last few years, cerebrospinal fluid (CSF) biomarkers for AD have gained increased attention, since CSF likely reflects the biochemical changes taking place in the brain, and a biomarker should have a direct relation to the central pathogenetic processes of the disease. As the molecular pathogenesis of AD is better known that those of other dementia disorders, research and development of CSF biomarkers for dementia have focused on AD. In this respect, the two CSF biomarkers that have been most extensively studied and have proved to have the highest clinical diagnostic potential are CSF total tau and CSF Aβ42 [1, 2]. CSF total tau is markedly increased in AD [3, 4] and its ability to discriminate between AD and normal ageing is above 80% in most studies; the increase of CSF tau in AD is found in the earlier stages of dementia, which makes this measurement useful for early diagnosis. CSF Aβ42 is consistently decreased in AD; its measurement reflects cerebral amyloid deposition. Combining the measurement of both biomarkers, the predictive value for AD is greater than 90% [5].

Besides the reports on research series, a very few studies have been published on the routine assessment of these parameters in clinical practice. We report here our experience from the Memory Clinic, Alzheimer Center, of a consecutive series of 119 subjects referred for diagnostic evaluation of cognitive impairment in the period January 2000 to January 2002. All underwent lumbar puncture after informed consent. All patients were diagnosed following the internationally accepted criteria. There were 61 patients with probable AD (25 male, 36 female, aged 60–80 years, 52% of the whole sample), 24 with mild cognitive impairment (MCI, 10 male, 14 female, aged 58–70 years, 20% of the sample), 14 with vascular dementia (VaD, 8 male, 6 female, aged 65–80 years, 12% of the sample), 11 with Lewy body dementia (LBD, 6 male, 5 female, aged 68–75 years, 9% of the sample), and 9 with fronto-temporal dementia (FTD, 5 male, 4 female, aged 60–75 years, 7% of the sample). The AD group was subdivided into mild AD (MMSE score >14, 35 patients) and severe AD (MMSE score <14, 26 patients). The level of CSF tau was determined routinely each week using an ELISA (Innotest h TAU-Ag, Innogenetics NV, Gent, Belgium), which measures both normal and phosphorylated tau. The level of CSF Ab42 was determined using an ELISA (Innotest β-amyloid $_{1–42}$, Innogenetics) specific for Aβ42. Values of CSF Aβ42 >1 200 pg/ml and values of CSF tau <200 pg/ml were considered normal.

Table 1 shows the mean values of CSF biomarkers in each group studied. CSF Aβ42 was significantly lower in mild AD compared with MCI and VaD, while the lowest values of this biomarker were found in severe AD and LBD. The highest values of CSF tau were observed in the two AD subgroups. All the groups considered (MCI, VaD, LBD,

Table 1 Mean values (±SD) of cerebrospinal fluid (CSF) biomarkers in the different groups studied

	mild AD (no. 35)	severe AD (no. 26)	MCI[a] (no. 24)	VaD (no. 14)	LBD (no. 11)	FTD (no. 9)
CSF Aβ42	397±100	255±97*	742±400**	550±200***	260±177	500±200
CSF tau	590±300	696±292	420±290*	357± 200***	270±190*	340±200*

AD, Alzheimer's disease; *MCI*, mild cognitive impairment; *VaD*, vascular dementia; *LBD*, Lewy body disease; *FTD*, fronto-temporal dementia
*p<0.05, **p<0.01, ***p<0.02 versus mild AD
[a]19/24 (79%) showed pathological values of both biomarkers

FTD) showed significantly lower values of CSF tau than mild AD. It is interesting that in MCI, which is not yet a dementia syndrome, 79% of subjects showed pathological values of both biomarkers.

Discussion

We evaluated CSF tau and CSF Aβ42 as diagnostic markers for AD in clinical practice. We found a statistically significant increase in CSF tau and a decrease in CSF Aβ42 levels in mild AD with respect to the other disorders considered. This finding is important, since it shows the sensitivity of these measurements in the early stage of the disease, improving diagnostic accuracy. Furthermore, two-thirds of individuals with MCI, which is a condition of cognitive decline not meeting the diagnostic criteria for dementia, have pathological levels of both biomarkers. This finding confirms a previous study reporting that 14 of 16 MCI subjects who converted to AD had baseline pathological values of these biomarkers [6]. This might be very important when drugs affecting the progression of the disease will be available.

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Neurol Sci (2003) 24:201–202
DOI 10.1007/s10072-003-0130-2

A paradoxical acute effect of levodopa in *de novo* parkinsonian patients: worsening of some bradykinetic components

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Abstract Reports of alterations of reaction times (RTs) in Parkinson's disease are often discordant, particularly when the aim of the research is investigation of the relationship between levodopa (LD) administration and RTs. Slowing of simple RT in a group of *de novo* parkinsonian patients 30–90 min after administration of LD (Madopar 250) was recently reported. This temporary phenomenon was attributed to a sedative effect of LD. Our present study aimed to repeat these investigations using Multiple Delayed Reaction Verbochronometry (MDRV). We conclude that such a slowing is not a temporary phenomenon but may represent the increased time necessary for the subject to adequately perform the reaction tasks.

Many studies have investigated the relationship between Parkinson's disease (PD) and reaction times (RTs). However, the results have often been discordant. In the majority of these studies, the experimental paradigm consisted of two types of immediate reaction. The first one is the Simple Reaction (SRT), in which the stimulus presented (in general visual or auditory) is always the same and the subject has to respond as soon as she/he perceives it. Usually, in order to respond, the subject presses a button or moves a lever. The second type is the Choice Reaction (CRT), in which one of two or more stimuli is presented randomly to the subject. The subject has to respond in different ways as soon as she/he perceives the different stimuli.

In PD, some researchers have found that slowing only relates to SRT [1]. They thus supposed a deficit in the movement programming. In contrast, other authors found that CRT are also slowed in PD [2]. They accordingly hypothesized a deficit of movement execution.

More-specific studies have tried to relate the administration of levodopa (LD+ID) to possible variations in RTs. While some researchers described an improvement of RTs in PD patients after administration of LD+ID [3], others did not [4].

Recently, Müller et al. [5] observed a slowing of SRT in a group of *de novo* parkinsonian patients 30–90 min after administration of LD (Madopar 250) plus ID. They attributed this temporary phenomenon to a sedative effect of LD.

Such heterogeneous results could depend on numerous factors: the use of different criteria to select the experimental samples, differences in types of stimuli and responses requested, inclusion of subjects with mild cognitive impairment or depression, or using other drugs in addition to LD.

Our study aimed to reproduce the observations of Müller et al. [5] with *de novo* patients in order to evaluate his interpretation of the RT slowing. We applied the Multiple Delayed Reactions Verochronometry (MDRV) [6], a methodology for studying verbal RTs. An acoustic warning signal (WS) is given and after 80–120 ms a picture ("wall" or "sea") appears for 2 s on a computer screen in front of the subject (at a distance of approximately 70–90 cm). The subject has to name the picture as soon as she/he perceives it. The signal acquisition system records the vocal response (acousticogram or ACG) acquired by a microphone near the subject's mouth (30 cm) and the electromyographic response (EMG) from the *orbicularis oris* muscle. For ACG and EMG, the system analyzes both latency time and duration. It also records the time between the disappearance of one picture and the pressing of the button (B), which starts the successive WS (Interstimuli Interval, Int Int).

Our experimental paradigm was constituted as follows: (1) SRT (24 steps with "wall" picture); (2) CRT (12 steps with "wall" picture plus 12 steps with "sea" picture in a randomized sequence). In these tests the start button (B) was pressed by the experimenter, leaving 2–3 s between one step and the next. We added a third task, namely "prolonged reactions with active determination of interstimuli interval" (50 steps with "wall" plus 50 steps with "sea" in a randomized sequence), in which it was the subject who had to press B each time as soon as possible. Each task was preceded by verbal instructions and by two learning steps (excluded from statistical analysis).

Using this methodology, we studied two *de novo* PD patients before, and at 1 and 5 h after administration of Madopar 250 (1 male, 75 years old; 1 female, 72 years old). One PD patient in the initial stages of LD therapy, after >12 h wash-out, was studied before, and at 1 and 5 h after placebo (male, 75 years old). Two chronic PD patients, on long-term LD monotherapy and after >12 h wash-out, were studied before, and at 1 and 5 h after administration of Madopar 250 (males, 61 and 73 years old). Two normal subjects were studied before and at 1 and 5 h after placebo (females, 74 and 33 years). One normal subject was studied before, and at 1 and 5 h after administration of Madopar 250 (male, 41 years). All PD patients showed an akinetic-rigid form of PD and were not taking drugs other than LD.

At baseline (i.e., before administration of Madopar 250), the two *de novo* patients showed slower RTs in SRT than in CRT. The parameter that was most variable under the different experimental conditions was Int Int in prolonged reactions. This increased significantly (t-test, $p<0.0001$) in the

most-serious *de novo* patient (UPDRS 50, stage 3) 1 h after ingestion of LD. The second *de novo* patient (UPDRS 35, stage 2.5) showed an increase of Int Int near the level of statistical significance ($p<0.066$). In both cases, the Int Int returned to baseline levels 5 h after ingestion of LD. In chronic PD patients we observed a decrease in Int Int 1 h after Madopar 250. In other conditions, Int Int did not show any increase or decrease.

De novo patients presented an increased EMG duration (only in prolonged reactions and 1 h after LD ingestion) due to the persistence of motor unit recruitment after the execution of the verbal response. On clinical examination, no subject showed increased akinesia.

Discussion

The performances of our *de novo* patients at baseline, with a greater worsening in SRT than in CRT, suggest that PD causes a deficit in programming of movement and not in its execution. To evaluate the correlation between RTs and administration of LD+ID, we analyzed the parameters Int Int and EMG duration. The increase of Int Int was observed only in *de novo* PD patients (not in chronic patients or in normal subjects), and it was not accompanied by a worsening of bradykinesia. Hence, this subclinical increase may be attributed only with difficulty to a generic sedative effect of LD.

The effect was most evident in the most-serious *de novo* patient. It seems likely, therefore, that the rate of the slowing is related to the degree of motor deficit at the start of pharmacological treatment.

The lengthening of EMG is similarly limited to *de novo* patients and to the prolonged reactions task. We can hypothesize that the increase of Int Int is a necessary consequence of the temporary increase of the time required (at a peripheral executive level, measured by EMG) to pass from one motor act to the next.

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Neurol Sci (2003) 24:203–204
DOI 10.1007/s10072-003-0131-1

Caregiving and Parkinson's disease

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Abstract Relatively little has been published in the international literature concerning the caregiving-related problems associated with Parkinson's disease. We therefore undertook two exploratory studies that have allowed us to identify the needs and specific problems perceived by such caregivers in both qualitative and quantitative terms.

Introduction

Only a little more than 1% of the published papers concerning Parkinson's disease refer to caregiving-related problems. These highlight the presence of tiredness, fatigue, and depression associated with the more-advanced phases of the disease [1–3], a qualitative worsening in conjugal relationships [4], and financial problems [5].

The aim of this study was to explore the problems perceived by the people looking after patients with Parkinson's disease regardless of the stage of the disease. Two samples were recruited: the first consisted of 25 spouse-patient couples, and the second of 57 caregivers, mainly consisting of the spouse/children of the patient.

