J Neural Transm (1998) 105: 497–505

Journal of Neural Transmission

Cerebrospinal fluid levels of transition metals in patients with Parkinson's disease

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Accepted December 17, 1997; received November 19, 1997

Summary. We compared CSF and serum levels of iron, copper, manganese, and zinc, measured by atomic absorption spectrophotometry, in 37 patients with Parkinson's disease (PD) and 37 matched controls. The CSF levels of zinc were significantly decreased in PD patients as compared with controls ($p < 0.05$). The serum levels of zinc, and the CSF and serum levels of iron, copper, and manganese, did not differ significantly between PD-patient and control groups.

There was no influence of antiparkinsonian therapy on CSF levels of none of these transition metals. These values were not correlated with age, age at onset, duration of the disease, scores of the Unified Parkinson Disease Rating Scale of the Hoehn and Yahr staging in the PD group, with the exception of CSF copper levels with the duration of the disease ($r = 0.38$, $p < 0.05$). These results suggest that low CSF zinc concentrations might be related with the risk for PD, although they could be related with oxidative stress processes.

Keywords: Parkinson's disease, transition metals, iron, copper, manganese, zinc, cerebrospinal fluid levels, serum levels.

Introduction

The pathogenesis of the neuronal degeneration of neurons in the pars compacta of the substantia nigra in patients with Parkinson's disease (PD) remains unknown. Several studies have shown data suggesting the presence of oxidative stress in the substantia nigra of PD patients (reviewed by Fahn and Cohen, 1992; Jiménez-Jiménez and Luquin 1996), although the significance of these findings is unclear. Increase of iron and other trace metals in the substantia nigra could hypothetically elicit these processes.

Iron (Halliwell and Gutteridge, 1988), copper (Halliwell and Gutteridge, 1985), and manganese (Donaldson et al., 1992) act as prooxidant agents. Paradoxically, copper is also essential for the antioxidant function of the protein ceruloplasmin (Dormandy, 1978), and copper and manganese are constituents of the cytosolic Cu/Zn- and the mitochondrial Mn-superoxide dismutases, respectively, which could protect against oxidative processes (Marttila et al., 1988; Saggu et al., 1989). Zinc has antioxidant activity (Dexter et al., 1991), is a constituent of the cytosolic Cu/Zn-superoxide dismutase (Marttila et al., 1988; Saggu et al., 1989), and has a stabilizing influence on membranes (Chvapil, 1976).

Many studies have shown increased iron concentrations in the substantia nigra of PD patients (for review see Gerlach et al., 1994; Jiménez-Jiménez and Luquin, 1996). In addition, it has been reported increased zinc (Dexter et al., 1989, 1991), decreased copper (Dexter et al., 1989, 1991; Riederer et al., 1989; Uitti et al., 1989) and normal manganese concentrations in the substantia nigra of PD patients (Dexter et al., 1991, 1992; Larsen et al., 1981).

There is little information concerning CSF concentrations of transition metals in patients with PD. The aim of this study was to assess the lumbar cerebrospinal fluid levels of iron, copper, manganese and zinc in patients with PD compared with a control population.

Patients and methods

We assessed the cerebrospinal fluid and serum levels of iron, copper, zinc, and manganese in 37 patients with PD recruited from outpatients attended in the Neurology Departments of 2 urban Hospitals. They fulfilled diagnostic criteria for PD (Hughes et al., 1992) and were evaluated with the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn et al., 1987) and the Hoehn and Yahr staging (1967). Eight patients were untreated, while the other 29 were treated with antiparkinsonian drugs alone or in combination including levodopa (26 cases), bromocriptine or pergolide (25 cases), and deprenyl (8 cases).

The control group comprised 37 "healthy" patients, who underwent lumbar puncture because of suspected (but not confirmed) subarachnoid hemorrhage or pseudotumor cerebri, oculomotor palsies or other indications in the usual neurological survey. Routine CSF analysis was normal in each patient or control. Informed consent was obtained in each case. The clinical features of both groups are summarized in Table 1.

