SHORT REPORT

Dopamine transporter SPECT in patients with mitochondrial disorders

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Background: Mitochondrial disorders may affect basal ganglia function. In addition, decreased activity of complex I of the mitochondrial electron transport chain has been linked to the pathogenesis of dopaminergic cell loss in Parkinson's disease.

Objective: To investigate the dopaminergic system in patients with known mitochondrial disorders and complex I deficiency.

Methods: Dopamine transporter density was studied in 10 female patients with mitochondrial complex I deficiency by ^{123}I -FP-CIT (N- β -fluoropropyl-2 β -carbomethyl-3 β -(4-iodophenyl)-nortropine) SPECT.

Results: No differences in ^{123}I -FP-CIT striatal binding ratios were observed and no correlation of the degree of complex I deficiency and striatal binding ratios could be detected.

Conclusions: These data argue against the possibility that mitochondrial complex I deficiency by itself is sufficient to elicit dopaminergic cell loss.

Mitochondrial disorders affect a broad spectrum of central nervous system functions, and parkinsonian signs have been described in Leigh's disease, Leber's hereditary optic neuropathy plus dystonia, and other mitochondrial encephalopathies.^{1,2} In addition, primary pathogenic mitochondrial DNA defects have been reported with parkinsonism.³ On the other hand, decreased activity of complex I of the mitochondrial electron transport chain, mitochondrial DNA damage in tissue samples, and complex I deficiency have been detected in the substantia nigra of patients with Parkinson's disease at necropsy.^{4,5} In addition, several compounds that inhibit complex I activity induce nigral neuronal cell death and parkinsonism in humans and non-human primates.^{6,7}

While parkinsonism is not a common feature of mitochondrial disorders, a moderate loss of dopaminergic neurones might have been missed. A loss of approximately 80% of striatal dopamine is required to elicit parkinsonian symptoms.⁸ Most patients with Parkinson's disease studied using dopamine transporter (DAT) SPECT show a loss of approximately 50–60% of putamen DAT early in the disease process.⁹

Data on the state of the dopamine system in patients with mitochondrial disorders are lacking. We therefore investigated the dopaminergic system in 10 female patients with known mitochondrial disorders and complex I deficiency, using ^{123}I -FP-CIT (N- β -fluoropropyl-2 β -carbomethyl-3 β -(4-iodophenyl)-nortropine) SPECT.^{10–12} ^{123}I -FP-CIT binds to membrane dopamine transporters and allows preclinical detection in as yet unaffected family members of patients with Parkinson's disease.¹⁰

METHODS

We studied 14 consecutive patients (10 women and four men) from our neuromuscular clinic with mitochondrial

disorders but without overt parkinsonian symptoms. A sex specific differential loss of DAT with age has been described previously.¹³ We therefore included only the women in our final data analysis (range 35 to 69 years, median 48 years) to avoid data inhomogeneity. Nine patients had "CPEO plus" (chronic progressive external ophthalmoplegia with proximal muscular weakness, ataxia, retinopathy, hypacusis or arrhythmia), two of whom fulfilled the criteria of Kearns-Sayre syndrome, and one patient had MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes). Histological examination, enzyme histochemical analyses, biochemical analyses, and molecular genetic studies were carried out on serial frozen sections and homogenised muscle tissue from all the patients. Muscle specimens showed ragged red fibres (modified Gomori's trichrome stain) and COX negative (cytochrome c oxidase stain) fibres in all patients with mitochondrial disorders. Diagnostic muscle biopsies from 21 neurologically normal patients without evidence of a myopathy were used as controls (range 38 to 72 years, median 54). Enzyme activity determinations (rotenone sensitive NADH:CoQ oxidoreductase, cytochrome c oxidase, and citrate synthase) and analysis of mitochondrial DNA (for detection of large scale deletions and the A3243G point mutation) was carried out as described previously.¹⁴

All patients were examined using a neuropsychological test battery covering general intellectual capabilities, verbal and visual memory, concentration, vigilance, attention, language, visuospatial perception, visual construction, abstraction, and flexibility.