Methods and results

The 25 couples underwent an individual, audiotaped semi-structured interview covering the following areas: the impact of the diagnosis and reactions to the disease; disease-induced changes in terms of work, everyday activities, and relationships with spouses, children, and other people; economic problems; drug management; and expectations. The 57 caregivers, mainly spouses (58%) and children (36.8%), 35.7% of which were the only caregiver, were administered questionnaires designed to assess emotional instability (as a personality trait), emotional distress, problems related to social involvement, the need to understand the disease, the quality of family relationships, thoughts of death, and the degree of satisfaction with their own lives [6, 7].

The results of the first study showed that the spouses and patients have similar needs (summarized in Table 1).

The statistical significances emerging from the correlational and comparative analyses in study 2 show that the need for more information is mainly felt by the caregivers who perceive greater emotional distress (p=0.05); positive family relationships correlate with a general satisfaction with one's own personal life (p=0.05) and therefore with greater flexibility in adjusting to modified roles.

Table 1 Categorization of the needs of couples (study 1)[a]

A need to elaborate the disease cognitively and affetively The need to be informed about the therapeutic and evolutionary aspects of the disease, and a simultaneous tendency to "live day by day" in order to adjust oneself gradually to the progression of the disease and protect oneself against the fear of worsening
A need to play a role The need to identify oneself and be identified as playing one's usual roles and the new roles related to the disease
A need to share and compare The need to meet people facing the same type of situation
A need for support The need for support expressed in socio-familial terms of feelings, esteem, and approval
A need for spirituality The need to trust in a dimension that transcends reality in order to be able to accept and hope

[a]After an analysis of the concordance between two independent observers

The subjects characterized by emotional instability perceive more problems during the process of caregiving: they are more anxious, more depressed, and have difficulties relating to social involvement (p=0.01).

Thoughts of death may be experienced with simultaneous feelings of fear and guilt. Female caregivers perceive a greater emotional burden than their male counterparts (p=0.002); no differences were found between the patients' spouses and children.

Discussion

Although the results of these exploratory studies are limited by the small size of the samples, they do make it possible to draw some general conclusions. The patients with Parkinson's disease and their spouses have similar needs: in particular, they would like to have an increasingly better knowledge of the disease (in relation to the real clinical situation of the patients), and seek the recognition and confirmation of their reciprocal roles. The use of validated questionnaires makes it possible to refine our knowledge perceived by the needs perceived by caregivers, thus highlighting a target population for more-economic socio-health interventions.

The differences found between male and female caregivers, and the absence of differences between spouses and children, must be considered with caution, because other studies have shown that male caregivers are more fragile over the long term and that caregiving spouses have more psychosocial problems than caregiving children [8].

Further studies must certainly consider the stage of the disease and identify more-precise relationships between the disease stage and caregiving problems. However, when mak-

ing the transfer, it is worth bearing in mind that the caregivers who have more difficulties are not always those who have to look after the most-impaired patients, and that socio-psychological screening independent from, but integrated with, a medical evaluation can improve the efficacy of interventions aimed at patients and their caregivers.

Acknowledgements The authors would like to thank Kevin Smart, Link SrL, for his linguistic help in the preparation of the text.

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Neurol Sci (2003) 24:205–206
DOI 10.1007/s10072-003-0132-0

Hedonistic homeostatic dysregulation in Parkinson's disease: a short screening questionnaire

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Behavioural abnormalities and cognitive impairment frequently complicate long-standing Parkinson's disease (PD), a neurodegenerative disorder usually characterized by its movement abnormalities. Several factors are implicated in the development of non-motor symptoms of PD, including the underlying neurodegenerative process related to the disease, psychological reactions to the physical illness, and the effect of medications. Hedonistic homeostatic dysregulation (HHD) is neuropsychiatric disorder recently described in PD [1]. It occurs mainly in young-onset male patients with PD, and it is linked to substance misuse and addiction to dopaminergic replacement therapy (DRT) [2]. The core clinical features of HHD are self-medication, drug hoarding, and alteration of the perception of the on state; these patients may develop violent and disabling but surprisingly well-tolerated drug-induced dyskinesia, which does not act as a deterrent to further increases in the level of DRT. Walkabouts are common in these patients, who also present a wide range of mood disorders, including depression, anxiety, hypomanic state, euphoria, and psychosis.

Behavioral disorders, such as pathological gambling [3], obsessional shopping, hypersexuality, aggression, and social isolation make the management of these patients more difficult and urgent, since they significantly affect their own and their families' quality of life.

HHD is not common and the only prevalence figure reported in literature (4%) [1] is probably biased, as the studied population belonged to tertiary and quaternary referral clinics. However, since milder forms with many features of the dysregulation may occur in PD, the real prevalence is difficult to ascertain. Hence, in order to study the prevalence and the characteristic features of HHD, we have designed a brief screening questionnaire (Table 1) that was administered for 6 months to consecutive PD patients attending our movement disorder unit.

The questionnaire consists of three parts: demographic data (name, surname, sex, age, duration of disease, Hoehn and Yahr score); presence of dyskinesias and percentage of the day taken up with dyskinetic periods; five questions (yes or no) about (1) self-medication and extra doses of DRT in the last 3 months, (2) mood disorders, (3) behavioral disorders with emphasis on aggressive and violent behavior, (4) compulsive behaviors, such as compulsive

Table 1 Hedonistic homeostatic dysregulation – short screening questionnaire

SURNAME . . . . . DATE OF BIRTH . . . . . / . . . . . / . . . . .

NAME . . . . . DATE OF VISIT . . . . . .

DIAGNOSIS . . . . . .

THERAPY . . . . . .

HOEHN AND YAHR SCORE: . . . . . .

DISEASE DURATION . . . . . .

DYSKINESIA:
NONE
PRESENT:
UP TO 25% . . . . . 50% . . . . . 75% . . . . . DAY-TIME . . . . . .

1. ARE DRUG HOARDING AND/OR AUTOMEDICATION PRESENT ?
IF YES, HOW MANY TIMES A DAY AND HOW MUCH

2. ARE MOOD DISTURBANCES PRESENT?

3. IS VIOLENT BEHAVIOR, AGGRESSION, OR SOCIAL ISOLATION PRESENT?

4. IS COMPULSIVE BEHAVIOR, SUCH AS COMPULSIVE SHOPPING AND/OR COMPULSIVE GAMBLING PRESENT?

5. HYPERSEXUALITY AND/OR SEXUAL INADEQUACY?

shopping and gambling, (5) hypersexuality, with special emphasis on inappropriate behaviour, exhibitionism, excessive use of sex phone lines, prostitution services, and sex shops. We aim to select patients according to the following criteria:

– Parkinson's disease with documented levodopa responsiveness
– Need for increasing doses of DRT in excess of those normally required to relieve parkinsonian symptoms and signs
– Pattern of pathological use of DRT

and

– Mood disorders: depression, anxiety, hypomanic state, euphoria

or

– Behavioral disorders: pathological gambling obsessional shopping, hypersexuality, aggression, social isolation

or

– Alteration of the perception of the on state, walkabouts

We will compare the clinical features of the identified patients with a group of control PD patients matched for age, sex and disease duration.

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Neurol Sci (2003) 24:207–208
DOI 10.1007/s10072-003-0133-z

Nocturnal anomalous movement reduction and sleep microstructure analysis in parkinsonian patients during 1-night transdermal apomorphine treatment

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Abstract This study analyzed the macrostructure and microstructure of sleep in 12 parkinsonian patients under basal conditions (T0) and during 1-night treatment (T1) with a new formulation of apomorphine. This new formulation consisted in a microemulsion of apomorphine administered by the transdermal route, able to provide a constant release of the drug over several hours (APO-TD). Sleep analysis at T1 compared with T0 revealed a 16% increment of total sleep time, a 12% increment of sleep efficiency, a 16% increment of stage 3 and 4 non-REM sleep, a 15% reduction of periodic limb movements index, a 22% reduction of arousal index, and a 23% reduction of cycling alternating patterns/non-REM. We conclude that APO-TD may be able to reduce nocturnal anomalous movements, akinesia, and rigidity in Parkinson's disease, and may reduce the disturbed sleep typical of Parkinson's disease.

Introduction

Sleep in Parkinson's disease is frequently disrupted. There may be several anomalous movements, such as periodic limb movements (PLMs), persistence of tremor and prolonged tonic contractions of limb muscles during non-REM sleep, prolonged elevations in muscle tone during REM sleep, REM behavior disorders, and reduction of normal body shifts during sleep. These sleep disorders may lead to awakening and microarousal, and may cause sleep fragmentation and excessive daytime somnolence [1, 2]. Analysis of sleep microstructure, including cycling alternating patterns (CAP), may provide an index of sleep maintenance and is an objective measure of disruption of sleep [3, 4].

The aim of this study was to analyze the anomalous movements during sleep and the alterations of sleep microstructure (CAP sequence analysis, microarousals) in a group of parkinsonian patients during 1-night treatment with a new formulation of apomorphine. This new formulation is a microemulsion-based drug delivery system, administered by an epicutaneous-transdermal route (APO-TD). This formulation provides a constant release of the drug for several hours, as demonstrated in vitro with hairless mouse skin [5], and may become particularly useful for night treatment of motor fluctuation in parkinsonian patients.

Materials and methods

We selected 12 consecutive patients with idiopathic Parkinson's disease, according to the following inclusion criteria: age between 55 and 75 years, stage III–IV Hoehn-Yahr, presence of long-term L-dopa syndrome characterized by "wearing-off" or predictable "off" periods, and a positive response to subcutaneous apomorphine test without severe side effects. The mean age was 62.3 years, the mean duration of illness 6.3 years, and the mean Hoehn-Yahr stage 3.2. The daily mean L-dopa dosage was 584 mg.

The patients underwent standard polysomnography under basal conditions (T0) and during nocturnal treatment with 50 mg of APO-TD applied to a 100-cm^2 cutaneous area on the chest, from 10 p.m. until 8. a.m. (T1). During this period blood samples were collected at 3-h intervals from 10 p.m. for 12 h. The apomorphine concentration was then analyzed by high-performance liquid chromatography. Anomalous sleep movements, and macrostructure and microstructure of sleep were analyzed; CAP rate (total CAP time/total non-REM time) was calculated.

Results

Pharmacokinetic analysis confirmed the absorption of apomorphine and the maintenance of therapeutic plasma levels for several hours (mean C_{max} 31.8±9.7 ng/ml, mean T_{max} 3.1±1.6 h, mean half-life absorption 1.2±1.4 h, mean half-life of elimination 8.8±1.9 h).

Sleep macrostructure analysis at T1 compared with T0 showed a 5% reduction of sleep onset latency (not significant), a 16% increment of total sleep time (p=0.03), a 12% increment of sleep efficiency (p=0.04), and a 16% increment of stage 3–4 non-REM duration (p=0.04). In contrast, no variation of stage 1–2 non-REM and REM phase duration was noticed.