The following exclusion criteria were applied to both patients and controls: A) Ethanol intake higher than 80 g/day in the last 6 months. B) Previous history of chronic hepatopathy or diseases causing malabsorption. C) Previous history of severe systemic disease. D) Atypical dietary habits (diets constituted exclusively by one type of foodstuff, such as vegetables, fruits, meat, or others, special diets because of religious reasons, etc). E) Previous blood transfusions, anemia, and policytemia, F) Intake of supplements of iron, copper, aluminum, zinc, or chelating agents, G) Therapy with chlorotiazides, ACTH, or steroids, H) Acute infectious disorders, traumatisms or surgery in the last 6 months.

Lumbar CSF and venous blood samples were taken from each fasted patient or control between 8.00 and 10.00 a.m. The blood samples were collected on ice and centrifuged. Traumatic spinal punctures were excluded from the study. The CSF and serum specimens were frozen at −30°C and protected from light exposure with aluminum foil until analysis. The determinations were performed blindly.

An atomic absorption spectrophotometer (AAS, model 3110) equiped with an electrothermal atomizer (model HGA 400) and an autosampler (all from Perkin-Elmer,

Table 1. Clinical data and results of PD patient and control groups. Data of quantitative variables are expressed as mean ± SD. *PD* Parkinson's disease, *ADL* activities of daily living

	PD-patients n = 37	Controls n = 37
Clinical data		
Age (years)	65.7 ± 8.8	62.4 ± 17.8
Female	23	21
Male	14	16
Age at onset of PD (years)	58.7 ± 9.8	
Duration of PD (years)	7.0 ± 6.4	
Scores of the Unified PD Rating Scale (UPDRS)		
Total (items 1–31)	39.3 ± 15.9	
ADL subscale (items 5–17)	16.8 ± 8.4	
Motor subscale (items 18–31)	21.6 ± 7.4	
Hoehn and Yahr stage	2.9 ± 1.1	
Transition metals levels		
Iron		
CSF (mg/l)	0.17 ± 0.17	0.21 ± 0.15
Serum (mg/l)	1.01 ± 0.33	0.95 ± 0.30
Copper		
CSF (μg/l)	104.9 ± 86.3	109.1 ± 88.2
Serum (mg/l)	1.06 ± 0.31	0.94 ± 0.27
Zinc		
CSF (mg/l)	0.10 ± 0.06*A	0.17 ± 0.14*A
Serum (mg/l)	0.82 ± 0.23	0.77 ± 0.17
Manganese		
CSF (μg/l)	1.20 ± 0.98	0.88 ± 0.76
Serum (μg/l)	0.93 ± 0.81	1.22 ± 0.59
Nutritional markers (serum)		
Retinol (μmol/l)	1.89 ± 0.53	1.84 ± 0.51
Total proteins (g/dl)	7.1 ± 0.5	7.2 ± 0.5
Albumin (g/dl)	4.4 ± 0.4	4.3 ± 0.5

*A: $p < 0.05$

Beaconsfield, Bucks, UK) were used for the analysis of iron, copper, and manganese in the CSF. The instrument parameters are shown in Table 2. The furnace operating conditions were slightly modified (Table 3) to eliminate matrix interference and to maximize data reproductibility and the life span of the graphite tubes. CSF specimens were centrifuged at 3,500 rpm for 5 minutes, and 500 μl of the supernatant were appropriately diluted with 0.5% nitric acid. Quadruplicate aspirations were averaged for calculations. CSF zinc concentrations were measured using a Perkin-Elmer 2380 spectrophotometer according to Meret and Henkin (1971).

Copper and zinc were determined in serum by AAS using a Perkin-Elmer 2380 spectrophotometer after the proper dilution (1/4). The iron analysis was carried out by AAS using a Perkin-Elmer 2380 spectrophotometer according to Olson and Hamlin (1969). The instrument parameters are shown in Table 4. Serum manganese levels were performed with the same working conditions than those used for the CSF analysis.

