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Regional distributions of manganese, iron, copper, and zinc in the brains of 6-hydroxydopamine-induced parkinsonian rats

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Abstract Time courses of changes in manganese, iron, copper, and zinc concentrations were examined in regions of the brain of a 6-hydroxydopamine (6-OHDA)-induced rat model of Parkinson's disease using inductively coupled plasma mass spectrometry (ICP-MS). The concentrations were simultaneously determined in brain section at the level of the substantia nigra 1, 3, 7, 10, 14, and 21 days after the 6-OHDA treatment and compared with those of control rats. The distributions of these elements were obtained for 18 regions of the sagittal section (1-mm thick). The ICP-MS results indicated that Mn, Fe, Cu, and Zn levels of the 6-OHDA-induced parkinsonian brain were observed to increase in all regions that lay along the dopaminergic pathway. In the substantia nigra, the increase in Mn level occurred rapidly from 3 to 7 days and preceded those in the other elements, reaching a plateau in the 6-OHDA brain. Iron and Zn levels increased gradually until 7 days and then increased rapidly from 7 to 10 days. The increase in the copper level was slightly delayed. In other regions, such as the globus pallidus, putamen, and amygdala, the levels of Mn, Fe, Cu, and Zn increased with time after 6-OHDA treatment, although the time courses of their changes were region-specific. These findings contribute to our understanding of the roles of Mn and Fe in the induction of neurological symptoms and progressive loss of dopaminergic neurons in the development of Parkinson's disease. Manganese may hold the key to disturbing cellular Fe homeostasis and accelerating Fe levels, which play the most important role in the development of Parkinson's disease.

Keywords Manganese · Iron · Copper · Zinc · Brain · Parkinson's disease

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Introduction

Parkinson's disease is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra [1]. In Parkinson's disease, large amounts of iron are known to concentrate in substantia nigra. Postmortem studies of the substantia nigra from parkinsonian patients have suggested that oxidative stress is an important factor in the pathology of the disease [1, 2]. Recently, some authors have demonstrated the link between oxidative stress and increased iron levels in the parkinsonian substantia nigra [3]. Brain Mn is also thought to play an important role in some neurodegenerative diseases and to lead to a neurological disorder resembling Parkinson's disease [4], but these theories are still controversial. Although many hypotheses have been proposed, the mechanisms responsible for Fe accumulation in the parkinsonian substantia nigra and the relationships between Fe and other elements remain unknown [5]. Quantitative information regarding the regional distributions of Fe and Mn in the brain will provide fundamental insight into such symptoms. On the other hand, Zn is known

to accumulate markedly and selectively in the substantia nigra of the brain in patients with Parkinson's disease. The excitatory effect of Zn may compensate for the loss of dopaminergic neurons in this region during development of the disease [6]. Copper levels were reported to be decreased in the substantia nigra in Parkinson's disease [7]. However, little is known systematically about the time course of changes in concentration and regional distribution of these elements in the brain in Parkinson's disease due to difficulties in reliably measuring very low concentrations of these elements in small brain samples. To resolve these issues simultaneously, methods are required for trace element analysis using inductively coupled plasma mass spectrometry (ICP-MS) and a parkinsonian rat model with a selective neurotoxin.

ICP-MS can be used for simultaneous multielemental analysis in small samples, as it has both excellent sensitivity and very low detection limits [8, 9]. Previously, we examined optimized parameters in ICP-MS and used the method for analysis of Mn, Fe, Cu, and Zn levels on the order of several hundred ng/g of thin brain slices [8]. In the present study, the ICP-MS technique was applied to determine the Mn, Fe, Cu, and Zn contents in the brain regions of a rat model of Parkinson's disease. A catecholamine neurotoxin, 6-hydroxydopamine (6-OHDA), was used to produce a rodent model of Parkinson's disease by stereotactic injection into the medial forebrain bundle or substantia nigra, which has been the standard model for many years [10].

In the present study, we determined the concentrations of Mn, Fe, Cu, and Zn in a series of brain regions simultaneously at various stages during the development of the parkinsonian model and discuss here the processes of regional accumulation of these elements, especially Mn and Fe, in relation to the neurodegenerative process of Parkinson's disease.

Materials and methods

Animal experiments

All animal experiments were carried out in compliance with the guidelines for the care and use of laboratory animals and approved by the Committee on Animal Experimentation of Kanazawa University.

Wistar rats were anesthetized with pentobarbital (50 mg kg^{-1}, i.p.), and unilateral lesions of the left medial forebrain bundle were made by injection of 15 μg of 6-OHDA hydrobromide (Sigma, St. Louis, MO, USA) in 5 μL of sterile saline containing 0.01% ascorbic acid. The stereotactic coordinates for the lesions were 3.2 mm rostral to the interaural line, 1.3 mm left of the midline, and 7.0 mm ventral to the dural surface. The incisor bar was set 2.4 mm below the level of the ear bars. The 6-OHDA solution was delivered by a microinjection pump at 1 μL min^{-1}, and the cannula was left in place for 5 min after the end of injection [11].

The 24 6-OHDA-treated Wistar rats (6-OHDA rats) and 24 control Wistar rats (control rats) used in this experiment were divided into six groups of four rats each on 1, 3, 7, 10, 14, and 21 days after treatment. Rats were housed five to six per cage in a temperature-controlled room under a light/dark cycle with free access to tap water (Kanazawa Water and Energy Center; Mn, Fe, Cu, and Zn concentrations in the water were 0.001, 0.03, 0.01, and 0.005 μg g^{-1}, respectively) and standard diet (Oriental Yeast Inc., Kyoto, Japan; Mn, Fe, Cu, and Zn concentrations in the diet were 77.8, 166, 9.2, and 69.4 μg g^{-1}, respectively) ad libitum prior to the experiments.

The 6-OHDA lesion of rats at 10, 14, and 21 days after lesioning was evaluated with methamphetamine (3 mg kg^{-1} i.p.) administration induced rotational testing. Rats were placed in a cylindrical container (30 cm in diameter), and left and right full-body turns were counted. Motor disturbance was assessed by full-rotations per min at 10-min intervals for the first 60 min after injection, and the mean number of rotations per minute was calculated. We have routinely excluded rats showing less than seven full turns per minute in the methamphetamine challenge rotational test from the experiment [12]. We can now prepare the parkinsonian model rats with a high level of confidence. However, due to partial lesions and considerable individual differences in the early stages, the rotational test was not easy to evaluate for rats at 1, 3, and 7 days after 6-OHDA treatment.

The 6-OHDA and control rats were anesthetized with ether and subjected to intracardiac perfusion with 0.9% saline, and were then dissected for excision of the brain. The brain samples were weighed and frozen. As shown in Fig. 1, a sagittal section 1-mm thick was obtained at the level of the substantia nigra and then the slice samples were divided into 18 regions. Each region was identified according to the stereotactic brain atlas of Paxinos and Watson [13] and Paxinos and Franklin [14]. The wet samples were weighed accurately and used for the experiments. The titanium instruments used for collecting tissue, weighing, and transportation were made from the same materials in all cases and care was taken to avoid contamination of samples during collection and treatment [15]. The weights of the brain regions ranged from 2 to 15 mg.