Persistence of tremor and prolonged tonic contractions of limb muscles during non-REM 1–2 sleep stages were present in a high percentage of patients (75% and 83.3%, respectively). All patients showed a reduction of these disorders at T1 compared with T0, although statistical significance was not reached; 66.6% of patients presented PLMs. In these

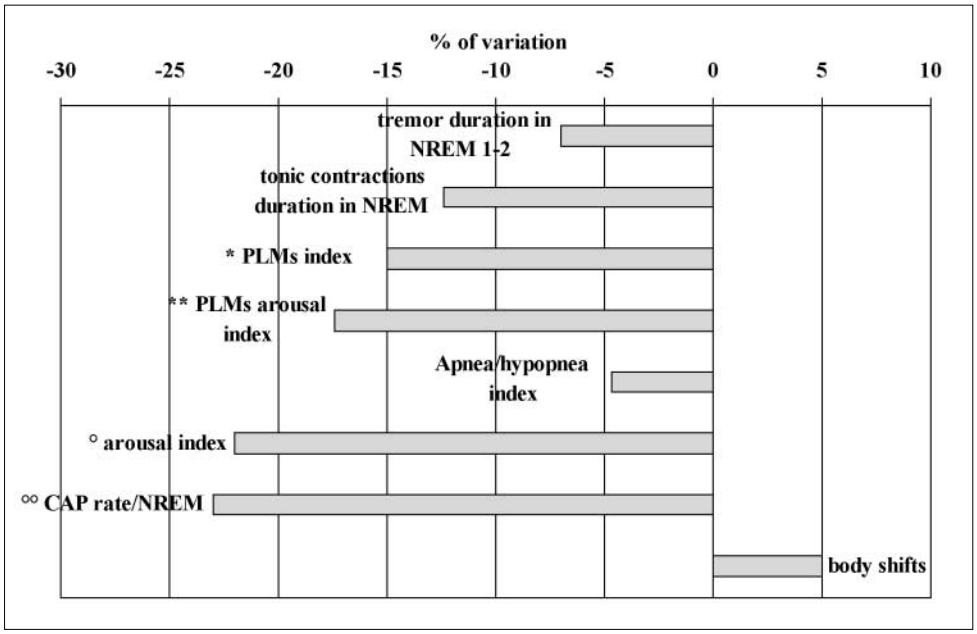

Fig. 1 Nocturnal anomalous movements reduction and sleep microstructure analysis during APO-MT treatment (T1) compared to basal condition (T0). **p*= 0.03; ***p*=0.02; °*p*=0.03; °°*p*=0.01

patients, the PLMs index and the PLMs index associated with arousals showed a significant reduction at T1 compared with T0 (15%, p=0.03 and 17%, p=0.02, respectively). Apnea/hypopnea index was below 5 at T0 in all patients, and did not show any significant variation at T1. Interestingly, during APO-TD treatment, arousal index and CAP rate/non-REM showed a significant reduction (22%, p=0.03 and 23%, p=0.01, respectively). Body shifts appeared to be increased (5%), but this variation did not reach statistical significance. In our study APO-TD overall tolerability was good. No hallucinations, vivid dreams, or nightmares occurred. Only 1 patient on awakening the following morning presented transient nausea controlled with domperidone. Five patients had a transient mild erythema at the site of APO-TD application, with complete regression within 48 h.

Discussion

Our study suggests that APO-TD treatment during the night may reduce anomalous sleep movements in Parkinson's disease. In our group of patients, persistence of tremor and prolonged tonic contractions of limb muscles during non-REM sleep were reduced, while body shifts were increased. This may be due to the prolonged dopaminergic stimulation provided by APO-TD, with a reduction of akinesia and rigidity during the night hours. Similarly the PLMs index showed a significant decrease, thus reducing one of the possible sources of broken sleep, as demonstrated by the reduction of the PLMs index associated with arousals.

This improvement of motor conditions during sleep may explain the significant decrease in the arousal index and CAP rate/non-REM, and the significant increment of sleep stage 3–4 non-REM and sleep efficiency. In particular, the CAP rate/non-REM decrease may be considered an objective index of the reduction of sleep instability in these patients.

In conclusion, our study suggests that nocturnal transdermal slow-release apomorphine treatment in Parkinson's disease, giving a constant absorption of the drug for at least 12 h, may be able to reduce nocturnal anomalous movements, akinesia, and rigidity. Hence it may be efficacious in reducing sleep instability typical of the fragmented parkinsonian sleep.

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Neurol Sci (2003) 24:209–210
DOI 10.1007/s10072-003-0134-y

Health-Related Quality of Life and sleep disorders in Parkinson's disease

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Abstract Parkinson's disease (PD) is a chronic, progressive, disabling movement disorder with a clear impact on Health-Related Quality of Life (HRQoL). We investigated the correlations between HRQoL and sleep disorders measured with the Parkinson's disease Sleep Scale (PDSS) and the motor and non-motor aspects of the disease. A correlation was found between HRQoL and the scores from PDSS, motor and depression scales. We conclude that more attention should be paid to the non-motor aspects of PD to attempt to improve HRQoL.

Introduction

Health-Related Quality of Life (HRQoL) refers to a diverse range of the patients' own perceptions and experiences of their disease and could be considered as the ultimate outcome measure of health care, beyond single symptoms. Parkinson's disease (PD) is a chronic, progressive, disabling neurodegenerative disorder that affects up to 2% of the population over 65 years, does not significantly shorten life expectancy, involves both motor and non-motor symptoms, has a high incidence of comorbidity, and for which only symptomatic treatment is currently available. Although drug therapy alleviates parkinsonian symptoms, this response is later complicated by motor fluctuations and dyskinesias. The progression of the disease and the long-term dopaminergic therapy leads to symptoms such as behavioral and mood disturbance, cognitive impairment, psychosis, sleep disorders, pain, and autonomic dysfunction. Up to 80% of patients with PD complain of sleep fragmentation, insomnia, and nocturia. About half of patients have excess daytime sleepiness and unintended sleep episodes [1]. Depression is also a major feature of PD, can affect 40% of patients, and can be a presenting symptom in up to 25% of cases [2].

HRQoL has, until recently, been a scarcely studied aspect in patients with PD [3] and little attention has been paid to night-time sleep disturbances. This prompted us to investigate the impact on HRQoL of sleep disturbance and depression in PD, beyond the purely motor aspects of the disease.

Patients and methods

The patients all attended the Parkinson's Disease Clinic of the University Hospital in Padua (Italy). They included 77 patients, 34 male and 33 female, aged between 53 and 78 years with a disease duration between 2 and 16 years and Hoehn and Yahr stages between 1.5 and 4. The inclusion criteria were those of the UK Parkinson's Disease Brain Bank.

The scales used were: Unified Parkinson's Disease Rating Scale (part III), Parkinson's disease Sleep Scale (PDSS) to investigate the night-time symptoms, Epworth Sleepiness Scale (ESS) for the daytime sleepiness, Hamilton Depression Rating Scale (HDRS) for the mood disturbance, MMSE for cognitive function, and Parkinson's Disease Questionnaire (PDQ) 39 for the HRQoL. This last scale is the only HRQoL scale that has been specifically designed to study HRQoL in PD.

PDQ-39 consists of 39 questions each with 5 different answers (never, occasionally, sometimes, often, always) and explores 8 different subgroups; mobility, activities of daily living, emotional well-being, stigma, social support, cognition, communication, and bodily pain [4].

PDSS consists of 15 questions on commonly reported sleep disturbances and differs by being a visual analogue scale. It surveys six overlapping domains, insomnia and sleep fragmentation, motor symptoms, neuropsychiatric symptoms, nocturnal restlessness, dystonia, pain, urinary symptoms, and daytime sleepiness [5]. For statistical analysis the Spearman correlation coefficient was used.

Results

When the correlation coefficients were analyzed, the following correlations were found: age correlated with Hoehn and Yahr stage (p=0.002, r=0.47), UPDRS (p<0.001, r=0.55), and MMSE (p<0.001, r=-0.52).

Disease duration correlated with Hoehn and Yahr stage (p<0.00001, r=0.71) and UPDRS (p<0.001, r=0.52). UPDRS motor scores correlated with MMSE (p=0.003, r=-0.46) and PDQ 39 scores (p=0.001, r=0.50) (Fig. 1).

HDRS scores correlated with Hoehn and Yahr stage (p=0.001, r=0.51) and UPDRS (p<0.001, r=0.47) and with PDQ 39 scores (p<0.003, r=0.47) (Fig. 2). A lower correlation was found between PDSS scores and PDQ 39 scores (p<0.05, r=-0,39) (Fig. 3).

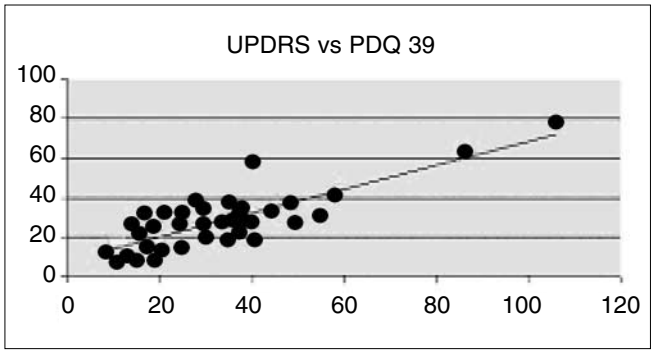

Fig. 1 UPDRS versus PDQ 39 scores

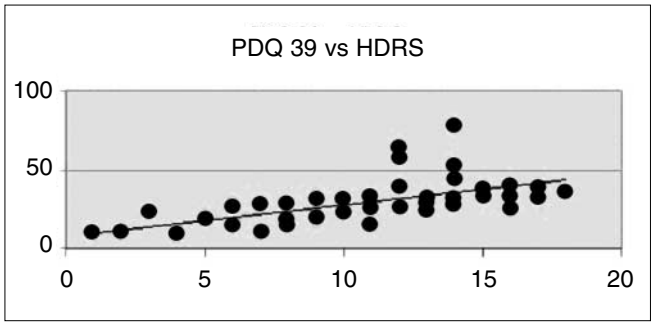

Fig. 2 HDRS versus PDQ 39 scores

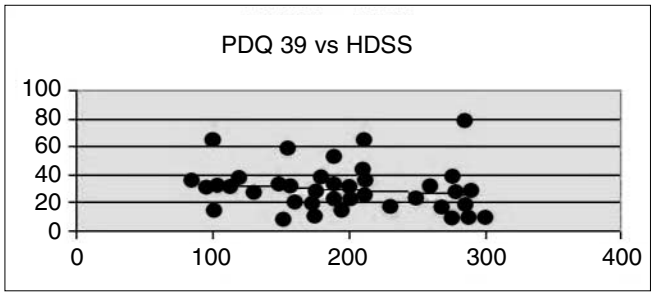

Fig. 3 PDQ 39 versus PDSS scores

Discussion

PD has a significant impact on HRQoL and its evaluation is complex. It is a mainly motor disorder and this is reflected by the results of our study, which show significant correlations between PDQ 39 scores and UPDRS motor scores, confirming the data reported in the literature and highlighting the importance of mobility. Nevertheless, non-motor symptoms are frequently underestimated. Depression has a higher than expected frequency and correlates with HRQoL scores.

The UPDRS motor scores correlated with the subscores of the PDSS, in which the motor aspects could play a role, such as the inability to remain asleep, due possibly to the lack of mobility, or the difficulty in reaching the bathroom during the night. This aspect is also reflected in the correlation between the PDQ 39 scores and the night-time urge incontinence. We acknowledge that the relatively small number of patients, from a single center, could bias the results and alter their statistical significance.

When the data from the single items of the PDSS were analyzed, few correlations with HRQoL scores were found. The item that correlated best with these scores was incontinence due to lack of mobility. This item, although giving a single score, is influenced by two domains, motor and urinary, reflecting both the patients' need to reach the bathroom and their difficulties in doing so. The fact that one question investigates more than one domain reflects the difficulty of formulating such a scale. One scale in not sufficient to cover all aspects of HRQoL, but increasing the number of questions would only make the questionnaires harder to complete by the patients. Other aspects, such as care-giver strain, which play an important role, especially in advanced disease, are not covered.