^{123}I -FP-CIT (DaTSCAN®, Nycomed Amersham, UK) SPECT imaging and evaluation protocol followed the procedure recommended by the ^{123}I -FP-CIT study group.¹⁵ All scans were done with a dual or triple headed gamma camera equipped with collimators optimised for iodine-123. Image acquisition started three to four hours after an intravenous injection of 185 MBq ^{123}I -FP-CIT with 120 projections at 3°, a 50 second acquisition time for each step, and a 128×128 matrix. To avoid a systematic bias the acquisition processes were adjusted between both camera systems, employing identical acquisition parameters, number of projections, projection angle, and projection time. The raw data of all patient studies were reconstructed using a low pass filter of seventh order with a cut off frequency of 0.38. Chang's attenuation correction, with an attenuation coefficient of 0.11, was applied to the reconstructed images. For further processing all datasets were coregistered to a mean template of the healthy controls, which had been coregistered to a normal brain magnetic resonance image (MRI), aligned according to the Talairach coordinates.¹⁶

Abbreviations: CPEO, chronic progressive external ophthalmoplegia; DAT, dopamine transporter; SPECT, single photon emission computed tomography

Semiquantitative analysis of striatal FP-CIT binding was done with a standardised three dimensional map of regions of interest (ROI) which had been established on the MRI scan aligned according to the Talairach coordinates. Striatal specific to non-specific binding ratios were calculated by subtracting the mean counts per pixel in an occipital background region of interest (ROI) from the mean counts per pixel in the basal ganglia ROI, and dividing the result by the mean counts per pixel in the background ROI. The specific binding ratios of patients were compared with 13 female control subjects without any clinical signs of Parkinson's disease (range 20 to 70 years, median 56). A striatal asymmetry index was also calculated according to the formula:

[striatum left – striatum right] / [striatum left + striatum right].

For statistical analysis unpaired Student's *t* tests, regression analyses, and covariance analyses were used where appropriate.

The study was approved by the local ethics committee and all subjects gave their informed consent.

RESULTS

Biochemical analysis of respiratory chain enzyme activities showed complex I and IV deficiency in all patients compared with measurements in healthy controls (table 1). The mitochondrial disorder was the result of heteroplasmic single large scale mitochondrial DNA deletions in seven of the 10 patients, with a deletion size ranging from 3.5 to 7.5 kB. The degree of heteroplasmy identified by Southern blot analysis varied from 16% to 78%. An A3243G point mutation in the tRNA^{Leu(UUR)} gene at position 3243 was found in two patients. In patient No 10, Southern blot analysis detected multiple deletions of the mitochondrial genome with a degree of heteroplasmy below 20%. Neuropsychological deficits were present in eight patients, indicating a significant impairment of central nervous system function, predominantly affecting the frontoparietal region.¹⁷ Specific striatal ¹²³I-FP-CIT binding was not significantly different between patients and controls (mean (SD): 3.12 (0.36) *v* 2.99 (0.41), *p* = 0.47, unpaired Student's *t* test). Analyses of striatal subregions also did not detect differences in specific ¹²³I-FP-CIT binding between patients and controls in putamen (2.93 (0.39) *v* 2.8 (0.48), *p* = 0.51) or caudate (3.04 (0.44) *v* 2.99 (0.39), *p* = 0.78).

Figure 1 Decline with age of specific striatal ¹²³I-FP-CIT binding in patients and controls.

DAT density assessed with various PET and SPECT ligands has been shown to decrease with age. Regression analyses showed a similar loss of ¹²³I-FP-CIT binding in caudate and putamen of both patients and controls (fig 1).