ICP–MS measurement conditions and sample preparation

ICP–MS was performed using a Seiko SPQ-9000 (Seiko Instruments Inc., Chiba, Japan), as described previously [8]. Quality control was performed routinely to ensure that the instrument satisfied the manufacturer's recommendations for performance criteria. To eliminate interference from polyatomic ions, especially the $^{40}Ar^{16}O^{+}$ ion on $^{56}Fe^{+}$, the ICP-MS was carefully set up in cold plasma conditions and optimized for the best

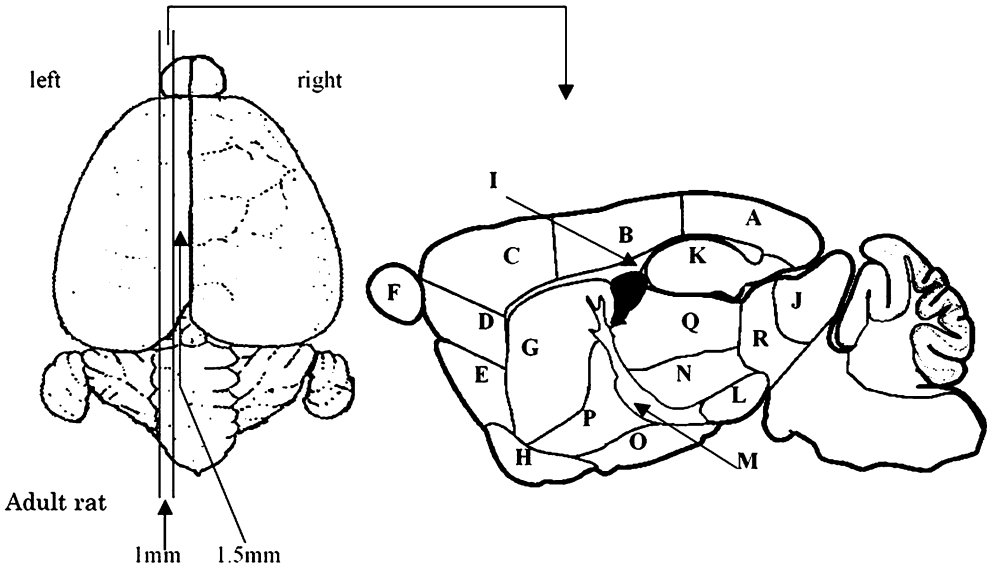

Fig. 1 Sagittal slice map. Sagittal slices 1 mm in thickness were divided into 18 regions (*A* visual cortex, *B* somatosensory cortex, *C* motor cortex, *D* agranular insular cortex, *E* piriform cortex, *F* olfactory bulb, *G* putamen (striatum), *H* olfactory tubercle, *I* corpus callosum, *J* the superior and inferior colliculus, *K* hippocampus, *L* substantia nigra, *M* internal capsule, cerebral peduncle, *N* thalamic nucleus, *O* amygdala, *P* globus pallidus, *Q* subthalamic nucleus, *R* mesencephalic nucleus, layer superior colliculus). This figure shows sections taken from the atlas reported by Paxinos and Watson [13] and Paxinos and Franklin [14]

combination of high sensitivity and low background noise [16]. The chamber gas pressure was increased and the radiofrequency power was lowered to obtain these optimized parameters. Stable ^{55}Mn, ^{56}Fe, ^{63}Cu, and ^{64}Zn isotopes were measured simultaneously with the parameters shown in Table 1. The procedural detection limits of Mn, Fe, Cu, and Zn were 8, 70, 27, and 30 pg g^{-1}, respectively. Each day before starting analysis and after all injections, the tubing used was conditioned by rinsing with HNO_3 (5%) to prevent contamination. The siphon was washed several times with high-purity water prior to use to avoid contamination of the metals from the previous measurements. Calibration was performed for six standards and the correlation coefficients (r) ranged from 0.9988 to 0.9999. Elemental concentrations are shown in μg g^{-1} wet weight. Values are shown as means ± SD (standard deviation) for four rats.

Table 1 ICP–MS instrumental operating parameters

Parameter	Value
Radiofrequency power	0.9 kW
Plasma gas flow	16.0 L min^{-1}
Nebulizer gas flow	1.0 L min^{-1}
Auxiliary gas flow	1.0 L min^{-1}
Chamber gas	30.0 kgf
Nebulizer	Cross flow
Spray chamber	Double pass
Sample uptake	1.0 mL min^{-1}
Scanning mode	Peak top
Dwell time	15 s
Sweeps/reading	3
Mn measurement	^{55}Mn
Fe measurement	^{56}Fe
Cu measurement	^{63}Cu
Zn measurement	^{64}Zn

Brain samples were subjected to closed-vessel microwave digestion to minimize sample contamination and analyte loss. A 0.7-mL aliquot of ultra-pure HNO_3 containing less than 10 ng L^{-1} of Mn, Cu, and Zn and less than 55 ng L^{-1} of Fe (Wako Pure Chemicals Industries Ltd., Osaka, Japan) was added to each sample of 2–15 mg in a Teflon/PFA vessel in the microwave digestion system [17]. Microwave-assisted digestion was carried out at 400 W for 20 s, and the solution was checked visually for completeness of digestion. If a clear solution was not achieved, the digestion was continued for another 20 s at 400 W. For ICP-MS measurements, the solution was then transferred carefully into acid-cleaned polypropylene tubes and diluted to 20 mL with high-purity water [18]. Multielemental external calibrating solutions (calibration range 0–10 μg L^{-1}) were prepared from multielement standard solution (100 mg L^{-1} Cd, Cr, Cu, Fe, Mn, Na, and Pb; Wako Pure Chemicals Industries Ltd.). High-purity water was used to dilute the samples and calibration solutions.

Statistical analysis

All data are expressed as means ± SD for the number ($n = 4$) of independent experiments performed. Statistical analysis of the data was performed using the Student's t-test. Values of $p < 0.05$ were taken as statistically significant.

Table 2 Concentrations of manganese in the brain regions of control and 6-OHDA-treated rats 1, 3, 7, 10, 14, and 21 days after treatment (values in μg g^{-1})