More attention should be paid to the treatment of depression, which along with the treatment of specific non-motor symptoms, should help to improve HRQoL in patients with PD.

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Neurol Sci (2003) 24:211–212
DOI 10.1007/s10072-003-0135-X

Progressive dysarthria: definition and clinical follow-up

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Abstract Progressive dysarthria is a common sign of several degenerative disorders of the central nervous system; it may also be a distinct nosographic entity. We identified nine patients in which progressive dysarthria remained the sole neurological sign for at least 2 years after onset. At least a year after hospital admission, the following diagnoses were made: two cases of corticobasal degeneration, one of frontotemporal dementia, one of primary progressive aphasia, one of motor neuron disease (MND)-dementia, one of ALS, and one of ALS-aphasia. In the remaining two patients progressive dysarthria remained the only neurological sign at latest examination. We conclude that in most cases progressive dysarthria is the presenting sign of an established neurodegenerative disease (generally degenerative dementia or motor neuron disease), although the possibility that progressive dysarthria is a distinct entity cannot be excluded. To clarify this issue, studies (probably multicenter) on more patients with longer clinical follow-up and pathological confirmation are required.

Progressive dysarthria may be a sign of several neurodegenerative diseases, but has been described as a unique disorder in a few cases [1, 2]. We retrospectively examined all patients admitted to our department over a 5-year period in which progressive dysarthria was the only sign for at least 2 years following onset. The patients were followed for at least a year following admission. We therefore excluded acute-onset cases, those associated with other neurological signs from onset, and those in whom dysarthria was due to peripheral lesions (flaccid dysarthria) or cerebral lesions confirmed by imaging.

We identified nine patients (six women, three men) complying with these criteria. On admission the mean age of these nine patients was 65.2±13.7 years (range 30–75 years) and mean duration of dysarthria 3.2±1.3 years (range 2–6 years). At first examination all had tongue hypomobility and dysprosody, and all but two had by then developed other neurological signs. These were bradykinesia, rigidity, and anomic language in two patients; mental decay (including aphasia, hemispatial neglect, and alien limb), behavioral alteration, and pseudobulbar signs in one patient; motor aphasia in another patient; and a combination of tongue hypotrophy, behavioral modification (mostly apathy), tongue and vocal tremor, hyperreflexia, and aphasia in three other patients. In spite of the additional signs in these seven patients the diagnoses were unclear at first examination.

At latest follow-up, at least a year after admission, two patients met the clinical criteria for possible corticobasal degeneration [3]. They had slightly asymmetric parkinsonism, initial mental decay, and aphasia. On cognitive examination, limb kinetic apraxia, orofacial apraxia, and nonfluent aphasia were evident, in addition to mild subcortical dementia. Cerebral magnetic resonance imaging (MRI) revealed symmetric fronto-parietal atrophy in one patient and symmetric parietal atrophy in the other.

Another patient was diagnosed with probable fronto-temporal dementia [4]. Clinically he had pervasive mental decay with aphasia and behavioral disturbances, however he also had pseudobulbar palsy and vertical oculomotor paresis. Neuropsychological examination demonstrated frontal dementia with ideomotor and orofacial apraxias, nonfluent aphasia, hemispatial neglect, and alien limb phenomenon. Brain MRI revealed asymmetric fronto-temporal-parietal cortical atrophy that was greater on the right.

Another patient was diagnosed with primary progressive aphasia [5]. In addition to dysarthria, he had moderate nonfluent aphasia and orofacial apraxia, but no other neurological signs or cognitive deficits. MRI was nonspecific, showing only mild intraparenchymal vascular alterations. Cerebrospinal fluid (CSF) was normal.

In three other patients we diagnosed motor neuron disease (MND) [6]. One of these patients developed disinhibition, poor judgement of what was appropriate, aphasia, and tongue hypotrophy with fasciculations and dysphagia. Neuropsychological examination revealed frontal dementia, perseverations, orofacial apraxia, and anomic aphasia. Brain MRI showed diffuse cortical and brainstem atrophy associated with bilateral putamen hypointensity, while electromyography (EMG) showed spontaneous activity on the first dorsal interosseous muscle and oral orbicular muscle. We decided on a diagnosis of ALS-dementia.

The second case of ALS had a long history of dysarthria and tongue/vocal tremor before developing the evident signs of motor neuron disease. Her neuropsychological evaluation was unremarkable, brain MRI showed hyperintensity (T_2-weighted image) of corticospinal tracts and the motor gyrus was also atrophic; EMG was consistent with motor neuron disease.

The last case of ALS developed aphasia [7] and pseudobulbar signs and palatal myoclonus a few years after the appearance of progressive dysarthria. He eventually also developed tongue hypotrophy and fasciculations. Brain MRI showed a T_2 hyperintense signal on the bulbar olives and superior cerebellar pedunculi consistent with concomitant palatal myoclonus (whose relation to ALS is unclear). Neuropsychological examination confirmed nonfluent apha-

sia and orofacial apraxia, but found no other cognitive defects. CSF was normal; EMG was consistent with motor neuron disease.

For the last two patients our diagnosis is still pending, as they have not developed neurological signs other than the dysarthria. Both their cognitive profiles are normal and brain MRI unrevealing (completely normal in one patient, mild and nonspecific ischemic alterations in the other). In the youngest of these patients (31 years) CSF was normal.

Discussion

As a result of the accumulation of genetic and pathological data, several neurodegenerative diseases are being re-classified. The diagnosis of these conditions is often complicated by the presence of overlapping syndromes or overlapping pathological findings; while other cases may have the clinical profile of one disease and the pathological findings of another [8].

It is debatable whether progressive degenerative dysarthria is a distinct nosological entity. Broussolle et al. [1] described eight patients followed for 6–10 years who exhibited dysarthria, dysprosody, and orofacial apraxia at onset; some progressed to muteness, frontal dysfunction, and involvement of pyramidal tracts [1]. They reported the clinical presentation of slowly progressive dysarthria, SPECT or PET findings of decreased cerebral blood flow and metabolism in the left postero-inferior frontal gyrus and premotor cortex, and nonspecific neuronal loss, gliosis, and spongiosis, mainly in the frontal lobes on autopsy (only two patients). They called the condition slowly progressive anarthria and suggested it should be considered as one of the focal cortical degenerative syndromes.

We identified nine patients with slowly progressive dysarthria, orofacial apraxia, and dysprosody. However, in seven of these other neurological signs eventually developed that permitted the (sometimes tentative) clinical diagnosis of various degenerative diseases.

Table 1 Neurodegenerative diseases that may present as slowly progressive dysarthria

ALS±dementia
ALS+aphasia
Frontotemporal dementia
Primary lateral sclerosis
Primary progressive aphasia
Corticobasal degeneration
Progressive supranuclear palsy
Other nosographic entities?

We suggest that the clinical manifestation of progressive dysarthria is due to alteration at a specific anatomical site during the course of various neurodegenerative changes (Table 1). Such sites may be identified by neuroimaging studies in conjunction with careful neurological examination. Most (6/9) of our patients developed nonfluent aphasia, orofacial apraxia, and dysarthria consistent with dysfunction of the anterior and frontal operculi (Brodmann areas 4, 6, and 44). However, in most of these cases this supposition was not confirmed by imaging studies.

To conclude, our study suggests that most patients with progressive dysarthria eventually develop manifestations permitting (at least tentative) clinical diagnosis of established neurodegenerative entities. We diagnosed corticobasal degeneration, frontotemporal dementia, ALS, and primary progressive aphasia. However, in two patients the diagnosis at present conforms to the "slowly progressive anarthria" proposed by Broussolle et al. [1]. We are continuing follow-up; signs may eventually appear to allow us to decide whether they have lobar atrophies or other degenerative conditions. Clearly longer observations on larger numbers of patients, supported by autopsies, are necessary to determine whether or not slowly progressive anarthria is a nosographically distinct entity.

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Neurol Sci (2003) 24:213–214
DOI 10.1007/s10072-003-0136-9

Distal-proximal differences in limb apraxia in corticobasal degeneration but not progressive supranuclear palsy

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Abstract Limb apraxia is an important diagnostic sign of cortico-basal degeneration (CBD), although it is also found in progressive supranuclear palsy (PSP). We investigated whether the severity of apraxia differed between proximal and distal arm movements in the two diseases, as suggested by their differing patterns of motor impairment. We studied 24 CBD patients, 25 PSP patients, and 19 healthy controls using a battery of cognitive tests and an ideomotor apraxia test that examined imitation of hand and of whole arm gestures separately. CBD and PSP patients did not differ in general characteristics or disability and were similarly impaired in cognitive performance. Within-group differences between distal and proximal gesture scores were significant only for CBD patients (p=0.007), in whom distal movements were more compromised. This finding suggests the presence of limb kinetic apraxia in CBD, perhaps in association with ideomotor apraxia.

Introduction

Cortico-basal degeneration (CBD) and progressive supranuclear palsy (PSP) are neurodegenerative parkinsonisms that do not generally respond to levodopa therapy and can be difficult to diagnose at disease onset. Limb apraxia is present in 70% of CBD patients [1] and is considered one of the most-characteristic signs of the disease, although it has recently been described in PSP patients [2]. Since motor impairment is known to be mainly distal in CBD and mainly proximal in PSP, we investigated whether limb apraxia in the two diseases was influenced by this differing distribution of motor defect. We therefore examined praxis in the whole upper limb, including shoulder (proximal movements), and praxis in the hand and fingers (distal movements) in CBD and PSP patients.

Patients and methods

Twenty-four patients with probable CBD and 25 with probable PSP, diagnosed following commonly accepted clinical criteria [3, 4], were studied. Magnetic resonance imaging findings of asymmetric fronto-parietal atrophy and mesencephalic atrophy were used to support the diagnoses of CBD and PSP, respectively. We also examined 19 normal control subjects matched with the patients in terms of age and education.

Motor disability in daily living was rated in patients using the Schwab and England scale. Depression was assessed in patients and controls using the Beck depression scale. A comprehensive battery of neuropsychological tests was administered to patients and controls to assess global cognitive functioning (MMSE), attention (visual search test), verbal long-term memory (short tale test), logical reasoning (Raven 47 test), visual spatial abilities (Benton's test), shifting and categorization (Nelson's test), and strategic ability to find words (verbal fluency test). To assess limb apraxia we used the De Renzi ideomotor apraxia test [5], which is well suited to evaluating distal versus proximal movements. It consists of 24 items, 12 of which assess hand and finger movements and 12 of which assess movements of the whole upper limb. A total score lower than 53 indicates apraxia, a score greater than 62 excludes apraxia, and a score between 53 and 62 is borderline. To avoid the confounding effect of the motor disorder, we examined limb apraxia in CBD in the less-compromised arm (which in 13 patients was the right and in 11 patients the left arm). In PSP patients (who by definition have relatively symmetric motor compromise) and in controls we tested the right arm in half the cases and the left arm in the remaining half.

Between-group differences were assessed using one-way analysis of variance and the post hoc Scheffè test, while within-group differences were tested using Student t-test for paired samples.