There were no differences in the striatal asymmetry indices between patients and controls (0.002 (0.03) *v* 0.0026 (0.03), *p* = 0.73). Striatal binding ratios were not correlated with the degree of complex I (F(crit) value 0.5), IV deficiency (F(crit) value 0.52), or the degree of heteroplasmy (F(crit) value 0.68). Further subgroup analyses of eight patients with cognitive impairment (3.11 (0.32) *v* 2.99 (0.40), *p* = 0.63) and four patients with the most severe complex I deficiencies (<3 SD) revealed no decrease of the striatal binding indices compared with controls (3.02 (0.34) *v* 2.99 (0.40), *p* = 0.88).

DISCUSSION

To investigate the integrity of the dopaminergic system in patients with mitochondrial disorders and complex I deficiency we used the iodine labelled tropane analogue ¹²³I-FP-CIT to determine the density of dopamine transporter terminals in vivo. As direct measurement of complex I activity in the brain, in particular in the substantia nigra, is not possible, we carefully selected patients who displayed CNS involvement clinically—that is, they had neuropsychological deficits, MELAS syndrome, or cerebellar ataxia. However, we did not detect a significant loss of dopaminergic striatal terminals in patients with mitochondrial disorders. Parkinsonism—especially dopa responsive parkinsonism—is not a common symptom in patients with mitochondrial disorders, and there are only case reports about patients with

Table 1 Specific striatal ¹²³I-FP-CIT binding: clinical data and biochemical data in skeletal muscle

Patient	Age (years)	Clinical diagnosis	Heteroplasmy of mtDNA mutation	CI/CS	COX/CS	NPD	Specific striatal ¹²³ I-FP-CIT binding
1	35	CPEO plus	16% deletion	0.088	0.26	No	3.59
2	40	KSS	78% deletion	0.004	0.05	Yes	3.57
3	40	KSS	62% deletion	0.017	0.08	Yes	2.87
4	43	CPEO plus	26% deletion	0.024	0.12	Yes	3.22
5	46	MELAS	75% A3243G point mutation	0.068	0.43	Yes	3.52
6	51	CPEO plus	53% deletion	0.005	0.10	Yes	3.09
7	53	CPEO plus	58% deletion	0.021	0.10	No	2.90
8	61	CPEO plus	23% deletion	0.071	0.33	Yes	3.09
9	62	CPEO plus	52% A3243G point mutation	0.042	0.17	Yes	2.83
10	69	CPEO plus	<20%, multiple deletions	0.044	0.22	Yes	2.50

Control values (n = 21; mean (SD)): CI/CS = 0.11 (0.03); COX/CS = 0.64 (0.18). CI, complex I; COX, cytochrome c oxidase; CPEO, chronic progressive external ophthalmoplegia; CS, citrate synthase; KSS, Kearns-Sayre syndrome; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; mtDNA, mitochondrial DNA; NPD, neuropsychological deficit.

multiple mitochondrial DNA deletions and parkinsonism as a symptom of mitochondrial disorders.¹⁸ With respect to the patient with multiple mitochondrial DNA deletions in our study, we must emphasise that the grade of heteroplasmy was low (<20%). Although it remains to be determined whether individual patients will reveal an abnormal loss of ¹²³I-FP-CIT SPECT on follow up, it is unlikely that the present result reflected insufficient sensitivity of the method, as FP-CIT readily allows the detection of dopaminergic terminal loss in clinically unaffected family members of patients with Parkinson's disease.¹⁰ Thus our data suggest that dopaminergic neurones are not exceptionally vulnerable to impairment of the mitochondrial electron transport chain. The data may argue against the hypothesis that mitochondrial defects play a primary role in the pathogenesis of dopaminergic cell death in Parkinson's disease, although we cannot exclude the possibility that the remaining DAT/dopaminergic terminals are functionally impaired. Indeed, despite ample experimental evidence that complex I inhibition damages dopaminergic neurones, epidemiological studies do not yet unequivocally identify corresponding environmental risk factors that might be responsible for the majority of sporadic cases of Parkinson's disease.¹⁹ Genetic or metabolic factors, in addition to impairment of the mitochondrial electron transport chain, are required for dopaminergic cell death and terminal loss in Parkinson's disease.

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