Region	Days					
	1	3	7	10	14	21
A						
Control	0.86 ± 0.13	0.84 ± 0.13	0.85 ± 0.13	0.84 ± 0.13	0.83 ± 0.12	0.85 ± 0.13
6-OHDA	0.79 ± 0.04	0.82 ± 0.05	0.87 ± 0.09	0.86 ± 0.05	0.90 ± 0.09	0.88 ± 0.09
B						
Control	0.92 ± 0.08	0.89 ± 0.08	0.91 ± 0.08	0.90 ± 0.08	0.88 ± 0.08	0.91 ± 0.08
6-OHDA	0.84 ± 0.03	0.81 ± 0.13	0.78 ± 0.06*	0.93 ± 0.09†	0.92 ± 0.06	0.84 ± 0.15
C						
Control	0.81 ± 0.06	0.79 ± 0.06	0.81 ± 0.06	0.80 ± 0.06	0.79 ± 0.06	0.81 ± 0.06
6-OHDA	0.83 ± 0.04	0.85 ± 0.07	0.76 ± 0.07	0.98 ± 0.10*†	0.91 ± 0.10	0.82 ± 0.11
D						
Control	0.92 ± 0.06	0.90 ± 0.06	0.91 ± 0.06	0.91 ± 0.05	0.89 ± 0.05	0.91 ± 0.06
6-OHDA	0.86 ± 0.02	0.76 ± 0.06† *	0.79 ± 0.07*	0.99 ± 0.12†	0.87 ± 0.07	0.86 ± 0.11
E						
Control	0.91 ± 0.05	0.8 ± 0.05	0.90 ± 0.05	0.89 ± 0.05	0.88 ± 0.05	0.90 ± 0.05
6-OHDA	0.81 ± 0.06	0.84 ± 0.06	0.83 ± 0.05	0.94 ± 0.03†	0.98 ± 0.07	0.89 ± 0.05
F						
Control	1.02 ± 0.05	1.00 ± 0.05	1.01 ± 0.05	1.00 ± 0.05	0.98 ± 0.05	1.01 ± 0.05
6-OHDA	0.93 ± 0.10	0.88 ± 0.09	0.98 ± 0.05	1.02 ± 0.12	1.09 ± 0.05*	1.09 ± 0.03*
G						
Control	1.01 ± 0.12	0.98 ± 0.12	1.00 ± 0.12	0.99 ± 0.12	0.97 ± 0.11	1.00 ± 0.12
6-OHDA	0.95 ± 0.12	1.15 ± 0.06*†	1.19 ± 0.02*	1.33 ± 0.07***††	1.43 ± 0.08***	1.00 ± 0.06†††
H						
Control	1.46 ± 0.17	1.44 ± 0.17	1.40 ± 0.08	1.33 ± 0.07	1.41 ± 0.17	1.44 ± 0.17
6-OHDA	1.35 ± 0.06	1.34 ± 0.05	1.22 ± 0.04**†	1.31 ± 0.05†	1.42 ± 0.04†	1.33 ± 0.05†
I						
Control	1.30 ± 0.17	1.27 ± 0.17	1.28 ± 0.08	1.13 ± 0.05†	1.24 ± 0.16	1.29 ± 0.17
6-OHDA	1.26 ± 0.06	1.27 ± 0.06	1.07 ± 0.04***†††	1.11 ± 0.06	1.01 ± 0.07*	1.17 ± 0.10†
J						
Control	1.01 ± 0.14	0.9 ± 0.14	0.99 ± 0.08	0.99 ± 0.14	0.97 ± 0.13	1.00 ± 0.14
6-OHDA	1.01 ± 0.09	1.12 ± 0.11	1.05 ± 0.06	1.18 ± 0.09†	1.13 ± 0.05	1.07 ± 0.06
K						
Control	1.04 ± 0.08	1.00 ± 0.08	1.02 ± 0.08	1.01 ± 0.08	0.99 ± 0.08	0.92 ± 0.01
6-OHDA	1.01 ± 0.06	1.05 ± 0.05	1.10 ± 0.08	1.23 ± 0.09*	1.31 ± 0.02***	1.36 ± 0.11***
L						
Control	1.15 ± 0.18	1.13 ± 0.18	1.18 ± 0.08	1.12 ± 0.17	1.09 ± 0.17	1.13 ± 0.02
6-OHDA	1.18 ± 0.02	1.24 ± 0.10	1.68 ± 0.03***†††	1.70 ± 0.04***	1.74 ± 0.02***	1.74 ± 0.06***
M						
Control	0.67 ± 0.06	0.64 ± 0.06	0.68 ± 0.06	0.66 ± 0.06	0.65 ± 0.06	0.68 ± 0.06
6-OHDA	0.62 ± 0.08	0.65 ± 0.09	0.70 ± 0.07	0.82 ± 0.07*†	0.79 ± 0.03**	0.70 ± 0.06†
N						
Control	0.85 ± 0.03	0.82 ± 0.03	0.84 ± 0.03	0.83 ± 0.03	0.82 ± 0.03	0.84 ± 0.03
6-OHDA	0.70 ± 0.06**	0.84 ± 0.10	0.81 ± 0.06	1.20 ± 0.06***†††	1.28 ± 0.07***	1.21 ± 0.12***
O						
Control	0.97 ± 0.03	0.95 ± 0.03	0.96 ± 0.03	0.96 ± 0.03	0.95 ± 0.03	0.96 ± 0.03
6-OHDA	0.91 ± 0.10	1.05 ± 0.07	1.23 ± 0.06***††	1.41 ± 0.09***†	1.51 ± 0.04***	1.50 ± 0.07***
P						
Control	1.02 ± 0.06	0.99 ± 0.06	1.00 ± 0.05	0.99 ± 0.05	0.97 ± 0.05	1.01 ± 0.06
6-OHDA	0.83 ± 0.05	0.95 ± 0.06†	1.03 ± 0.05	1.27 ± 0.04***†††	1.38 ± 0.06***†	1.19 ± 0.08**††
Q						
Control	0.81 ± 0.05	0.79 ± 0.05	0.80 ± 0.05	0.79 ± 0.05	0.78 ± 0.05	0.80 ± 0.05
6-OHDA	0.86 ± 0.07	0.90 ± 0.07	0.73 ± 0.05†	0.89 ± 0.04*†††	0.93 ± 0.04***	0.81 ± 0.05
R						
Control	0.85 ± 0.08	0.83 ± 0.08	0.84 ± 0.08	0.84 ± 0.08	0.82 ± 0.08	0.85 ± 0.08
6-OHDA	0.85 ± 0.04	0.89 ± 0.05	0.83 ± 0.02	0.83 ± 0.10	0.89 ± 0.09	0.88 ± 0.04

The concentrations of each element are indicated in μg g^{-1} wet weight. Values represent means ± SD for four rats.
Statistical significance was evaluated using the Student's *t*-test; *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively, as compared to the control value; †, ††, and ††† indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively, as compared to the value at the previous time point

Results

Methamphetamine (3 mg kg^{-1}, i.p.)-induced strong ipsilateral rotation was observed with scores of more than seven full-body turns per minute in all 12 6-OHDA-lesioned rats at 10, 14, and 21 days after treatment. Therefore, we had a high level of confidence that the other 12 rats at 1, 3, and 7 days after 6-OHDA

Table 3 Concentrations of iron in the brain regions of control and 6-OHDA-treated rats 1, 3, 7, 10, 14, and 21 days after treatment (values in μg g^{-1})