Results

The three groups did not differ in general characteristics and the two patients groups were of similar motor compromise. Mean age was 65.0±8.1 years in CBD patients, 66.1±7.1 in PSP patients, and 64.0±6.4 in control subjects; mean years of education were 8.4±4.4 in CBD patients, 6.6±3.9 in PSP patients, and 9.6±4.9 in controls; illness duration was 3.3±1.3 years in CBD patients and 3.1±1.4 in PSP patients. There was no difference in handedness between the groups. Schwab and England motor disability was 55.5±19.2 in CBD patients and 61.9±16.0 in PSP patients. Both patient groups had a mean Beck depression scale score greater than controls (p=0.01), although still in the normal range (≤ 14), and none had major depression according to DSM IV clinical criteria.

Both patient groups were significantly more compromised in all cognitive tests than control subjects (p<0.002), with PSP patients more impaired than CBD patients in the visual orientation test of Benton and in the categorization test of Nelson (p<0.05). Total apraxia score was significantly worse in CBD than PSP and control groups. CBD patients also had worse apraxia subscores for whole upper limb and hand/finger movements than PSP patients and controls. PSP and control subjects did not differ significantly in any of

Table 1 De Renzi ideomotor apraxia test scores in cortico-basal degeneration (CBD) patients, progressive supranuclear palsy (PSP) patients, and controls

	CBD	PSP	Controls	*p*
Upper limb	25.23±10.16*‡	31.04±5.23‡†	35.63±0.76*†	<0.0001
Hand	21.77±13.43*‡	29.88±5.65‡†	35.95±0.23*†	<0.0001
Total score	47.00±23.19*‡	60.92±9.73‡†	71.58±0.84*†	<0.0001

$p<0.05$ (*CBD vs. C; ‡CBD vs. PSP; †PSP vs. C)

these measures (Table 1). Within-group differences between distal and proximal gesture scores were significant only for CBD patients ($p=0.007$), in whom distal movements were more compromised.

Discussion

Our CBD and PSP patients did not differ in motor disability and had pervasive cognitive impairment in all domains. However, PSP patients were more impaired than CBD patients in the category test of Nelson and visuo-spatial orientation test of Benton, consistent with the greater frontal dysfunction and oculo-motor alteration in PSP in relation to prominent frontal deafferentation and mesencephalic damage.

Ideomotor apraxia scores were significantly worse in CBD than PSP patients and controls. Furthermore, in CBD patients the severity of apraxia differed significantly between distal and proximal forelimbs, a difference not present in PSP patients or controls. The possibility that the bilateral premotor and supplementary motor areas may be involved in control of proximal movements [6], so that the less-affected hemisphere can compensate for defects in the most-affected hemisphere, may explain the relatively minor impairment of proximal compared with distal gestures in CBD.

It has been suggested that limb kinetic apraxia is the most-frequent type of apraxia in CBD and can coexist with ideomotor apraxia [7]. Limb kinetic apraxia consists of impaired, coarse mutilated execution of simple movements of the arm contralateral to the cortical lesion [8]. When the patient is required to make a gesture, the movements are well selected although imprecise; the disorder is more evident when testing single finger movements, and whole movements of the hand are more or less retained. This definition is consistent with our finding that the execution of distal gestures was more impaired than that of proximal gestures in CBD patients. Alternatively, limb kinetic apraxia may be the expression of minor paresis due to damage to the primary motor area and cortico-spinal tracts. Unfortunately, no tests can distinguish minor paresis from ideomotor apraxia or elementary motor defects. In spite of this difficulty in characterizing limb kinetic apraxia, it is common clinical experience that gesture impairment in CBD cannot be explained simply as due to dystonia, rigidity, paresis, or bradykinesia, but appears to be of praxic nature, as suggested by the prominent involvement of premotor as well as motor areas.

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Neurol Sci (2003) 24:215–216
DOI 10.1007/s10072-003-0137-8

Italian Huntington disease patients-data and tissue bank

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Abstract We have collected clinical and genetic data on Huntington disease (HD) patients and their families over the last 5 years at the Unit of Neurogenetics, IRCCS Neuromed of Pozzilli (IS), Italy. Data on 854 mutation carriers are included in the data bank, together with a large number of DNA samples, blood, and other tissues. In particular, lymphoblastoid cell lines from 100 patients, including subjects carrying very rare genetic conditions (CAG mutation homozygosity, juvenile and infantile onset, pre-mutations) have been established. For all these initiatives ethical approval from the bioethics committee was obtained. We wish to extend this initiative to all families, investigators, and institutions within and, possibly outside, the Italian border in an attempt to enlarge the bank and to institute a HD Research Roster.

Introduction

Huntington disease (HD) is a hereditary neurodegenerative illness dominantly transmitted and caused by an expanded mutation beyond 35 CAG repeats, with a low penetrance at the low edge of the pathological range (36–41 CAG) [1, 2]. The disease is characterized by behavioral changes, movement disorders, and cognitive decline, with a variable age of onset ranging between 2 and over 90 years. The phenotype at onset is often characterized by subtle, soft, and unusual motor symptoms, sometimes preceded by severe psychiatric manifestations [2, 3]. The age at onset is influenced by the mutation length, as well as by factors other than CAG, of either genetic or environmental origin [4, 5]. The accurate collection of clinical data therefore represents an essential procedure for allowing genotype-phenotype correlations. We have been collecting data and biological samples from patients with HD and their families for 5 years and have established a computerized clinical, genetic data, and tissue bank at the Neurogenetics Unit of Neurological Institute IRCCS Neuromed in Pozzilli, (IS) Italy.

Methods and results

Patients and families data bank

The patients are currently seen in the outpatient service at Neuromed. Due to the cooperation of the HD Association of Families "Associazione Italiana Corea di Huntington" (A.I.C.H.-Neuromed) (http://strudel.di.unipi.it:8180/sitoweb/index.html), HD families have easy access to association sites in Northern (Milan), Middle (Rome), and Southern (Pozzilli) Italy for collection of information on HD and for medical and counselling support. We review the patients' clinical records and interview their relatives to collect detailed information about the manifestations at onset and clinical course of the disease. The age at onset is calculated according to the first motor symptoms. The age at occurrence of severe psychiatric manifestations modifying the normal life style is also reported in the database. The first symptoms are classified with a code number according to their presentation (either motor or psychiatric). We use the Unified Huntington Disease Rating Scale (UHDRS) for assessment of motor and cognitive behavior, independence, and progression rate [6]. The database includes the following items: date of birth, date of death, patient code, pedigree code, biological sample code (position in the bank, type of specimen), geographic origin, age of onset (motor onset symptoms) and of psychiatric symptoms, difference in years between onset of psychiatric and motor symptoms, sex, affected parent's sex, affected grandparent's sex, age at onset of the affected parent. For reasons of privacy, we do not report the patient's name in the database and assign two different progressive code numbers to each subject defining the subject's family and his/her exact position within the pedigree. An identical code is assigned to the biological samples in the tissue bank. The same code is also reported on the patient's clinical record.

Neuroimaging

A HD neuroimage bank has been set up. Magnetic resonance imaging and FDG-positron emission tomography are currently used to longitudinally study patients at different disease stages. To date, 50 mutation carriers, at either a presymptomatic or affected stage, have undergone these procedures.

Tissue bank

The DNA is extracted from leukocytes and held in TE buffer. The DNA samples of each subject (about 1 μg/μl, samples of 100 ng each) are stored at -20°C in the DNA bank. Lymphocytes are isolated from peripheral blood, immortalized by infection with Epstein-Barr virus, and suspended in RPMI 1640 medium plus 10% fetal bovine serum. Fibroblast and myoblast cell lines are obtained after skin and muscular biopsy.

Ethical approval

We established a predictive testing program in 1998 according to international ethical guidelines [7]. This was formally approved by the bioethics committee. Informed consent was obtained for genetic tests and for blood withdrawal or tissue biopsy. Specific informed consent is required to establish lymphoblastoid cell lines. The bioethics committee formally approved the tissue bank.

Over the last 5 years we have collected data and biological samples from 854 mutation carriers from about 600 unrelated families. The families come from every region of Italy. Therefore the sample is representative of the whole country. The mean expanded CAG repeat number was 45.8±6.2, (range 37–120 CAG, mode 44 CAG) and the mean age at onset was 43.2±13.5 years (range 3–89, mode 44 years). To date we have collected lymphoblastoid cell lines from 100 patients and performed skin biopsy in 10 patients, including subjects heterozygous and homozygous for the HD mutation. Data from 14 homozygous subjects and their unrelated families have been collected worldwide [6] and DNA samples were available from 6 of these, lymphoblasts were obtained from 5, fibroblasts from 2, and myoblasts from 1.

Discussion

We have collected data on HD patients and their families over the last 5 years and established a database and tissue bank. The tissue bank at the Neurogenetics Unit had the support of the Italian Ministries of Health (Finalizzato 2001) and of Research (Cluster 02). Clinical details concerning age at onset and disease progression rate were reported in the database following direct interview of patients and their relatives. We believe that this strategy may better contribute to explain some obscure aspects of the natural history of HD than studies performed using questionnaires [8]. The atypical onset symptoms (i.e., psychiatric and unusual motor manifestations), the disease duration, and the effects of familial and genetic factors on the phenotype [9] still represent inexplicable features that are difficult to investigate by retrospective analyses only. We also included in the database information regarding at-risk subjects [7] to study possible adverse events after genetic testing in the presymptomatic stage. We wish to extend this initiative to all families, investigators, and institutions within and, possibly, outside the Italian border in an attempt to enlarge the bank and to institute a HD Research Roster. The HD Research Roster may include information about the history of HD in the family (family trees) and other related data, and identify HD patients and families who are interested in participating in research projects.

Acknowledgements We thank all specialists and institutions within and outside Italy who collaborated and supported the Italian Huntington Disease Data and Tissues Bank. We especially thank patients and their relatives for their constant support, the Associazione Italiana Corea di Huntington-Neuromed, and the "Mauro Emolo" Foundation.

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Neurol Sci (2003) 24:217–218
DOI 10.1007/s10072-003-0138-7

Optimizing levodopa pharmacokinetics in Parkinson's disease: the role of COMT inhibitor

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Introduction

After more than 30 years of clinical use, levodopa therapy still offers the best symptomatic control of Parkinson's disease (PD), and all patients will require it during the course of their disease [1, 2]. However, with each year of levodopa treatment, about 10% of patients will develop motor complications [3, 4]. There is considerable evidence that abnormal intermittent or pulsatile activation of brain dopamine receptors leads to the development of motor complications in PD, through the induction of plastic changes in striatal neurons and altered neuronal firing patterns [5, 6]. These observations suggest that long-acting dopaminergic agents that provide more-continuous stimulation of dopamine receptors might be associated with a reduced risk of inducing motor complications [7, 8]. For example, long-acting dopaminergic agents are associated with a reduced frequency and severity of motor complications in MPTP monkeys [9, 10]. The same short-acting dopamine agonist also induces gene changes in striatal neurons and dyskinesia when it is administered intermittently, but not when it is infused in a continuous manner [11, 12]. Prospective double-blind clinical trials in PD patients similarly demonstrate that the risk of inducing motor complications is markedly reduced if therapy is initiated with a long-acting dopamine agonist compared with a short-acting formulation of levodopa [13, 14].

It is less clear if motor complications can be reversed with long-acting or continuous dopaminergic therapies. We recently demonstrated in a prospective, randomized trial, that patients randomized to receive treatment with continuous subcutaneous daytime infusions of the dopamine agonist lisuride experienced significant improvement in both "off" time and dyskinesias compared with patients randomized to receive oral levodopa treatment with or without other medical therapies [15]. Continuous infusions of levodopa, apomorphine, or lisuride have been shown to consistently reduce "off" time and the severity of motor fluctuations [16–19]. In some patients, this improvement in "off" time was associated with a marked reduction in the severity and duration of dyskinesia [18].