Table 2. Instrumental conditions of the electrothermal atomic absorption spectrophotometer 3110, equiped with HGA 400

	Manganese	Iron	Copper
Wavelenght (nm)	279.5	248.3	324.8
Slit width (nm)	0.2	0.2	0.7
Mode	Peak area	Peak area	Peak area
Inert gas	Argon	Argon	Argon
Lamp current (mA)	30	30	30
Integration time (s)	6	6	6
Sample volume (μl)	20	20	20
Background correction	Deuterium lamp	None	None

Table 3. Furnace operating conditions of the electrothermal atomic absorption spectrophotometer 3110

Step	Temperature (°C)			Ramp time (sec)			Hold time (sec)			Argon flow-rate (ml/min)
	Mn	Fe	Cu	Mn	Fe	Cu	Mn	Fe	Cu	
1. Dry	110	100	110	10	20	15	30	10	20	3
2. Charring	1,400	1,400	900	10	20	15	10	20	20	3
3. Atomization	2,200	2,400	2,000	1	1	0	5	4	4	0
4. Cleaning	2,650	2,650	2,650	1	1	1	2	2	3	3

Table 4. Instrumental conditions of the flame atomic absorption spectrophotometer 2380

	Iron	Copper	Zinc
Wavelenght (nm)	248.3	324.8	213.9
Slit width (nm)	0.2	0.7	0.7
Lamp current (mA)	30	30	30

The lamps were of hollow-cathode type. The standard curves were prepared by using standard solutions (Titrisol, E. Merck, Darmstadt, Germany) under the same conditions than those used for the samples. All the analyses were performed by quadruplicate. The recoveries for iron, copper, zinc, and manganese were, respectively, 97.5 ± 2.3, 95.3 ± 1.7, 98.0 ± 4.1 and 96.3 ± 5.4%. The interday coefficients of variations, both for CSF and serum, were 12.05, 3.09, 3.51, and 3.53%; and the within-day coefficients of variation 0.17, 0.03, 0.36 and 0.29%, respectively.

The results were expressed as mean ± SD. The statistical analysis used the Biostatistical Packet of "R-Sigma Data Base" (Horus Hardware) (Moreu et al., 1990), and included the two-tailed student's t test, ANOVA, and calculation of Pearson's correlation coefficient when appropriate.

Table 5. Influence of antiparkinsonian treatment on CSF levels of transition metals (mean ± SD)

	Iron (mg/l)	Copper (μg/l)	Zinc (mg/l)	Manganese (μg/l)
Levodopa				
Yes (n = 26)	0.19 ± 0.17	113.6 ± 90.4	0.10 ± 0.05	1.02 ± 0.75
No (n = 11)	0.12 ± 0.17	83.5 ± 75.8	0.11 ± 0.10	1.62 ± 1.33
Dopamine agonist				
Yes (n = 25)	0.17 ± 0.15	115.8 ± 91.9	0.10 ± 0.04	1.06 ± 0.89
No (n = 12)	0.18 ± 0.21	78.3 ± 68.0	0.12 ± 0.09	1.49 ± 1.12
Deprenyl				
Yes (n = 8)	0.17 ± 0.23	117.8 ± 109.8	0.11 ± 0.05	1.51 ± 1.21
No (n = 29)	0.17 ± 0.16	102.4 ± 83.5	0.10 ± 0.07	1.11 ± 0.91

Results

The results are summarized in Table 1. The mean CSF and serum levels of iron, copper, zinc, and manganese did not differ significantly from those of controls, with the exception of CSF zinc levels, that were significantly decreased in PD patients as compared with controls. There was also no significant differences in the serum levels of a number of nutritional markers (retinol, total proteins, albumin), between the two study groups. Antiparkinsonian therapy did not influence significantly the CSF levels of the metals that were measured (Table 5).

There was no significant correlation in PD patients between the CSF or serum levels of iron, copper, zinc, and manganese and the following values: age, age at onset of PD, duration of PD, scores of the UPDRS (total and subtotals of Activities of Daily Living and motor examination), and the Hoehn and Yahr staging, with the exception of CSF copper concentrations and duration of the disease ($r = 0.38$, $p < 0.05$).