Region	Days					
	1	3	7	10	14	21
A						
Control	23.61 ± 28	23.43 ± 3.28	23.03 ± 3.18	23.34 ± 3.25	22.89 ± 3.17	23.42 ± 3.21
6-OHDA	23.19 ± 1.48	24.21 ± 1.63	21.57 ± 1.49	29.93 ± 1.85*†††	33.26 ± 0.77***†	31.25 ± 2.65**
B						
Control	25.85 ± 2.43	25.67 ± 2.43	25.14 ± 2.35	25.53 ± 2.38	25.00 ± 2.34	25.59 ± 2.42
6-OHDA	23.64 ± 1.06	24.32 ± 2.55	22.40 ± 1.28	30.64 ± 1.43*†††	34.92 ± 1.69***††	32.48 ± 2.29**
C						
Control	24.38 ± 1.75	24.20 ± 1.75	23.90 ± 1.70	24.17 ± 1.72	23.74 ± 1.70	24.12 ± 1.73
6-OHDA	23.99 ± 1.97	24.97 ± 2.04	22.60 ± 2.53	30.92 ± 2.69**†††	33.13 ± 2.07***	31.89 ± 3.00***
D						
Control	26.94 ± 2.09	26.76 ± 2.10	26.27 ± 2.02	26.62 ± 2.05	26.10 ± 2.01	26.57 ± 2.06
6-OHDA	25.50 ± 1.79	22.90 ± 1.05*†	24.05 ± 1.99	32.80 ± 2.12**†††	31.88 ± 1.77**	33.99 ± 2.85**
E						
Control	23.47 ± 0.77	23.28 ± 0.77	22.86 ± 0.75	23.21 ± 0.76	22.73 ± 0.75	23.27 ± 0.79
6-OHDA	23.14 ± 2.20	24.10 ± 2.32	23.80 ± 1.42	31.64 ± 1.46***	34.63 ± 2.56***	34.91 ± 1.79***
F						
Control	22.28 ± 1.99	22.09 ± 1.99	21.63 ± 1.92	22.01 ± 1.95	21.51 ± 1.92	22.15 ± 1.99
6-OHDA	24.20 ± 3.00	25.48 ± 3.43	26.42 ± 3.20*	30.50 ± 1.45***	35.05 ± 1.89***††	39.39 ± 3.12***
G						
Control	18.90 ± 1.35	18.67 ± 1.35	18.40 ± 1.29	18.74 ± 1.32	18.31 ± 1.29	18.94 ± 1.37
6-OHDA	17.61 ± 0.83	22.82 ± 2.74*†	28.03 ± 2.65***†	41.98 ± 3.78***†††	47.75 ± 2.64***†	44.07 ± 3.63***
H						
Control	21.52 ± 2.33	21.33 ± 2.33	21.02 ± 2.28	21.31 ± 2.31	20.89 ± 2.28	21.29 ± 2.33
6-OHDA	20.69 ± 1.17	21.50 ± 1.16	18.60 ± 0.72††	25.22 ± 1.02*†††	28.64 ± 1.22***††	27.74 ± 0.94***
I						
Control	28.62 ± 1.57	28.45 ± 1.58	27.53 ± 1.43	28.10 ± 1.49	27.39 ± 1.42	28.43 ± 1.56
6-OHDA	23.97 ± 1.69**	25.49 ± 3.84	20.66 ± 2.42***	22.42 ± 1.79***	25.53 ± 1.94	24.42 ± 1.89*
J						
Control	27.43 ± 1.18	27.26 ± 1.18	26.54 ± 1.18	27.01 ± 1.19	26.38 ± 1.20	27.14 ± 1.26
6-OHDA	24.66 ± 1.90*	26.68 ± 5.73	25.53 ± 2.04	26.82 ± 1.33	36.55 ± 1.58***†††	31.61 ± 0.79***†††
K						
Control	17.41 ± 1.46	17.12 ± 1.46	16.80 ± 1.39	17.23 ± 1.43	16.73 ± 1.39	17.66 ± 1.49
6-OHDA	19.89 ± 1.09	21.98 ± 2.84	22.38 ± 3.26*	23.32 ± 1.83***	26.56 ± 1.78***†	22.17 ± 2.23*†
L						
Control	19.00 ± 0.73	18.76 ± 0.72	18.31 ± 0.71	18.73 ± 0.73	30.55 ± 1.00†††	30.78 ± 1.38
6-OHDA	18.50 ± 0.84	28.60 ± 3.70***†††	79.44 ± 4.20***†††	238.36 ± 21.52***†††	272.02 ± 21.02***	224.92 ± 10.81***††
M						
Control	24.89 ± 0.84	24.70 ± 0.84	24.06 ± 0.85	24.53 ± 0.85	23.93 ± 0.85	24.78 ± 0.86
6-OHDA	22.49 ± 2.26	23.66 ± 2.62	25.40 ± 1.24	28.02 ± 1.05***†	28.10 ± 0.61***	25.44 ± 0.65†††
N						
Control	21.88 ± 1.45	21.69 ± 1.45	21.33 ± 1.38	21.66 ± 1.41	21.21 ± 1.38	21.72 ± 1.43
6-OHDA	17.79 ± 1.96*	21.46 ± 2.91	30.47 ± 4.75*†	34.30 ± 4.69***	37.84 ± 3.77***	37.95 ± 6.63***
O						
Control	18.19 ± 0.79	18.02 ± 0.79	17.94 ± 0.78	18.11 ± 0.79	17.84 ± 0.78	18.04 ± 0.7
6-OHDA	17.62 ± 2.49	20.60 ± 1.93*	30.05 ± 4.59***	45.95 ± 2.25***†††	53.57 ± 1.91***†††	52.69 ± 3.25***
P						
Control	25.97 ± 1.66	25.80 ± 1.67	25.03 ± 1.59	25.52 ± 1.62	24.88 ± 1.58	25.72 ± 1.64
6-OHDA	23.72 ± 1.41	27.09 ± 1.55†	30.51 ± 3.69*	33.99 ± 0.89***	38.84 ± 1.56***†††	39.40 ± 3.89***
Q						
Control	18.11 ± 1.11	17.92 ± 1.10	17.73 ± 1.09	17.97 ± 1.10	17.61 ± 1.09	17.94 ± 1.12
6-OHDA	19.09 ± 1.61	19.94 ± 1.69	16.14 ± 0.87††	22.56 ± 0.79***†††	25.51 ± 0.84***†††	20.73 ± 1.18*†††
R						
Control	19.36 ± 2.02	19.16 ± 2.02	18.87 ± 1.97	19.18 ± 2.00	18.77 ± 1.97	19.26 ± 2.03
6-OHDA	19.02 ± 0.92	19.76 ± 1.07	18.38 ± 0.39	21.81 ± 1.02***†††	27.57 ± 0.70***†††	19.55 ± 0.51†††

The concentrations of each element are indicated in μg g^{-1} wet weight. Values represent means ± SD for four rats.
Statistical significance was evaluated using the Student's *t*-test; *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively, as compared to the control value; †, ††, and ††† indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively, as compared to the value at the previous time point

treatment also had the same predispositions (to develop parkinsonism).

The ICP–MS analysis enabled the simultaneous measurement of the concentrations of Mn, Fe, Cu, and Zn in all brain region samples as reported previously [8]. The concentrations of Mn, Fe, Cu, and Zn for the brain regions at 1, 3, 7, 10, 14, and 21 days after injection in the control and 6-OHDA-treated rat brains are listed in

Table 4 Concentrations of copper in the brain regions of control and 6-OHDA-treated rats 1, 3, 7, 10, 14, and 21 days after treatment (values in μg g^{-1})