Practical methods of providing continuous dopaminergic stimulation with levodopa

Based on the studies described above, it has been proposed that the development of motor complications associated with levodopa is primarily related to its short half-life, rather than to some unique property of the molecule itself. Since levodopa is the most-effective antiparkinsonian therapy, there has been interest in extending its elimination half-life to reduce its side-effect profile. Benefits observed with continuous parenteral infusion of levodopa support this hypothesis [20, 21], but this approach is impractical for patients, physicians, and caregivers. Therefore, there has been interest in the development of more-practical ways to extend the half-life of levodopa, which might enable patients to enjoy its antiparkinsonian effects without the risk of potentially disabling side-effects. One such strategy involves the use of COMT inhibitors to block the peripheral metabolism of levodopa, thereby extending its plasma half-life and potentially reducing the risk of pulsatile receptor stimulation. Recent pharmacokinetic studies performed in advanced PD patients suggest that the clinical advantages associated with continuous levodopa infusion are primarily related to the elimination of the low trough plasma levels that occur with present oral formulations of the drug.

Optimizing oral levodopa therapy

Establishing the number and frequency of daily levodopa doses is expecially difficult in patients with a stable motor response. However, there is increasing evidence that the levodopa plasma profile induced by levodopa doses in stable patients may lead to the development of motor fluctuations and dyskinesias. Preliminary results of a large pharmacokinetic study suggest that administration of oral formulations of levodopa/carbidopa combined with a COMT inhibitor at 3-h intervals provides a plasma profile that avoids low trough levels, and is similar to that obtained with levodopa infusion. In contrast, low trough levels can not be avoided with regular formulations of levodopa/carbidopa, even when administered at hourly intervals (Fig. 1). Further studies to test this hypothesis and other approaches that might reduce the motor complications associated with levodopa are clearly warranted.

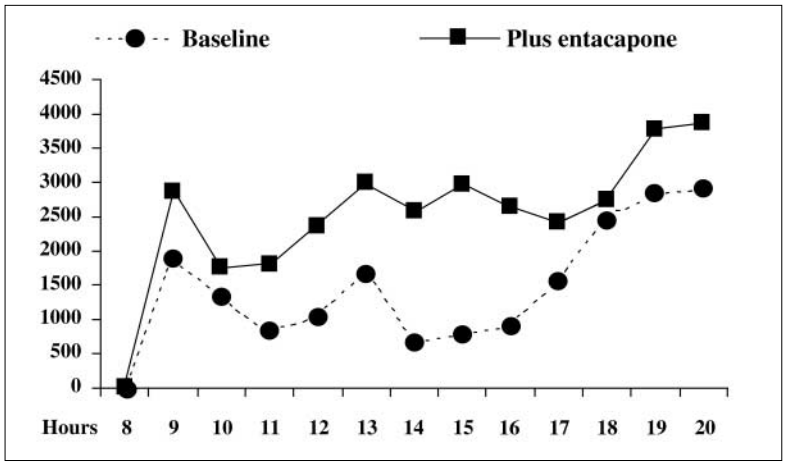

Fig. 1 Levodopa plasma profile in a representative patient before and after administration of entacapone. Levodopa was administered with 3 hours interval and entacapone was added to all levodopa doses

Discussion

Despite the problems of treatment-associated motor complications, levodopa still offers the best symptomatic control, and comprises the cornerstone of any effective treatment strategy for PD. It is therefore essential to provide the best schedule of levodopa therapy possible. This includes ensuring optimal levodopa absorption and providing smoother, more-continuous stimulation of dopamine receptors. This may be achieved with COMT inhibitors in combination with levodopa.

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Neurol Sci (2003) 24:219–220
DOI 10.1007/s10072-003-0139-6

Acute akinesia or akinetic crisis in Parkinson's disease

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Abstract In 22 patients with idiopathic Parkinson's disease we observed a sudden worsening of motor symptoms and severe akinesia during hospitalization because of infectious diseases, bone fractures, surgery for gastrointestinal tract diseases, and iatrogenic causes. Of these patients, 12 recovered completely, 6 had a partial recovery, and 4 died. Treatments included subcutaneous apomorphine/lisuride infusion and dantreolene (with a creatine phosphokinase level higher than 200 IU). In all patients a definite refractoriness to therapy was shown with a transient lack of response to apomorphine.

Introduction

Acute akinesia or akinetic crisis [1, 2] is an ill-defined complication occurring during the course of Parkinson's disease (PD) with infectious diseases, bone fractures, and gastrointestinal tract diseases. There is acute worsening of parkinsonian symptoms and transient unresponsiveness to current treatments or to increments of dopaminomimetic treatments. The major clinical symptoms are represented by a severe akinetic state with frequent cognitive and/or psychotic disturbances, and dysphagia and aphonia, with opportunistic infections in the most-severe forms. We report our experience with 22 patients who were being followed in our Movement Disorder Unit.

Patients and methods

In order to simplify descriptions we divided our patients into three categories.

Category 1: probable absorption deficit-akinetic crisis
This included 3 patients, 2 men and 1 woman , with a disease duration of 9, 8, and 5 years, Hoehn/Yahr stage (H/Y) 4, 4, and 2.5 [3] who were hospitalized because of acute akinesia, with UPDRS score increment (+) of 21, 37, and 27 points, confusioned state, incontinence, pyrexia (37.5–39.9°C), raised creatine phosphokinase (CPK, 628–2,502 IU), and increased myoglobinuria (Myo) 420-1,310 ng/ml, due to acute disturbances of the gastrointestinal tract (2 gastric stasis because of duodenal ulcers and jejunal volvulus). All patients were treated with 150-200 mg/day apomorphine (apo) subcutaneously for 6–3 days and pre-treated with ondansetron (4–8 mg/day, in 1 60 mg/day); dantreolene was added for 3 days. In 2 patients the recovery was slow, 14 days, and not complete; 1 patient recovered completely in 13 days.

A 73-year-old women, with a disease duration of 13 years and H/Y stage3, had pneumonia with pyrexia and dysphagia [CPK 1,660 IU, Myo 99.80 ng/ml, white blood cell count (WBC) 17,000/mm^3] with acute akinesia (UPDRS increased by 41 points) and mental confusion. She was treated with 150-200 mg/day apo subcutaneously and dantreolene 50 mg/day. After 5 days the motor score improved by only 7 points and on day 7 she died because of pulmonary embolism.

Category 2: without deficit of absorption—pure akinetic crisis
Three patients presented with acute akinesia (UPDRS increased by 26–34 points) with increased body temperature up to 40.5°C (CPK 801–2,870 IU, Myo up to 1,430 ng/ml, WBC 15,000–18,500/mm^3) 3–4 days after the onset of a flu-like syndrome. Two patients (a 71-year-old male, with a disease duration of 8 years, H/Y stage 3 and a 74-year-old women, with a disease duration of 11 years, H/Y stage 4) were treated with 100-200 mg/day apo subcutaneously. One patient partially recovered after 10 days, 1 died after 18 days with pneumonia. In the 3rd (a 75-year-old male, with a disease duration of 9 years, H/Y stage 3) the total L-dopa dosage was increased by 25%; recovery was complete in 7 days.

Two patients had acute akinesia (UPDRS increased by 22–26 points) in the course of broncho-pneumonia with pyrexia (38.9°C, 39.0°C, CPK 772 IU, 1,200 IU, Myo 423.80 ng/ml, 693.30 ng/ml, respectively). The 1st patient (a 74-year-old male, disease duration 9 years, H/Y stage 4, UPDRS increase 45), with altered consciousness, was treated with 75-200 mg/day apo; he died after 14 days. The 2nd patient (a 69-year-old male, disease duration 9 years, H/Y stage 2.5) recovered completely after 4 days on 75 mg/day apo therapy.

In 8 patients (67–74 years old, 3 male, disease duration 6–14 years, H/Y stage 2.5-4), acute akinesia (UPDRS increase 20–29) was observed 3–4 days after surgical treatment of femoral and/or hip fractures. Four had pyrexia (CPK 751-2,392 IU, Myo 339–1,023 ng/ml), 4 patients were apyretic, with CPK and Myo within normal ranges. Patients with pyrexia were treated with subcutaneous apo or lisuride [4, 5] One patient died after 13 days; in the 3 other patients UPDRS scores improved from day 12 to day 21, with complete recovery. The 4 afebrile patients recovered completely in 3–6 days; 2 patients were treated with L-dopa increases of 20%; 2 patients were given adjunct apo subcutaneously 50–100 mg/day.

One man (65 years old, disease duration 3 years, H/Y stage 2.0) had acute akinesia (UPDRS increase 21) during colecystitis. L-Dopa dosage was increased by 40%, with remission of symptoms in 8 days.

Category 3: iatrogenic akinetic crisis-neuroleptic malignant-like syndrome
A 76-year-old male, with a disease duration of 11 years and H/Y stage 4 had acute akinesia (UPDRS increase 26) with hyperthermia (40.1°C, CPK 2,109 IU, Myo 998.60 ng/ml), because of amantadine withdrawal. Amantadine was reintroduced; akinesia persisted for 6 days.

In a 68-year-old women (disease duration 8 years, H/Y stage 2.5), acute akinesia (UPDRS increase 21, CPK 344 IU, Myo 320.30 ng/ml) appeared 12 h after a rapid change from ropinirole 24 mg/day to pramipexole 2.1 mg/day. L-Dopa dosage was increased by 40%; recovery was observed after 6 days.

A 68-year-old male (disease duration 4 years, H/Y stage 2) had acute akinesia with hyperthermia (40.9°C, CPK 2,454 IU, Myo 1,233.80 ng/ml), because of administration of risperidone, 1 mg for 5 days. Risperidone was withdrawn and 70 mg/day apo was added to the therapy. He recovered in 2 days.

A 75-year-old male (disease duration 16 years, H/Y stage 4) had acute akinesia (UPDRS increase 25) with pyrexia (38.5°C, CPK 829 IU, Myo 560.00 ng/ml) due to risperidone (1.5 mg/day) for 1 week. Apo 50-150 mg/day was added to therapy; risperidone was withdrawn; partial recovery occurred in 10 days.

Statistics
Differences in continuous variables were evaluated with analysis of variance. Manthel-Haenzel and Fisher tests were used to assess categorical data. ANCOVA was used to verify differences in H/Y stage at follow-up between complete and partial recovery, adjusting for baseline and disease duration. Log-rank test was performed to identify survival variables (SAS 8.1).