Discussion

In the last decade there has been an increasing interest for the possible role of transition metals in the pathogenesis of PD. Since the first report by Dexter et al. (1987), many investigators found increased iron concentrations in the substantia nigra (for revision see Gerlach et al., 1994; Jiménez-Jiménez and Luquin, 1996). In the present study we found normal CSF iron concentrations, as it was previously reported (Gazzaniga et al., 1992; Pall et al., 1987; Pan et al., 1997; Takahashi et al., 1994). In addition, CSF ferritin (Dexter et al., 1990; Kuiper et al., 1994; Pall et al., 1990) and transferrin concentrations are normal (Loeffler et al., 1994). However, the results on serum iron levels are controversial. In the present and other previous study by our group (Cabrera-Valdivia et al., 1992), using stringent exclusion criteria, we found normal serum iron concentrations, a finding that is in agreement with other authors

(Chen et al., 1992; Pan et al., 1997, Takahashi et al., 1994). Moreover, 24 hour urinary excretion of iron has reported to be normal by our group (Cabrera-Valdiviva et al., 1994). In contrast with these data, Abbot et al. (1992), and Logroscino et al. (1997), the latter in a recent population study, reported decreased serum iron levels.

Pall et al. (1987), and more recently Pan et al. (1997) reported raised copper concentration in the CSF of patients with PD. Pall et al. (1987) suggested that this metal might be raised in brain. However, two groups reported decreased levels of copper in the substantia nigra of parkinsonian patients (Dexter et al., 1989, 1991; Uitti et al., 1989). Serum levels of copper (Campanella et al., 1973; Jiménez-Jiménez et al., 1992; Pall et al., 1987) and ceruloplasmin (Campanella et al., 1973; Jiménez-Jiménez et al., 1992) were normal in previous studies with the exception of decreased copper levels in a single study (Pan et al., 1997). In agreement with our results other groups reported normal CSF copper (Gazzaniga et al., 1992; Takahashi et al., 1994) and ceruloplasmin concentrations (Loeffler et al., 1994).

Dexter et al. (1989, 1991) reported increased zinc levels in the substantia nigra, lateral putamen, and caudate nucleus in patients with PD. The authors related this increase of zinc to an attempt of protection against oxidative stress. Other authors have not found alterations in zinc levels in parkinsonians' brain (Riederer et al., 1989; Uitti et al., 1989). Serum zinc levels have been reported to be normal (Jiménez-Jiménez et al., 1992) or decreased (Abbot et al., 1992; Pan et al., 1997). In this study, we found decreased CSF zinc levels, in contrast with the normal ones found in other previous shorter studies (Pan et al., 1997; Takahashi et al., 1994), but serum zinc levels were normal.

Finally, brain (Dexter et al., 1991; Dexter et al., 1992; Larsen et al., 1981), CSF (Gazzaniga et al., 1992; Pall et al., 1987; Pan et al., 1997), and serum manganese levels were normal (Jiménez-Jiménez et al., 1995; Pan et al., 1997) in previous studies, as it was the case in this report.

A recent epidemiological study have shown that occupational study to copper, manganese, and to the combinations lead-copper, lead-iron, and iron-copper, were associated with the risk for PD (Gorell et al., 1997). In contrast, dietary habits of PD patients regarding consumption of foodstuffs rich in iron, copper, zinc, and manganese, were similar to those of their spouses (Ayuso-Peralta et al., 1997).

The results of the present study showed as the main result a decrease of CSF levels of zinc, and there was no correlation between the CSF iron, zinc, copper, and manganese levels and the analyzed clinical features of PD. These results suggest that low CSF zinc concentrations might be related with the risk for PD, although they also could be related with oxidative stress processes and with the pathogenesis of this disease.

Acknowledgments

This work was supported in part by the grants FIS 97/1262, 97/5227, 97/5228, and 97/5578 and by the "Fundación Neurociencias y Envejecimiento".

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