Region	Days					
	1	3	7	10	14	21
A						
Control	2.67 ± 0.21	2.65 ± 0.21	2.66 ± 0.21	2.67 ± 0.21	2.64 ± 0.21	2.72 ± 0.21
6-OHDA	2.55 ± 0.18	2.66 ± 0.20	2.37 ± 0.20	2.97 ± 0.29	2.88 ± 0.34	2.66 ± 0.21
B						
Control	2.99 ± 0.11	2.98 ± 0.11	2.97 ± 0.11	2.99 ± 0.11	2.95 ± 0.12	3.04 ± 0.12
6-OHDA	2.75 ± 0.22	2.82 ± 0.29	2.60 ± 0.27*	2.86 ± 0.32	3.18 ± 0.29	2.87 ± 0.22
C						
Control	2.95 ± 0.20	2.93 ± 0.20	2.93 ± 0.20	2.95 ± 0.20	2.91 ± 0.20	2.97 ± 0.20
6-OHDA	2.86 ± 0.12	2.97 ± 0.12	2.69 ± 0.19†	2.87 ± 0.08	3.08 ± 0.10†	2.96 ± 0.14
D						
Control	3.22 ± 0.13	3.20 ± 0.13	3.18 ± 0.13	3.21 ± 0.13	3.16 ± 0.13	3.23 ± 0.13
6-OHDA	2.95 ± 0.06**	2.65 ± 0.20***†	2.78 ± 0.09***	3.17 ± 0.12†††	2.94 ± 0.22	3.07 ± 0.05
E						
Control	2.91 ± 0.20	2.90 ± 0.20	2.89 ± 0.19	2.91 ± 0.20	2.87 ± 0.19	2.96 ± 0.20
6-OHDA	2.71 ± 0.23	2.82 ± 0.25	2.78 ± 0.07	3.01 ± 0.13†	3.12 ± 0.22	2.97 ± 0.06
F						
Control	3.43 ± 0.14	3.42 ± 0.14	3.39 ± 0.14	3.42 ± 0.14	3.36 ± 0.14	3.48 ± 0.15
6-OHDA	3.94 ± 0.06***	3.53 ± 0.20††	3.30 ± 0.35	3.40 ± 0.33	3.87 ± 0.25*	3.71 ± 0.16
G						
Control	4.02 ± 0.13	4.01 ± 0.14	3.95 ± 0.13	3.99 ± 0.13	3.93 ± 0.14	4.07 ± 0.15
6-OHDA	3.73 ± 0.21	3.67 ± 0.08***	4.49 ± 0.24**†††	4.73 ± 0.30***	5.52 ± 0.15***†††	5.33 ± 0.18***
H						
Control	3.54 ± 0.03	3.53 ± 0.03	3.50 ± 0.03	3.53 ± 0.03	3.47 ± 0.03†	3.55 ± 0.03†
6-OHDA	3.29 ± 0.18*	3.41 ± 0.17	2.95 ± 0.10***†††	3.35 ± 0.14†††	3.61 ± 0.18	3.22 ± 0.13***†
I						
Control	3.33 ± 0.07	3.32 ± 0.07	3.29 ± 0.07	3.32 ± 0.07	3.27 ± 0.07	3.43 ± 0.06†
6-OHDA	2.86 ± 0.16***	3.00 ± 0.18*	2.44 ± 0.08***†††	3.01 ± 0.40†	2.72 ± 0.34*	2.60 ± 0.22***
J						
Control	3.24 ± 0.04	3.22 ± 0.04	3.20 ± 0.05	3.23 ± 0.05	3.18 ± 0.05	3.29 ± 0.06†
6-OHDA	2.86 ± 0.10***	3.07 ± 0.11*†	3.05 ± 0.18	3.62 ± 0.27†	3.68 ± 0.29*	3.10 ± 0.15
K						
Control	2.38 ± 0.06	2.36 ± 0.06	2.39 ± 0.06	2.41 ± 0.06	2.37 ± 0.05	2.53 ± 0.05††
6-OHDA	2.33 ± 0.20	2.16 ± 0.19	2.61 ± 0.29†	3.06 ± 0.16***†	3.06 ± 0.17***	3.10 ± 0.24***
L						
Control	3.10 ± 0.10	3.10 ± 0.04	3.28 ± 0.12†	3.32 ± 0.11	3.26 ± 0.12	3.42 ± 0.13
6-OHDA	3.11 ± 0.15	3.11 ± 0.27	3.41 ± 0.42	3.63 ± 0.22*	4.09 ± 0.26***†	3.99 ± 0.18***
M						
Control	2.00 ± 0.11	1.98 ± 0.11	2.03 ± 0.11	2.04 ± 0.11	2.02 ± 0.11	2.13 ± 0.12
6-OHDA	1.83 ± 0.24	1.93 ± 0.26	2.07 ± 0.19	2.57 ± 0.14***††	2.26 ± 0.18†	2.07 ± 0.15
N						
Control	2.93 ± 0.22	2.91 ± 0.22	2.91 ± 0.21	2.93 ± 0.22	2.89 ± 0.21	2.97 ± 0.22
6-OHDA	2.57 ± 0.10*	2.73 ± 0.14	3.01 ± 0.30	3.64 ± 0.15††	3.45 ± 0.12***	3.16 ± 0.11††
O						
Control	1.94 ± 0.09	1.92 ± 0.09	1.94 ± 0.09	1.95 ± 0.09	1.93 ± 0.09	1.96 ± 0.09
6-OHDA	2.16 ± 0.34	2.21 ± 0.21*	2.81 ± 0.33†	4.13 ± 0.20†††	4.57 ± 0.24***†	4.34 ± 0.22***
P						
Control	2.30 ± 0.15	2.28 ± 0.15	2.30 ± 0.14	2.32 ± 0.15	2.29 ± 0.14	2.40 ± 0.15
6-OHDA	2.27 ± 0.22	2.13 ± 0.06	2.40 ± 0.22	3.01 ± 0.09†††	3.02 ± 0.05***	3.14 ± 0.10***
Q						
Control	3.22 ± 0.21	3.20 ± 0.21	3.18 ± 0.21	3.20 ± 0.21	3.16 ± 0.21	3.22 ± 0.22
6-OHDA	3.46 ± 0.24	3.62 ± 0.26*	2.93 ± 0.14*†††	3.35 ± 0.23†	3.28 ± 0.16	3.28 ± 0.09
R						
Control	3.13 ± 0.28	3.12 ± 0.28	3.10 ± 0.28	3.12 ± 0.28	3.08 ± 0.28	3.17 ± 0.29
6-OHDA	3.17 ± 0.24	3.29 ± 0.26	3.06 ± 0.09	2.95 ± 0.30	3.17 ± 0.16	3.25 ± 0.06

The concentrations of each element are indicated in μg g^{-1} wet weight. Values represent means ± SD for four rats.
Statistical significance was evaluated using the Student's *t*-test; *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively, as compared to the control value; †, ††, and ††† indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively, as compared to the value at the previous time point

Tables 2, 3, 4, and 5. Figure 2 compares the time courses of Mn, Fe, Cu, and Zn concentrations of the putamen (G), amygdala (O), substantia nigra (L), and globus pallidus (P) in the control and 6-OHDA rats.