Results

Table 1 summarizes the demographics, H/Y stage, and UPDRS motor scores before, during, and after the recovery from acute aki-

Table 1 Demographics and follow-up evaluation (*CPK* creatine phosphokinase, *Myo* myoglobinuria)

	Total group (22 patients)	Category 1 (4 patients)	Category 2 (14 patients)	Category 3 (4 patients)
Age (years)	73.86±1.1	72.5±2.5	74.9±1.3	71.8±2.2
Sex (F/M)	7/15	1/3	5/9	1/3
Duration of disease	11.81±0.9	13.5±1.9	11.9±1.1	9.7±2.5
H/Y baseline	3.2±0.2	2.7±0.9	3.1±0.3	3.1±0.3
H/Y akinetic state	4.5±0.2	4.6±0.3	4.3±0.2	4.8±0.3
H/Y recovery	3.1±0.2[a]	3.5±0.5[b]	3.0±0.3[c]	3.1±0.3
UPDRS III baseline	35.6±3.5	37.5±9.6	34.5±4.6	37.6±5.2
UPDRS III akinetic state	63.1±3.7	69.0±5.6	60.9±5.3	64.6±6.3
UPDRS III recovery	38.7±4.7[a]	50.0±17.3[b]	35.9±6.3[c]	37.8±5.3
Temperature (°C)	38.6±0.6	38.5±0.6	38.3±0.4	39.1±0.9
CPK	1,281.3±403.3	1,385±438	1,025±268	1434±504
Myo	704.7±186	793±218	543±133	778±207
Recovery in days	9.2±2.3	9.5±2.8	9.6±1.5	8.6±2.7

[a]Only 18 surviving patients; [b]Only 3 surviving patients; [c]Only 11 surviving patients

nesia, and duration of acute akinesia. All patients were on dopaminomimetic therapy and all had been visited 1–8 months before the occurrence of acute akinesia. Complete hematological parameters were monitored throughout acute akinesia, up to recovery or eventual complications. No correlations were observed with the acute akinesia course.

UPDRS motor scores and H/Y stage during acute akinesia compared with values obtained prior to acute akinesia and scores after recovery were statistically significant ($p<0.01$). H/Y stage and UPDRS score (baseline versus outcome $p=0.05$) indicated modest worsening after acute akinesia. Age, disease duration, and H/Y stage differentiated patients with partial from patients with complete recovery ($p=0.02$, $p<0.001$, $p=0.01$), with significant correlation between disease duration and H/Y stage ($t=23.1$, $p<0.001$) and outcome ($t=23.1$, $p=0.05$). None of the variables was able to predict the survival after acute akinesia.

Sixteen patients were treated with apo; 4 patients died because of complications without significant reductions of UPDRS scores, 12 improved from 2 to 21 days after the initiation of treatment. Six patients were treated with an increase of dopaminergic treatment and recovered in 2–6 days. The disease duration and UPDRS score before and after acute akinesia were significantly different ($p<0.05$) between patients treated or not with apo.

Discussion

Our report evidences a relevant heterogeneity of possible causes of acute akinesia, as in the few reports in the literature [1, 2, 6]. At least three precipitating factors could be defined. In 11 patients a possible common etiology could be identified in altered gastric absorption or inadvertent withdrawal of treatment or administration of antidopaminergic drugs. In 11 patients, however, there was no reason to suspect an abnormal absorption and acute akinesia appeared 2–3 days after flu-like symptoms, colecystitis, or bone fracture, when hematological parameters were within normal limits and alterations of fluid compartments could not be considered a causative factor. In all patients acute akinesia lasted for several days after tentative treatments, thus evidencing a transient unresponsiveness to dopaminomimetic agents.

We suggest that a sudden worsening of the UPDRS motor score by 20 or more points, accompanied by unresponsiveness to the same drug regimens that adequately corrected symptoms before the appearance of acute akinesia, could constitute a working definition of the akinetic crisis. Furthermore, the definition highlights the presence of transient unresponsiveness to dopaminomimetic treatment, which should constitute the fundamental element of the crisis.

German literature suggests [1, 6, 7] that amantadine sulfate (available in Austria, Germany, and Hungary) at an intravenous dose of 500 mg/day my resolve aphagia; some authors also hypothesized that apo might be useful in acute akinesia [5], but our studies indicate that acute akinesia outlasts the apo administration by several days (2–10 days before the reduction of UPDRS and H/Y stage was observed) and 4 patients died despite treatment. The patients treated with apo were significantly more ill then patients not treated with apo. Due to delayed recovery in 12 patients and the death of 4 patients, we hypothesized that apo was useful mostly in avoiding the possible complication of dopaminergic drug withdrawal.

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Neurol Sci (2003) 24:221–222
DOI 10.1007/s10072-003-0140-0

Use of antiparkinsonian drugs in the Umbria Region

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Abstract Discussion of differences between recommended guidelines and clinical practice is very useful in medicine. The use of a computerized database of all prescriptions of antiparkinsonian drugs enabled us to perform an epidemiological population-based study with low cost and in a relatively short time. We have identified 2 827 parkinsonian patients in November 2000 and estimate the number of patients taking dopaminoagonists aged >75 years, antidepressive drugs, and atypical neuroleptics.

In the last few years many guidelines for the treatment of Parkinson's disease have been published [1, 2]. Algorithms in the early and late stages of disease have been proposed for motor and non-motor disturbances (depression, psychotic and behavioral symptoms). However, data on use of antiparkinsonian (AP) drugs in clinical practice are scarce. The aim of this study was to identify subjects exposed to AP drugs in the Umbria region (404 951 males and 430 537 females), to characterize the use on the basis of consumption of dopaminoagonists, and to consider coprescription with antidepressive drugs and atypical neuroleptics.

Methods

All residents in the Umbria region exposed to AP drugs (code N04 in accordance with Anatomical Therapeutic Chemical classification) have been identified in the years 1997–2000. The high specificity and the length of treatment for parkinsonism permitted the use of AP drugs as tracers of the disease. A subject was defined as a case of parkinsonism, and included in the study if he/she had received "specific" and "consistent" therapy in the years under investigation.

A subject was selected if he/she had received a "specific" treatment with at least one prescription of levodopa and/or selegiline and he/she was treated for a period of 6 months or more (even if not consecutive). The consistency of therapy was defined as a continuous treatment, with at least 30% period covered by AP prescriptions. Moreover, we selected among patients who did not have a "consistent" therapy those with a percentage of months of levodopa and/or selegiline treatment higher or equal to 70% of the total number of months with AP therapy. A case was defined as prevalent in November 2000 if his/her prescription history included the month of November 2000.

This method of identification of parkinsonism cases has been validated by record-linkage with the database of the Outpatient Center for Parkinson's Disease and extrapyramidal disorders of the University "La Sapienza" (sensitivity of 83.6%) and has allowed estimation of the prevalence of parkinsonism in the province of Rome [3].

Results

In the Umbria region, 2,827 patients (1,316 male and 1,511 female) were defined as prevalent cases of parkinsonism in November 2000. The crude prevalence rate of parkinsonism is 368.8 cases per 100 000 inhabitants. The standardized rate for the Italian population in 2001 is 279.4 cases per 100 000 inhabitants (95% confidence interval 234.3–379.6). The age- and sex-specific rates are reported in Fig. 1. In the age group <50 years, the prevalence rate was 5.2 cases per 100 000 inhabitants. Patients treated with dopaminoagonists represent 29.5% (834/2 827) and the distribution was higher for the age-group 70–74 years.

Among 834 patients treated with dopaminoagonists, 29.9% were >75 years. Pramipexole is the most used dopaminoago-

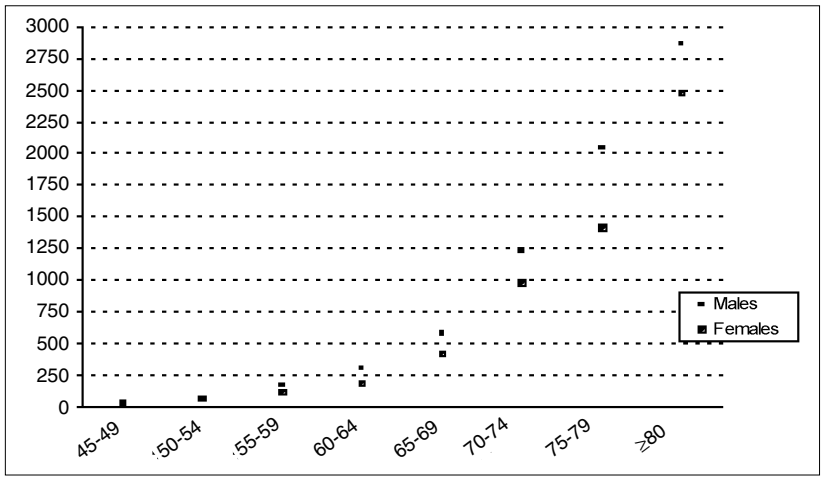

Fig. 1 Age-group prevalence rates for parkinsonisms (x100 000 inhabitants), Umbria 2000

nist (38.4%), followed by ropinirole (36.7%), pergolide (16.9%), bromocriptine (7.3%), and apomorphine (0.7%).

A total of 700 parkinsonian patients (24.8%) took one or more antidepressive drugs. In particular, 167 patients were treated with non-selective inhibitors of monoamine reuptake (code ATC NO6AA) (desipramine, imipramine, clomipramine, trimipramine, amitriptyline, nortriptyline, dosulepine, maprotiline), 304 with selective inhibitors of serotonin reuptake (code ATC N06AB) (fluoxetine, citalopram, paroxetine, sertraline, fluvoxamine), and 331 with other antidepressive drugs (code ATC No6AX) (oxitriptan, mianserin, tradozone, nefazodone, mirtazapine, venlafaxine, reboxetine). Finally, the use of atypical neuroleptics was 1.9% (54 patients). Risperidone was the most used atypical neuroleptic (1.3%), followed by clotiapine (0.3%), olanzapine (0.2%), and quetiapine (0.1%).

Discussion

The estimated prevalence of parkinsonism in the Umbria region is in accordance with European data [4]. The slight differences observed in the prevalence rates are probably due to both the number of incident cases not treated with drugs and to different diagnostic criteria. The epidemiological approach based on prescription history of AP drugs may supply data regarding the use of these drugs, only or in combination with others, in clinical practice.

In this study the use of dopaminoagonists in patients aged >75 years was frequent, contrary to recommended guidelines and in accordance with an observational study [5].

The estimated prevalence of depression in parkinsonism patients is between 25% and 70%; in our study the estimated incidence of patients treated with antidepressive drugs was 25%. The number of parkinsonian patients with psychotic symptoms is about 26% [6]; in patients of the Umbria region there were 1.9% treated with atypical neuroleptics.

It is necessary to emphasize that for atypical neuroleptics (clotiapine, quetiapine, risperidone, and olanzapine) the therapeutic indication of the National Health Service is only for schizophrenia, while the guidelines for Parkinson's disease recommend their use [1, 2].

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Neurol Sci (2003) 24:223–224
DOI 10.1007/s10072-003-0141-z

Association of *tau* gene polymorphism with Parkinson's disease

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Abstract We investigated the segregation of the dinucleotide GT repeat polymorphism in the intron between exons 9 and 10 of the *tau* gene in 300 patients with Parkinson's disease (PD) and in 197 normal controls. The *A3* allele was more frequent in cases than in controls (30% versus 16%, $p<0.001$), and individuals carrying at least one *A3* allele in their genotype had an increased risk of developing PD (odds ratio 2.78, 95% confidence interval 1.81–4.29). No significant differences were found between patients by considering the age at onset and the presence of family history or dementia. Our findings suggest a possible involvement of the *tau* gene in the pathogenesis of PD.