Manganese concentrations showed the lowest values: 0.64–1.46 μg g^{-1} in the control and 0.62–1.74 μg g^{-1} in the 6-OHDA brain. In the control brain, Mn concentrations showed almost no changes after treatment, and

Table 5 Concentrations of zinc in the brain regions of control and 6-OHDA-treated rats 1, 3, 7, 10, 14, and 21 days after treatment (values in μg g^{-1})

Region	Days					
	1	3	7	10	14	21
A						
Control	29.34 ± 4.49	29.21 ± 4.51	28.73 ± 44.37	29.01 ± 4.45	28.44 ± 4.34	28.91 ± 4.37
6-OHDA	25.45 ± 0.44	26.56 ± 0.45	23.67 ± 0.42†††	33.94 ± 0.55†††	34.71 ± 0.52*	26.56 ± 0.54†††
B						
Control	30.40 ± 3.19	30.27 ± 3.20	29.71 ± 3.10	30.04 ± 3.13	29.41 ± 3.08	29.90 ± 3.16
6-OHDA	25.44 ± 1.65*	26.07 ± 1.34	24.13 ± 2.22*	34.06 ± 2.16†††	35.07 ± 2.33*	26.58 ± 1.59†††
C						
Control	26.24 ± 1.74	26.08 ± 1.74	25.84 ± 1.69	26.04 ± 1.72	25.58 ± 1.69	25.86 ± 1.71
6-OHDA	24.58 ± 2.18	25.58 ± 2.25	23.16 ± 2.67	32.72 ± 3.02**†††	31.68 ± 2.21***	25.49 ± 2.30††
D						
Control	27.91 ± 1.49	27.77 ± 1.50	27.37 ± 1.45	27.64 ± 1.47	27.10 ± 1.44	27.44 ± 1.48
6-OHDA	25.60 ± 2.31	22.94 ± 0.49***	24.16 ± 2.69	34.03 ± 3.26*†††	29.84 ± 1.89	26.64 ± 2.54
E						
Control	29.97 ± 2.43	29.84 ± 2.44	29.31 ± 2.36	29.63 ± 2.39	29.01 ± 2.34	29.49 ± 2.40
6-OHDA	24.24 ± 2.53*	25.24 ± 2.61*	24.97 ± 2.49*	34.29 ± 3.06†††	33.93 ± 3.92	26.61 ± 2.26†
F						
Control	23.28 ± 1.18	23.14 ± 1.18	22.81 ± 1.17	23.07 ± 1.18	22.53 ± 1.16	23.00 ± 1.20
6-OHDA	24.99 ± 2.78	25.90 ± 2.70	27.20 ± 4.31	32.32 ± 1.99***	33.57 ± 2.30***	25.26 ± 1.29*†††
G						
Control	24.60 ± 2.28	24.46 ± 2.29	24.10 ± 2.22	24.37 ± 2.25	23.80 ± 2.20	24.33 ± 2.28
6-OHDA	25.93 ± 0.63	31.53 ± 1.88***†††	38.83 ± 3.10***††	46.45 ± 2.39***††	50.12 ± 3.45***	49.36 ± 1.21***
H						
Control	27.02 ± 2.19	26.87 ± 2.19	26.50 ± 2.15	26.76 ± 2.17	26.23 ± 2.14	26.56 ± 2.19
6-OHDA	24.90 ± 0.74	25.88 ± 0.59	28.60 ± 1.84†	31.39 ± 1.21**†	32.21 ± 1.12***	29.09 ± 0.29†††
I						
Control	33.83 ± 1.10	33.78 ± 1.12	32.77 ± 1.00	33.26 ± 1.04	32.40 ± 0.98	33.31 ± 1.08
6-OHDA	36.26 ± 1.46*	37.89 ± 2.16	30.52 ± 2.49†††	34.33 ± 2.82	35.33 ± 3.17	28.28 ± 1.58***††
J						
Control	24.04 ± 3.50	23.90 ± 3.50	23.52 ± 3.44	23.80 ± 3.47	23.24 ± 3.42	23.73 ± 3.51
6-OHDA	21.13 ± 1.59	22.03 ± 3.49	21.24 ± 2.08	23.08 ± 2.19	28.41 ± 2.43*†	26.18 ± 2.55
K						
Control	39.29 ± 2.15	39.37 ± 2.17	37.67 ± 2.04	38.35 ± 2.08	37.22 ± 2.02	38.53 ± 2.13
6-OHDA	40.05 ± 1.48	37.98 ± 2.23	45.80 ± 3.75*†	49.46 ± 1.35***	50.94 ± 1.81***	52.43 ± 2.89***
L						
Control	38.23 ± 2.00	38.26 ± 2.02	36.74 ± 1.96	37.37 ± 1.98	36.31 ± 1.96	37.39 ± 2.08
6-OHDA	39.54 ± 1.85	44.41 ± 4.50*	50.45 ± 2.67***	74.35 ± 2.68***†††	72.97 ± 4.95***	63.69 ± 2.82***
M						
Control	17.64 ± 1.64	17.45 ± 1.64	17.44 ± 1.62	17.61 ± 1.63	17.17 ± 1.61	17.62 ± 1.65
6-OHDA	18.38 ± 2.21	18.64 ± 2.27	20.73 ± 1.19*	23.69 ± 2.16***	21.42 ± 1.20**	20.78 ± 1.30*
N						
Control	20.55 ± 1.03	20.39 ± 1.03	20.24 ± 0.99	20.43 ± 1.00	19.99 ± 0.97	20.33 ± 1.01
6-OHDA	18.87 ± 0.60*	22.72 ± 1.37*†††	22.11 ± 1.89	31.44 ± 1.30***†††	31.89 ± 1.77***	28.06 ± 2.03***†
O						
Control	19.00 ± 0.94	18.85 ± 0.94	18.83 ± 0.93	18.95 ± 0.94	18.66 ± 0.93	18.78 ± 0.94
6-OHDA	18.57 ± 2.56	21.71 ± 1.95*	30.49 ± 0.54***†††	49.96 ± 2.76***†††	53.64 ± 3.48***	46.91 ± 3.44***†
P						
Control	40.77 ± 0.63	40.77 ± 0.64	39.35 ± 0.58†	39.96 ± 0.60	38.94 ± 0.58	39.89 ± 0.62
6-OHDA	36.29 ± 1.98**	41.56 ± 3.29	48.72 ± 1.46***††	53.87 ± 3.07***†	55.62 ± 3.73***	48.76 ± 4.00***†
Q						
Control	21.98 ± 1.13	21.83 ± 1.13	21.62 ± 1.12	21.82 ± 1.12	21.39 ± 1.12	21.63 ± 1.14
6-OHDA	23.30 ± 1.88	24.34 ± 1.97	19.71 ± 0.97*††	28.46 ± 0.90***†††	29.08 ± 0.86***	28.31 ± 0.71***
R						
Control	21.62 ± 2.45	21.47 ± 2.46	21.25 ± 2.39	21.46 ± 2.42	20.99 ± 2.38	21.36 ± 2.43
6-OHDA	22.10 ± 2.22	22.96 ± 2.43	21.31 ± 1.14	26.16 ± 2.24*††	29.83 ± 1.51***†	30.73 ± 0.81***

The concentrations of each element are indicated in μg g^{-1} wet weight. Values represent means ± SD for four rats.
Statistical significance was evaluated using Student's *t*-test; *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively, as compared to the control value; †, ††, and ††† indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively, as compared to the value at the previous time point

high Mn concentrations were observed in the olfactory tubercle (H), corpus callosum (I), and substantia nigra (L) relative to the cortexes (A–C) (Table 2). In the 6-OHDA brain, Mn concentrations increased significantly after treatment in the hippocampus (K), substantia nigra (L), hypothalamus (N), amygdala (O), and globus pallidus (P) (Table 2). Manganese concentrations in the substantia nigra (L) were higher than those in the other regions, and increased rapidly from 3 to 7 days, reaching a plateau at 7 days after 6-OHDA treatment (Fig. 2).

Iron concentrations showed a marked change several days after 6-OHDA treatment. In the control rats, however, Fe concentrations showed almost no change during the 3-week observation period (Table 3). In the substantia nigra (L) of the 6-OHDA rats, Fe concentrations increased gradually until 7 days, then increased rapidly from 7 to 10 days, and reached a plateau and a level 14-fold higher than the level of control rats (Table 3, Fig. 2). In other regions, such as the putamen (G), globus pallidus (P), and amygdala (O), which lay along the dopaminergic pathway, the Fe concentrations showed marked increases of 2.6, 1.5, and 3.0-fold, respectively (Fig. 2).

Changes in Cu levels were not so large in all measured regions, except in the amygdala (O) during the 3-week observation period after 6-OHDA treatment. In some regions, the Cu concentrations in the 6-OHDA brains showed slight but significant decreases in comparison with those of the control brains (Table 4). In the substantia nigra (L), Cu concentrations increased gradually

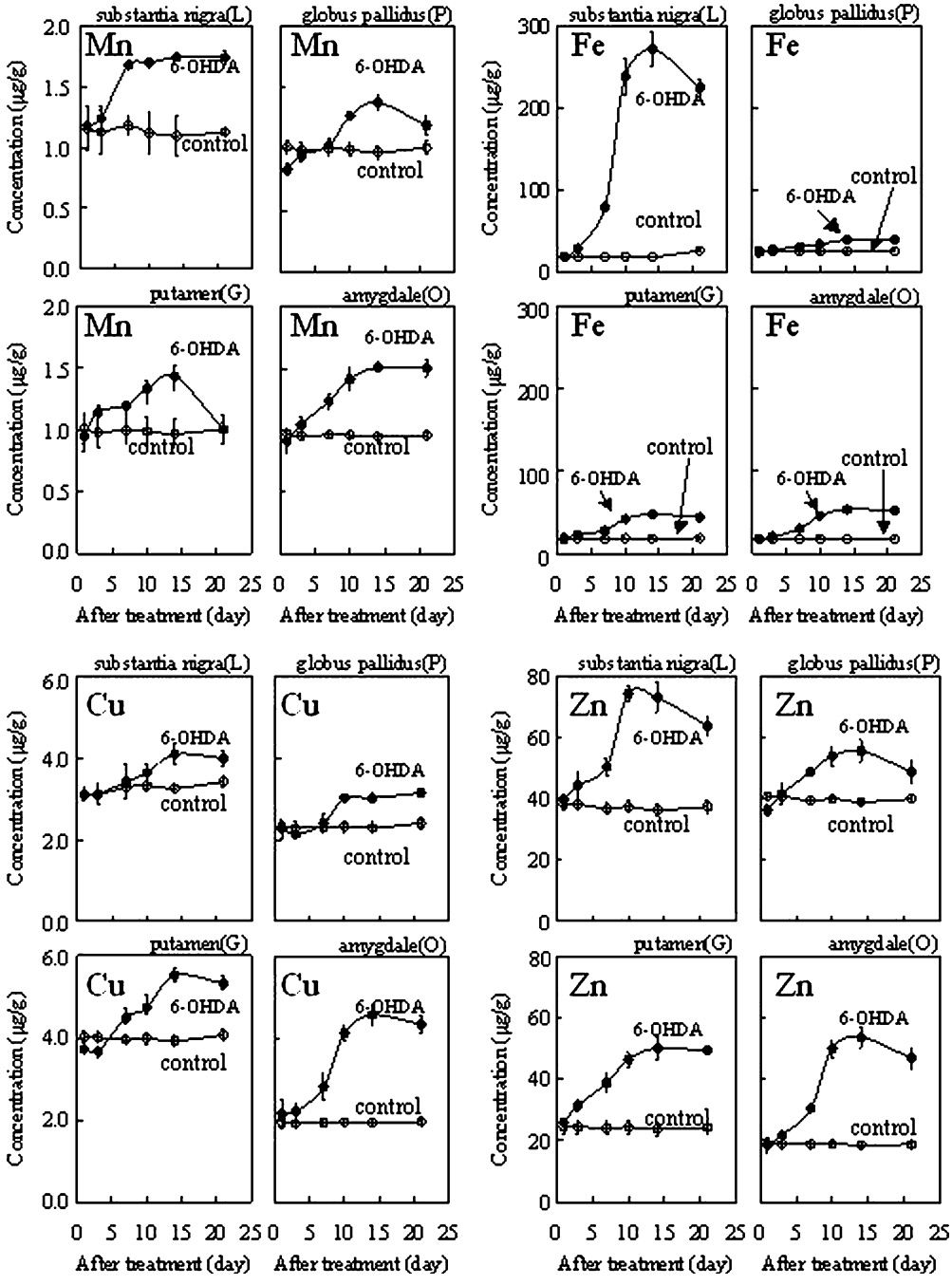

Fig. 2 Changes in Mn, Fe, Cu, and Zn concentrations in the substantia nigra (*L*), globus pallidus (*P*), putamen (*G*), and amygdala (*O*) regions of control and 6-OHDA-treated rats 1, 3, 7, 10, 14, and 21 days after treatment

and reached a plateau at 14 days after treatment (Fig. 2).

In the control brains, high Zn concentrations were observed in the hippocampus (K), substantia nigra (L), and globus pallidus (P) relative to the other regions (Table 5). In the 6-OHDA brains, the Zn levels also increased in those regions and the putamen (G), gradually up to 7 days and then rapidly from 7 to 10 days, and reached a plateau at 14 days after treatment (Table 5, Fig. 2).

Discussion

Parkinson's disease is a progressive neurological disorder characterized by the impairment of motor function, including bradykinesia, resting tremor, rigidity, gait abnormalities, and postural instability. Changes taking place after unilateral 6-OHDA lesion of the dopaminergic pathway in rats have been studied by performing spontaneous, amphetamine-induced and apomorphine-induced rotational behavior testing and tyrosine hydroxylase (TH) and Fos protein immunohistochemistry [19]. We estimated the degree of parkinsonian rat, which was termed the "neurodegenerative ratio" (NDR) by Labandeira-Garcia et al. [19] and He et al. [20]. Figure 3 compares this with the relative concentration ratios (RCRs) of Mn, Fe, Cu, and Zn, which were calculated from the following equation:

$$\mathrm{RCR} = \frac{(\text{concentration at time of interest after treatment})}{(\text{maximum concentration})} \times 100$$

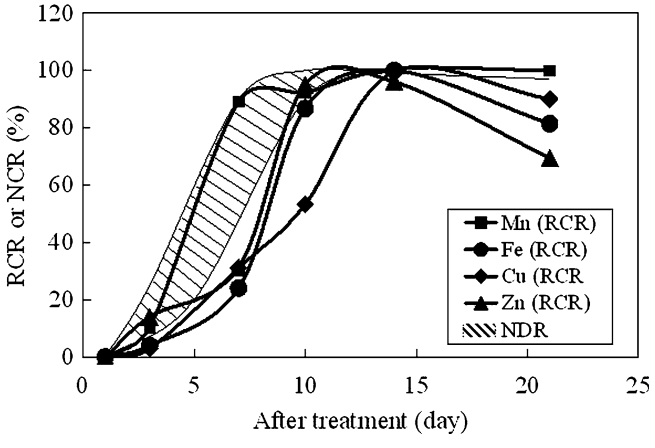

Fig. 3 Time course of changes in the relative concentration ratios (RCRs) of Mn, Fe, Cu, and Zn and in the neurodegenerative ratio (NDR) in the substantia nigra (*L*) of the treated rats, where the 6-OHDA was injected. *NDR* indicates the degree of lesion by 6-OHDA, as estimated by Labandeira-Garcia et al. [19] and He et al. [20]. *RCR* refers to the relative concentration ratios of Mn, Fe, Cu, and Zn, which were calculated by using the following equation: *RCR* = (concentration at time of interest after treatment)/(maximum concentration)×100

We now discuss the relevance of our results to severe and progressive neuron loss in the dopaminergic pathway (in the injected substantia nigra (L) and other globus pallidus (P) and putamen (G) sites).