Introduction

Deposits of the microtubule-associated protein tau are considered the neuropathological hallmark of some neurodegenerative diseases, defined as tauopathies, and dysfunction of the *tau* gene may lead to these disorders. Indeed, mutations in the *tau* gene are found in cases of familial frontotemporal dementia with parkinsonism linked to chromosome 17 [1] and studies have shown an association between *tau* polymorphisms and other sporadic tauopathies, such as progressive supranuclear palsy [2] and corticobasal degeneration [3]. Also in Parkinson's disease (PD), which is not primarily considered a tauopathy, the *tau* gene may have a role in the pathogenesis, because a complete genomic screen in families with PD provided evidence for linkage in the region containing the *tau* gene on chromosome 17q [4]. Moreover, a significant association has been reported between PD and the *A0/A0* genotype of the dinucleotide GT repeat polymorphism, located in the intron between exons 9 and 10 of the *tau* gene [5]. An earlier study with smaller sample size, however, did not show such an association [6]. In view of these conflicting findings in populations with different ethnic background, we conducted a case-control study to determine whether the *tau* gene dinucleotide polymorphism confers susceptibility to the development of PD in a population originating from southern Italy.

Materials and methods

Blood was collected from 300 patients with PD (180 men and 120 women, aged 66.3±8.9 years, mean±SD) and 197 healthy control subjects (92 men and 105 women, aged 74.9±7.5 years). All cases and control subjects were born and had resided in Calabria, southern Italy. All patients had a clinical diagnosis of definite PD according to the UKPDSBB criteria, and were consecutively selected starting from January 1999 until December 2001 from among the outpatients attending the Institute of Neurology of the University "Magna Græcia" of Catanzaro, Italy. Subjects with no or minimal improvement on levodopa were excluded. We also excluded patients with other causes of parkinsonism, or with unexplained signs of more-extensive neurological involvement (dementia or mild dysautonomia were allowed if they occurred after the 1st year of motor symptoms). Of the 300 patients with PD, 236 denied having first- or second-degree relatives with PD and were considered to have sporadic PD (SPD), whereas 64 patients reported a first- or second-degree relative with a physician's diagnosis of PD and were considered to have familial PD (FPD). Moreover, 61 patients reported an age at onset of 50 years or younger (44.2±5 years) and were considered to have early onset PD (EOPD), whereas 239 subjects reported an age of onset older than 50 years (62.7±6.9 years) and were considered to have late-onset PD (LOPD). The cognitive conditions were assessed with the Mini Mental State Examination (MMSE) in 292 PD patients and the scores were adjusted for age and education: 217 patients had normal cognitive functions (NCF, MMSE 26.2±2.7) and 75 patients had impaired cognitive functions (ICF, MMSE 18.7±5.8).

Genomic DNA was extracted in all subjects from peripheral blood leukocytes according to standard procedures. The intronic dinucleotide GT repeat polymorphism of the *tau* gene was amplified by polymerase chain reaction as described previously [2]. Five polymorphic variants were identified, defined as *A0*, *A1*, *A2*, *A3*, and *A4*, and characterized by the occurrence of 11, 12, 13, 14, or 15 GT repeats, respectively. Allele and genotype frequencies were compared between cases and controls using cross tabulation and χ^2 statistic, under the assumption of the Hardy-Weinberg equilibrium. The relative risk was estimated through calculation of odds ratios (OR) with 95% confidence intervals (CI). The ORs were adjusted for sex and age using the logistic regression model.

Results

Allele and genotype distributions for the *tau* GT repeat polymorphism are given in Table 1. The observed genotype frequencies did not differ from the expected frequencies according to the Hardy-Weinberg equilibrium for both the control group ($p=0.139$) and PD patients ($p=0.430$). Nevertheless, allele and genotype distributions were signifi-

Table 1 Allelic and genotype frequencies of the intronic dinucleotide GT repeat polymorphism of the *tau* gene. Number in each group with percentages in parentheses

	PD	Controls	χ^2	*p* value
Alleles	n=600	n=394		
A0	381 (63.5)	307 (77.9)	27.771	<0.0001
A1	33 (5.5)	19 (4.8)		
A2	4 (0.6)	3 (0.8)		
A3	181 (30.2)	63 (16.0)		
A4	1 (0.2)	2 (0.5)		
Genotypes	n=300	n=197		
A0/A0	114 (38.0)	120 (60.9)	37.652	<0.0001
A0/A1	21 (7.0)	14 (7.1)		
A0/A2	4 (1.3)	1 (0.5)		
A0/A3	127 (42.4)	51 (25.9)		
A0/A4	1 (0.3)	1 (0.5)		
A1/A3	12 (4.0)	5 (2.6)		
A2/A3	0 (0.0)	2 (1.0)		
A3/A3	21 (7.0)	2 (1.0)		
A3/A4	0 (0.0)	1 (0.5)		

PD, Parkinson's disease

cantly different between cases and controls. The risk for PD was increased (OR 2.78, CI 1.81–4.29) for the subjects who were carrying in their genotype at least one *A3* allele (*A3*+ PD 160/300, 53.3%; controls 61/197, 31%) compared with subjects without any *A3* allele (*A3*- PD 140/300, 46.7%; controls 136/197, 69%). No significant differences were found in *A3*+ genotype distributions when cases were stratified according to family history of PD (SPD 124/236, 52.5%; FPD 36/64, 56.3%), or according to age at onset (EOPD 31/61, 50.8%; LOPD 129/239, 54%), or according to cognitive functions (NCF 110/217, 50.7%; ICF 46/75, 61.3%).

Discussion

We have demonstrated that the presence of the *A3* allele in the intronic GT repeat polymorphism of the *tau* gene is associated with an increased susceptibility for PD in a southern Italian population. Our findings are at variance with a previous report showing an overrepresentation of the *A0* allele in PD patients from a Spanish population [5]. This discrepancy may be due to genetic heterogeneity between populations with different ethnic backgrounds. Indeed, the normal Spanish controls [5] had frequencies (*A0* 71%, *A3* 24.3%) quite different from the frequencies observed in our southern Italian normal controls (*A0* 77.9%, *A3* 16%). Interestingly, the allelic frequencies of our normal controls were similar to those reported for the normal subjects in another Italian population (*A0* 76.4 %, *A3* 18%) [7], thus suggesting genetic homogeneity for the intronic *tau* polymorphism in Italian healthy subjects from different studies.

It is unknown how the *tau* gene may contribute to PD pathogenesis. Nevertheless, there is recent evidence that protein tau co-localizes with α-synuclein in Lewy bodies of patients with PD [8], thus suggesting that the *tau* gene may be involved in the molecular pathways responsible for the neuropathological hallmark of PD. Future studies will be helpful in clarifying the relationship of the intronic *tau* polymorphism with neuropathological features.

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Neurol Sci (2003) 24:225–226
DOI 10.1007/s10072-003-0142-y

Fatigue in Parkinson's disease

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Abstract Fatigue is a recognized problem in Parkinson's disease and other clinical conditions. We characterized this symptom in 19 patients and 19 age- and sex-matched controls, using the Multidimensional Fatigue Inventory (MFI) and the Geriatric Depression Scale. Fatigue may be an independent symptom in Parkinson's disease, frequently associated with depression. Our analysis showed the usefulness of the MFI in discriminating between different dimensions of fatigue for a better therapeutic approach.

Although motor symptoms are important in Parkinson's disease (PD), several non-motor problems may have impacts on the lives of these patients. During the last few years much effort has been expended in the assessment of these problems. Amongst the symptoms that have received increasing attention is fatigue [1]. This is distinguished from symptoms of depression and is described as lack of energy, sense of tiredness, or a feeling of exhaustion. Fatigue is a common symptom in other neurological diseases, such as multiple sclerosis [2] and amyotrophic lateral sclerosis (ALS) [3], or in disease such as systemic lupus erythematosus [2], and reduces the quality of life of PD patients. Few studies have considered fatigue in PD, and have administered a variety of questionnaires [2, 4]. Multidimensional Fatigue Inventory (MFI) measures five dimensions of fatigue independently, including general fatigue, physical fatigue, reduced motivation, reduced activity, and mental fatigue [4]. We used the MFI, previously reported by Lou et al. [5], with the Geriatric Depression Scale (GDS) to characterize and better define fatigue in PD and explore the possible correlation with depression.

Methods and results

Nineteen PD patients followed in the Movement Disorder Clinic of our department and 19 age- and sex-matched controls were recruited. The control group included 6 patients admitted to our neurological clinic (1 with seizures, 1 with a mononeuropathy of the 6th cranial nerve, and 4 with blepharospasm). All participants filled out the MFI and GDS questionnaires. The GDS is widely used to assess depressive symptoms among the elderly, and has been tested for reliability and validity. To assure that all subjects could fill out the questionnaires independently, patients were recruited with Hoehn and Yahr stage from 1 to 3. We excluded patients with cognitive or psychiatric disorders or other medical conditions, such as heart failure, endocrine disorders, pulmonary disease, chronic renal failure, anemia, arthritis, chronic fatigue syndrome, stroke, neuromuscular disease, multiple sclerosis, and fibromyalgia.

Student's *t*-test was applied to compare each dimension of fatigue in patients and controls. Multivariate analysis was performed to evaluate the influence of some characteristics (age, sex, education, sleep disturbances) with each dimension of fatigue.

Patients scored higher than controls on all five dimensions in the MFI and on the GDS scale ($p<0.001$). Multivariate analysis (Table 1) showed that all five dimensions of fatigue are independent of age, sex, education, and sleep disturbances. Only depression influenced some dimensions of fatigue. Physical fatigue and reduced activity are independent of depression.

Discussion

In our study each dimension of fatigue measured with MFI was higher in patients than age- and sex-matched controls. MFI has been tested for its psychometric proprieties and validated in cancer patients, patients with chronic fatigue syndrome, psychology students, medical students, army recruits, and junior physicians [4]. Lou et al. [5] applied this questionnaire in PD patients and found higher scores for each dimension of fatigue in PD than controls. The application of MFI to PD patients in this study showed that physical fatigue and mental fatigue are

Table 1 Multivariate analysis

Characters:	GF (O.R., *p*)	PF (O.R., *p*)	RA (O.R., *p*)	RM (O.R., *p*)	MF (O.R., *p*)
None	1.40 (0.002)	1.53 (0.005)	1.66 (0.002)	1.25 (0.022)	1.24 (0.027)
Age	1.39 (0.005)	1.62 (0.006)	1.63 (0.003)	1.24 (0.03)	1.34 (0.02)
Sex	1.46 (0.002)	1.55 (0.005)	1.66 (0.002)	1.24 (0.025)	1.24 (0.031)
Education	1.37 (0.006)	1.51 (0.011)	1.58 (0.004)	1.18 (0.089)	1.27 (0.032)
Sleep disturbances	1.41 (0.005)	1.52 (0.011)	1.58 (0.004)	1.22 (0.069)	1.24 (0.047)
Depression	1.28 (0.65)	1.36 (0.047)	1.46 (0.041)	1.11 (0.31)	1.03 (0.79)

GF, general fatigue; *PF*, physical fatigue; *RA*, reduced activity; *RM*, reduced motivation; *MF*, mental fatigue

two independent symptoms in PD. In our study only physical fatigue and reduced activity were not influenced by depression and are independent of the other dimensions analyzed. These two groups of symptoms may have different etiologies and therefore may need to be managed separately. Levodopa may be helpful in reducing physical fatigue and reduced activity, but may not be useful for treating general fatigue, reduced motivation, and mental fatigue. These latter symptoms may be treated with drugs for depression. MFI showed general fatigue and physical fatigue as the predominant dimensions of fatigue in patients with ALS [3]. The authors demonstrated the usefulness of MFI for characterizing fatigue in ALS patients. We confirmed MFI as a reliable means of assessing fatigue in PD patients and separating the dimensions supposedly due to different etiologies.

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