Oxidative stress has been suggested to be important in the development of Parkinson's disease, either as a primary causal factor or as a secondary contributory factor [21]. The observation that Fe homeostasis is altered in Parkinson's disease is pivotal to this hypothesis. In the present study, Fe concentration was found to increase rapidly from 7 to 10 days after 6-OHDA treatment in the substantia nigra (L) (Fig. 2). The increase in Fe level was considered to be the result of the marked reduction in total aconitase activity. In general, aconitase is known to catalyze reactions involved in mitochondrial energy production and functions in the regulation of cellular Fe homeostasis [22]. On the other hand, Mn exposure significantly inhibits mitochondrial aconitase activity in the brain regions associated with parkinsonism. In this study, Mn increased rapidly from 3 to 7 days after 6-OHDA treatment in the substantia nigra (L) (Fig. 2). Our data corroborate the observation that the increase in Mn inhibits total aconitase activity, destroys the cellular Fe homeostasis, and then accelerates the increase in Fe level. Moreover, the increasing trend in the relative Mn concentration ratios (Mn RCRs) is similar to the pattern of NDR (Fig. 3). The result may prove the importance of Mn in the development of Parkinson's disease. On the other hand, the Fe increase was delayed a little from the NDR pattern, and reached a plateau later than the NDR increase.

The main Fe storage protein in all living species is ferritin, which protects tissues from the toxic effects of Fe [23]. 6-OHDA is a potent oxidant, and reactive oxygen species may be formed during its auto-oxidation. Moreover, 6-OHDA has been shown to induce the release of Fe from ferritin, resulting in Fe accumulation, and the free Fe contributes to the formation of hydroxyl radicals [20]. The protein lactoferrin may act as an Fe scavenger at these active sites, where it is synthesized and accumulated [24]. The ferritin levels were reported to reduce in the substantia nigra (L) and globus pallidus (P) in Parkinson's disease [7]. However, 7–10 days after 6-OHDA treatment, the total concentrations of Fe were also found to increase rapidly in both regions (Table 3), suggesting that a great deal of loosely bound Fe released from ferritin moves and accumulates, and is scavenged and retained in parts of these regions. Oxidative stress will be triggered by the Fenton reaction. It is thought that the Fenton reaction may be initiated by a small amount of loosely bound Fe, which may cause damage to the neurons. The initiation of the reaction and the damage to neurons may be due to other causes. It is not surprising that in the substantia nigra (L) and other dopaminergic regions, high levels of Fe could play an important role in the loss of dopaminergic neurons.

It is widely thought that Mn is linked to some induced Parkinson's disease-like syndromes, but is not a

risk factor for idiopathic Parkinson's disease. The concentration of Mn was found to increase in the early stage (3–7 days) after 6-OHDA treatment and then reached a plateau more rapidly than other elements in the substantia nigra (L) of the treated brain, although it showed no changes in the control brain (Fig. 2). Rapid Mn accumulation in the substantia nigra (L) could be importantly related to the development of Parkinson's disease, because the disease is a progressive neurological disorder associated with selective degeneration of the dopaminergic neurons in these regions. Moreover, the Mn accumulation may stimulate DA auto-oxidation within the dopaminergic neurons in this region, a process accompanied by an increase in the formation of quinones and protein-bound cysteinyl dopamine and cysteinyl dihydroxyphenylacetic [25]. The levels of Mn in the substantia nigra (L) were 1.18 $\mu g\ g^{-1}$ in the control brain but had increased to 1.68 $\mu g\ g^{-1}$ in the 6-OHDA brain 7 days after treatment (Table 2). Short-term exposure to a sufficiently high concentration of Mn is thought to be sufficient to induce secondary toxic mechanisms, such as a marked reduction in total aconitase activity. In the substantia nigra (L), the Mn level increased rapidly from 3 to 7 days (earlier than the other elements), reaching a plateau in the 6-OHDA brain. Iron and Zn levels increased gradually up to 7 days and then increased rapidly from 7 to 10 days. The increase in the copper level was slightly delayed (Fig. 3). Our results suggested that in the substantia nigra (L), increasing levels of Mn accelerate increases in levels of Fe and other elements and lead to a neurodegenerative disorder.

In the other regions, like the globus pallidus (P), putamen (G), and amygdala regions (O), the levels of Mn, Fe, Cu, and Zn increased with time after 6-OHDA treatment, although the time courses were region-specific (Fig. 2). This suggests that the rapid Mn accumulation and subsequent Fe increase in the 6-OHDA-treated substantia nigra (L) may lead to some neurotoxic effects in the globus pallidus (P) and putamen (G) regions, some variations in concentrations of enzymes, and simultaneous increases in the levels of Mn, Fe, Zn, Cu, and trace elements, and result in progressive loss of dopaminergic neurons in these regions. In a human study, chronic Mn intoxication was reported to cause degeneration of the basal ganglia with marked gliosis of the globus pallidus (P) and putamen (G) [26]. It has been reported that Zn accumulates in the substantia nigra (L) of the brain, where Parkinson's disease develops [6]. In the present study, in almost all regions of the 6-OHDA-treated brain, Zn concentrations were also shown to increase markedly and in a region-specific manner after Mn from 7 to 10 days after treatment (Fig. 2). However, Cu concentrations fluctuated from region to region after treatment (Fig. 2). Simultaneous information on Mn, Fe, Zn, and Cu behavior along the dopaminergic pathway is expected to lead to a much better understanding of neurodegenerative loss of dopaminergic neurons.

Conclusions

Simultaneous multielemental measurements provided new information about the process of accumulation and transport of these essential elements during the development of Parkinson's disease. In the 6-OHDA-treated substantia nigra (L), increases in the concentration of Mn occurred earlier than those in the other elements, reaching a plateau, and then a marked increase in Fe level was triggered. In the other regions, such as the globus pallidus (P) and putamen (G), which occur along the dopaminergic pathway, the levels of Mn, Fe, Cu, and Zn increased with time after 6-OHDA treatment, although their time courses were region-specific. These findings add to our understanding of the relationship between Mn and Fe in the induction of neurological symptoms and progressive loss of dopaminergic terminals. In particular, Mn plays the most important role in disturbing cellular Fe homeostasis and accelerating the increase in Fe levels in the substantia nigra (L).

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